UK Strategy for Research and Development on Human and Animal Health Aspects of Transmissible Spongiform Encephalopathies

2005-2008
Preface

Since the announcement in 1996 of a possible link between BSE in cattle and vCJD in humans two strategy documents for research and development into transmissible spongiform encephalopathies (TSEs) have been produced by the UK government. TSE research has advanced significantly during the past few years, and as senior representatives of the UK public funders of TSE research, we considered it timely to review the current understanding of these diseases and set out a new research strategy that reflected the changes in knowledge.

This document represents the first UK joint human and animal health strategy for TSE research and addresses issues that affect livestock, the food chain and public health. It highlights the improved co-ordination that has taken place between government departments since the publication of the Phillips Report and reflects the overlapping issues of relevance to both human and animal health.

In the production of this document, we have drawn on a wide range of advice from all of the UK’s major funders of TSE research and from members of specialist advisory committees such as SEAC, which are detailed within. We have also undertaken wide consultation in the UK and overseas in an attempt to ensure the accuracy of the information included. We are particularly indebted to Professor Chris Bostock (formerly director of the Institute for Animal Health) for his 2004 review of the science, which forms the backbone of this document.

With numbers of BSE cases continuing to fall and vCJD cases apparently stabilising in the UK, it would be easy to be complacent. However, the increasing incidence of chronic wasting disease in the USA reinforces the concern that TSEs are an international problem and one that will need to be monitored carefully for a number of years. In the UK, there remain key issues that continue to pose threats to animal and human health. Not least is the possibility of a non-symptomatic carrier state or the emergence of BSE-like illness in another species such as sheep.

While every attempt is made to reduce exposure to disease it is not always possible to eradicate it completely. Disease management is thus often based on risk assessments of potential exposure and these are based on the latest scientific results.

This document aims to highlight the major scientific uncertainties relating to TSEs and how these will be addressed by the research and development strategies of the major UK funders. The necessary experience of managing BSE and producing scientific evidence upon which to base control policies has led to the development of an extensive UK research base in human and animal health aspects of TSEs.

The UK has a special responsibility to share its expertise in TSEs with Europe and the rest of the world to minimise the effects of TSE infection in other countries.

Although considerable progress has been made, further studies are required to fill in the many gaps in our basic understanding of TSEs and in our knowledge of how TSE epidemics can be effectively controlled. It is therefore vital that the UK research base is maintained and that a co-ordinated and effective funding strategy is adopted for new research. Science develops and inevitably this strategy will require regular reassessment.
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Executive Summary

1. Transmissible spongiform encephalopathies (TSEs) have been present in human and animal populations for centuries. Interest in them has intensified since BSE became significant in British cattle in the 1990s and especially since its human version became a hazard to the UK population.

2. The number of human fatalities from TSEs in the UK is relatively small, in the low hundreds, and on this criterion TSEs currently appear to present a smaller risk to public health risk than vascular disorders such as heart disease or strokes, which are the UK's biggest killers, or cancers.

3. However, the impact of TSEs has been high and they remain important for a number of reasons. There may be human TSEs with longer latency periods than those that have appeared clinically to date. This could mean a further cost in terms of human life and suffering and to the healthcare system, where considerable resource has already been committed to importing blood products and improving safety measures such as the decontamination of surgical instruments. Identification of another TSE in another species (e.g. BSE in sheep) would affect consumer confidence in British produce, which would add a further burden to a slowly recovering confidence in farming and meat industry.

4. The five major UK funders of TSE research each have their own remits, strategies and approaches. The Department of Health and the Department for Environment, Food and Rural Affairs are government departments with responsibility for human and animal health, the environment and the agricultural industry. The Food Standards Agency is a public body established to protect the public from food hazards. The Medical Research Council and the Biotechnology and Biological Sciences Research Council are non-departmental public bodies that fund research in human and animal biology and health.

5. These bodies have different but related interests in TSE research. Although the research councils pay attention to the applicability of the research they fund, they have a strong interest in pursuing excellent science independently of its possible use. The three government departments have responsibilities for human and animal health, and for the environmental and economic ramifications of TSEs, and require sound scientific information on which to base their policies.

6. These organisations are currently (2004/05) spending approximately £35M per year on TSE research. The programmes supported by the five funders are coordinated and discussed with other interested organisations, including devolved UK administrations, the voluntary sector, industry and organisations outside the UK, including the European Commission and research groups in the USA. Central to this cooperation is the TSE R & D Funders Co-ordination Group.

7. Research on TSEs will continue to be necessary in part because of their economic consequences for agriculture, an industry that is already under pressure from a variety of sources. The National Scrapie Plan, which involves eliminating scrapie from the UK sheep flock by removing sheep with lower levels of genetic resistance to it, is costly and requires an adequate base of research knowledge. Furthermore, as a large proportion of the UK population may have been exposed to BSE-contaminated material, the implications for public health and the additional burden on the NHS could be severe. Consequently it is imperative that accurate estimates of the size of the human epidemic are obtained, methods to prevent its spread are continually updated and potential treatments investigated.
8. Research in this area, especially that funded by the research councils, forms part of the general process of advancing knowledge as well as being of practical value. It has been informed by, and in turn informs, advances in our understanding of protein and gene science, and knowledge of human and animal epidemiology. It illuminates important areas of knowledge such as the strength and nature of species barriers and infection processes, including species-to-species and possible mother-to-offspring transmission.

9. Currently, TSEs are one of the rare disease groups where infection leads invariably to death. For this reason, possible treatments are a target for both human and animal TSE research. These might take the form of drug therapy or immunological interventions such as vaccines. If developed for the human population, such interventions would have a high value for patients and their families despite the small number of people affected.

10. TSE research is a growing international field in which the UK has made a significant contribution. The UK is likely to be a valued partner in TSE research for other nations and for international organisations. TSE research has featured in previous European Commission framework programmes for research and is likely to be further funded under the sixth framework programme. The UK is a leader in TSE science and in the cross-agency organisation needed to support the full range of TSE research.

11. The approach to TSE research described in this report includes a number of short-term targets. One of the most pressing is the need for a preclinical diagnostic test for humans and animals. Also important is work on the safety of medical instruments, which is needed by the Department of Health and the UK National Health Service. Other health-related research is directed towards the needs of agencies such as the National Blood Service, which has an interest in blood transfusion hazards. Research is also being pursued on methods for monitoring food for TSE hazards, on animal feed hazards and on animal slaughter practices. This is of interest to Defra and the FSA. Defra has a strong interest in research to support the National Scrapie Plan, to investigate the possibility of BSE in sheep and to establish the risk of TSEs in other farmed animals.

12. The aim of this research is to uncover the science of TSEs, in particular their nature and means of transmission; to develop countermeasures at many points in the food chain; to protect the health of the UK population; and to engage the public in the research and its application.
Introduction

1.1 Background to the new strategy

1.1.1 The main scientific documents setting out the basis for UK research into TSEs – detailed below – have been overtaken by time and technical progress. Hence the present report, which sets out in detail the current strategy of the UK funders of TSE research and places it in its scientific context.

1.1.2 TSE research developed from the mid 1980s with the discovery of BSE in UK herds, and rapidly expanded after the identification of a closely related human disease, vCJD, in 1995.

1.1.3 Previous research and development strategies produced by the UK public funders, relating to the human and animal health aspects of TSEs, were published in November 1996 and July 1998 respectively. Many other reports and reviews have been published since then.

1.1.4 At EU level, the European Commission invited Professor Charles Weissmann to chair a group whose aim was to produce an inventory of the state of knowledge of BSE, and to propose future research priorities. Professor Weissmann’s report was published in October 1996 and formed the basis of a communication from the Commission to the European Council (COM (96) 582) proposing an Action Plan on TSEs. Since then the EU, with the assistance of a scientific steering committee, has produced almost 270 opinions on TSE, consulted more than 200 experts from 25 countries and provided the scientific basis for more than 30 legislative proposals.

1.1.5 In the UK, the BSE Inquiry, published in October 2000 and chaired by Lord Phillips of Worth Matravers, reviewed the history of the emergence and identification of BSE and vCJD, and of the action taken in response to it up to 20 March 1996. The Government’s response followed.

1.1.6 Since that time a number of additional reviews and reports have been published. Most notable for research were

- a joint working group of the councils of the Royal Society and of the Academy of Medical Sciences which published a report in 2001 reviewing six specific topics. These were gaps in knowledge, management of research, diagnostic tests, BSE in sheep and eradication of TSEs from all food animals, disposal of infective material, and therapeutic agents;

- the Horn Report produced in July 2001 by a committee set up by government and chaired by Sir Gabriel Horn (University of Cambridge) to assess the current state of understanding of the origin of BSE;

- on disease management, the Review of BSE Controls (2000); and a SEAC subgroup report on Research and Surveillance for TSEs in sheep (1999) which has had a significant influence on Defra’s research programme.

1.1.7 Reviews, such as those by Collinge\(^1\) and Aguzzi et al.\(^2\) have also been published in the scientific literature. Professor C Bostock’s 2004 review of current knowledge about TSEs formed an important part (Section 2) of the development of this strategy document.
1.2 The importance of research and development

1.2.1 The discovery of a new animal disease, especially one that is found to be transmissible to both animals and humans, raises concerns about animal and human health. Uncertainty about the risk factors makes management difficult and can result in preventative measures to protect public health being too cautious, or worse, inadequate. These measures can have a significant impact on daily living and place a large financial burden on both the public and private sectors, nationally and internationally. This means that there is a need for a good understanding of the nature of the agent, the mechanisms of disease causation, disease transmission and successful methods of containment. Early identification by good surveillance, coupled with thorough scientific investigation, has been shown to provide a powerful route to containment.

1.2.2 New variant CJD, now called vCJD, was first observed by the National CJD Surveillance Unit (NCJDSU) in 1995 and a possible link to BSE was subsequently made. The broad implications of BSE for public health were identified at this stage.

1.2.3 Further research has enabled preventative measures to be fine-tuned to be more effective. Examples have included:

i. Pathogenesis studies in which cattle and sheep have been challenged with BSE and tissues collected at various points in the course of disease development have determined which tissues should be classed as specified risk material (SRM) and kept out of the food chain.

ii. Epidemiological analysis of case data has produced evidence to show that cross-contamination of ruminant feed with pig and poultry feed containing meat and bone meal was responsible for the feed ban not being fully effective. This led to tighter feed controls and a total ban on the use of processed animal proteins in the feed of animals kept for food production.

iii. Studies on the genetic make-up of sheep with scrapie have provided the foundation for the National Scrapie Plan, which is intended to eliminate scrapie from the national flock by selective breeding with rams of a resistant genotype.

iv. Studies in sheep have demonstrated that TSE infectivity can be detected in blood, and recent experiments in transfusing large volumes of blood from infected animals have shown that infectivity can be transmitted by blood transfusion. These experiments, coupled with the death from vCJD in 2003 of a blood transfusion recipient who had received blood from a donor who subsequently died of CJD, demonstrate the wisdom of the UK blood services’ decision to leukodeplete blood donations and to source plasma from overseas for treatment of the very young and for the production of pharmaceuticals. The UK blood services have taken the further precautionary step of excluding recipients of blood transfusions from donation.

v. Bioassay identification of PrP Sc infectivity in some human tissues confirmed the possible risk of secondary infection from some surgical procedures. Research demonstrating that it may be possible to remove prions from surgical instruments without damaging the instruments will play a significant part in reducing that risk.

vi. Analysis of archived appendix tissue has demonstrated the presence of PrP Sc, indicating that a number of individuals in the UK population, who have not demonstrated clinical symptoms, may be carrying prion infection. Wider studies for collecting and screening tonsils that have been removed in the UK during routine tonsillectomies may provide a more accurate estimate of that number. Surveillance for human prion disease thus remains important, as do the measures for preventing secondary infection.
1.3 The funders of UK TSE research

1.3.1 Funding

1.3.1.1 Most TSE research in the UK is supported by five public funders: the Biotechnology and Biological Sciences Research Council (BBSRC), the Department for Environment, Food and Rural Affairs (Defra), the Department of Health (DH), the Food Standards Agency (FSA) and the Medical Research Council (MRC). Defra and DH are government departments, FSA is an independent food safety watchdog set up by an Act of Parliament in 2000 to protect the public’s health and consumer interests in relation to food, and BBSRC and MRC are national, non-departmental public bodies established under Royal Charter to fund scientific and medical research.

1.3.1.2 Up to and including the financial year 2004/05 these bodies had spent over £308M on research into transmissible spongiform encephalopathies on a programme that has developed progressively since the beginning of the BSE epidemic in cattle in 1986. More than 400 projects have been funded over this period ranging from 12-month pilot studies to 16-year bioassay studies in cattle. Full details of the respective funders’ annual spends can be found in the table and figure below.

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1 MAFF was reorganised to form Defra and FSA in 2000
2 BBSRC was established in 1994 taking over the TSE research supported by the former AFRC
1.3.1.3 This programme funds research to improve understanding at a variety of levels ranging from the biochemical, genetic and cellular aspects of these diseases to epidemiology and public health studies.

1.3.1.4 In 1999, UK devolution resulted in the establishment of the Scottish Executive, the Welsh Assembly and the Northern Ireland Executive. These devolved administrations have differing authorities, but probably the most notable funding contribution to the field is that provided to the National CJD Surveillance Unit by the Scottish Executive. The Scottish Executive is also funding a research project looking at prion proteins in vCJD patients.

1.3.1.5 TSE research is also funded by charities, in particular the Wellcome Trust. Others fund research in their areas of interest, for example the Human BSE Foundation has contributed to work in the area of palliative care. Some research is also undertaken in-house by government agencies such as the National Blood Service and the Health Protection Agency.

1.3.1.6 In addition, the EU has sponsored research into TSEs since 1990 under an action plan on two levels. The primary aim has been to encourage interdisciplinary research between member states, focusing on harmonisation of data collection and diagnostic criteria. The secondary aim has been to stimulate research at the Community level and to mobilise new and complementary expertise to attain European critical mass in the field.

Details of EU funded TSE research can be found on the Europa website.

1.3.2 Aims and missions

1.3.2.1 The aims and missions of the five UK public funders are complementary. The government departments (Defra and DH) and to some extent, the Food Standards Agency (FSA), primarily use research to inform and implement policy, to contribute to the identification of future policy options, to provide scientific foresight and most importantly to control disease. Their aims are to estimate the size of current and future epidemics, to create policies to prevent primary infection and secondary transmission, to develop treatments, and to present risk assessments to the community to facilitate informed decision making. Their emphasis is to control disease and to improve the health and well being of people and animals. The work is far reaching – ranging from providing specialist guidance to health practitioners, animal keepers, food processors and suppliers, to promoting healthier lifestyles and living.
1.3.2.2 The research councils (BBSRC and MRC) aim to encourage and support high-quality basic, strategic and applied research, and promote postgraduate training. Their mission is to develop the fundamental understanding of biological systems that will underpin maintenance and improvement of animal and human health, and they see TSE research in this context. Promoting public understanding and engagement in the biological sciences and its applications to medical research are also primary aims of the Councils.

1.3.3 Mechanisms of funding research

1.3.3.1 The five public funders of TSE research receive their funds from Her Majesty’s Treasury. DH and FSA have ring-fenced budgets to support research and development into TSEs and these are reconsidered annually. Defra used to operate similarly, but now TSEs are considered alongside its other departmental research priorities. The research councils do not as a rule ring-fence budgets for research, but fund TSE science in open competition with other demands on their recurrent budgets and according to their strategic priorities.

1.3.3.2 The mechanisms for supporting research vary from funder to funder. They all use rigorous peer review (often including overseas experts) to ensure that the science they fund is of a high competitive standard and is likely to achieve its objectives.

1.3.3.3 The government departments consider applications for support of research from universities, their own agencies and commercial companies both within the UK and abroad. Departments also issue specific calls for proposals and in specialist areas may consider commissioning research directly to provide answers to urgent issues.

1.3.3.4 Unlike the other funders, the FSA does not have an open door policy for research proposals submitted ad hoc. The research it commissions addresses specific research requirements. The agency’s research is normally funded through open competition, but research can be supported following an invitation to submit a tender for research on a limited or single tender basis if one or only a few contractors could do the work, or if an urgent need for the results precludes a prolonged open competition.

1.3.3.5 The principal mechanisms for delivering research council support are via: sponsored institutes and units (long term direct support), researcher-initiated grant funding to universities and research institutes (short term indirect support), and awards made to individuals to assist in their career development. Proposals from industry or from overseas (MRC only) are considered in exceptional circumstances. Councils highlight areas of key strategic interest and on occasions may issue specific calls for proposals. Councils are open to receiving proposals to undertake research at any time. Research competitions and requirements are usually published on individual funders’ websites (see Annex 1) or in the scientific press. Where topics of mutual interest are identified, joint calls for proposals from funders are issued. Examples have included the call for proposals on TSE Diagnostics (2001) involving all funders and the call to explore the potential of Nuclear Magnetic Resonance (NMR) and Mass Spectrometry (MS) for analysis and confirmation of TSE infection (2003) involving BBSRC, Defra and FSA.

1.3.3.6 BBSRC supports basic and strategic research into the biology of the TSEs that underpins the policy needs of DEFRA, DH and FSA. Since the early 1990s, the Council has funded several phases of a major programme of research into the biology of the spongiform encephalopathies (BSEP), building on earlier work at its Neuropathogenesis Unit in Edinburgh, part of the Institute for Animal Health (IAH). There is also a significant body of TSE research at IAH's Compton Laboratory, near Newbury, and the biology of the TSEs is currently a priority area for the Council's grant-awarding committees.

1.3.3.7 All science funded by the public funders is closely monitored and a number of mechanisms are in place to ensure high quality research is undertaken. Progress and
final reports are requested on most grants, and all grant holders are required to present their work at a biennial funders’ meeting as well as publishing it at scientific conferences and in peer reviewed journals. Research council institutes and units, which receive direct support, are all subject to quadrennial or quinquennial reviews. Research institutes and universities that receive indirect support are randomly audited to ensure that good management practices are in place to foster the best quality research. In addition, funders make monitoring visits and sometimes request interim reports, which may often be presented to scientific advisory committees for assistance in interpretation. Where there are difficult scientific challenges or complex ethical issues, the research is often overseen by dedicated steering groups. Current examples include research being undertaken to ensure the decontamination of surgical instruments from prions, and the national surveillance of lymphoid tissue for PrP\textsuperscript{Sc}.

1.3.3.8 Public funds support four main centres of TSE research in the UK:

**The Institute for Animal Health** (IAH) is one of eight BBSRC-sponsored research institutes, and is a world centre for research and training in fundamental, strategic and applied research on infectious diseases of farm animals. It advances knowledge on the etiology, pathogenesis, epidemiology and control of existing and new diseases and develops control measures which enhance farm animal welfare, increase the efficiency of agriculture and protect the environment. The Institute has particular interests in food-borne zoonoses for improving food quality and safety, exotic diseases which could spread to the UK, and in TSEs and endemic diseases. It is able to bring multidisciplinary teams to bear and has links worldwide.

TSE research is carried out at the IAH’s Neuropathogenesis Unit in Edinburgh and at its Compton Laboratory near Newbury. The Institute’s programme includes studies of TSE pathogenesis and neuropathology, the genetics of TSEs in mice and sheep, the epidemiology of scrapie, and inactivation of TSEs.

IAH is supported by core funding from BBSRC, and also receives research grants from BBSRC and other funding bodies.

Web site: [http://www.iah.bbsrc.ac.uk/index.html](http://www.iah.bbsrc.ac.uk/index.html)

**The MRC Prion Unit** was formally established in 1999 and is embedded within the Institute for Neurology in London. The principal focus of the Unit is on the human prion diseases and its research strategy is aimed at both rapid developments to target these areas of public health concern and a long term approach to the understanding of prion disease and the wider relevance of prion-like molecular mechanisms in pathobiology. The Unit provides a key training resource in this unique area of biology and medicine. The research philosophy is to combine basic and clinical research: many of the key contributions towards understanding the basic biology of these diseases have come from clinical and neuropathological observations and efficient translation of these basic studies to the clinic is crucial.

Research programmes include: molecular genetic studies of disease susceptibility; mapping of disease incubation loci; transgenic modelling of human prion diseases and studies of the species barrier to transmission; investigation of the function of normal cellular PrP; analysis of strain variation; structural studies on normal and mutant prion proteins; clinical research into diagnostics and therapeutics. The Unit has developed the infrastructure and patient base in partnership with the NHS and MRC Clinical trials Unit to permit effective therapeutic trials.

Web site: [http://www.prion.ucl.ac.uk/](http://www.prion.ucl.ac.uk/)

**The National CJD Surveillance Unit** was established in 1990 in the Western General Hospital in Edinburgh and serves the whole of the United Kingdom. The Unit is responsible for monitoring the characteristics of all forms of CJD to identify patterns, trends and risk factors; and, for informing the appropriate body so that prompt, effective action can be taken to protect public health. The National CJD Surveillance Unit has
cerebrospinal fluid and genetics laboratories which analyse samples from patients with suspected CJD as an aid to clinical diagnosis. The Unit is responsible for the national pathological surveillance of CJD, involving both histological studies on brain and all other tissues, and protein analysis. The Unit undertakes research on the clinical and diagnostic features of CJD, epidemiology (including the national Case Control Study of risk factors for sporadic and variant CJD, and the investigation of geographically associated cases of CJD), and histopathology and protein analysis of brain and other tissues and fluids. The Unit houses a large bank of brain tissue, cerebrospinal fluid and blood samples which are used for diagnosis and research both within the Unit and with external research groups.

The NCJDSU also administers the National Care Package for patients and families with CJD, which co-ordinates care for such patients and their carers throughout the United Kingdom. Staff in the National CJD Surveillance Unit are involved in major programmes of research (much of it in collaboration with other centres in this country and abroad), and co-ordinate the European CJD Surveillance Network. They also provide advice and expert input to the work of government departments, scientific committees and other organisations and agencies. The Unit is a WHO Collaborative Centre for Reference and Research on the Surveillance and Epidemiology of Human Transmissible Spongiform Encephalopathies.

Web site: <http://www.cjd.ed.ac.uk/>

The Veterinary Laboratories Agency (VLA) is an Executive Agency of the Department for the Environment, Food and Rural Affairs. It provides a specialist veterinary resource to the UK Government to help it fulfil its aims and objectives in veterinary public health and in developing sustainable agriculture and industries. Scientists at the VLA work collaboratively to deliver veterinary research, surveillance, consultancy and laboratory testing services through a series of integrated science programmes. Many of its services are also available to the private sector.

The VLA’s TSE programme aims to provide the primary source of high quality, scientifically based, advice to the Government via Defra and the Food Standards Agency on all aspects of TSEs, with special reference to BSE and scrapie. The programme consists of approximately 100 diverse research and surveillance projects which cover the following broad areas - surveillance and epidemiology, pathology and transmission, diagnostics and molecular biology. This TSE programme is one of the largest in the world and has several unique characteristics. The VLA also maintains the TSE Archive.

Web site: http://www.defra.gov.uk/corporate/vla

1.3.4 Working relationships

1.3.4.1 The multi-agency nature of TSE research means that it is supported by formal and informal interactions between the five funders. The government departments operate TSE-specific research management teams, responsible for research contracts, as well as policy teams that coordinate risk assessment, produce operating guidelines and monitor and enforce control measures. Close co-ordination is maintained between these groups in order to ensure that policy is underpinned by a scientific evidence base. In addition, since TSEs raise points of concern relevant to other disease areas, close collaboration between these groups and other sections within their departments is important. Project and programme management committees and boards perform this role.

1.3.4.2 Funders interact with many stakeholders in the course of their strategic planning, including the private sector (the agriculture, food, healthcare and biotechnology industries), medical charities, and academic researchers from a range of scientific disciplines, patient support groups and members of the public.

1.3.4.3 Research concordats are used to promote formal interactions and these exist between many government agencies. For example, all UK Health Departments (HDs) have long standing concordats with the MRC. Similar concordats have now been agreed
between the UK Health Departments and the other research councils, including BBSRC. Others are currently being formalised between Defra and other funders and environment agencies.

1.3.4.4 DH oversees the research of a number of Non-Departmental Public Bodies. The most important for the TSE field are the Health Protection Agency (incorporating the former PHLS, NRPB, NFCI and CAMR), NIBSC, and the National Blood Authority (NBA). In addition, DH has close links with the Medicines and Healthcare Products Regulatory Agency.

1.3.4.5 Interactions with the devolved administrations are frequently achieved by inviting representation on appropriate committees, regular meetings of the four UK Chief Medical Officers and the involvement of all the devolved administrations in projects of mutual interest (e.g. the national tonsil archive).

1.3.4.6 Other formal interactions include inviting people from user communities and from other funders to serve on key standing committees. Research Councils invite such members onto their strategic research boards, and SEAC (see 1.3.5) has lay representation. In addition, disease specific cooperations are often established as the need arises, on an ad hoc basis. Members of CJD support groups (the Human BSE Foundation and the CJD Support Network) are invited as members onto steering groups and advisory committees.

1.3.4.7 Interactions with industry and the encouragement of technology developments are promoted through the Office of Science and Technology's Foresight and Open LINK programmes. UK TSE public funders support these programmes. The LINK scheme aims to promote high-quality, pre-competitive research collaborations between academia and industry in the cause of innovation and increased UK industrial competitiveness.

1.3.4.8 Significant TSE research is funded by the EU under the EC Framework Programmes. A recent example includes a successful bid to Framework Programme 6 that has been co-ordinated by a French laboratory and involves large number of European laboratories. The funding will support a Network of Excellence in the "Prevention, control and management of prion diseases" to facilitate the exchange of TSE information and expertise between the participating laboratories.

1.3.4.9 To assist co-ordination of TSE research the Commission has also established a TSE Expert Group composed of representatives from member states, associated countries, members of the Scientific Steering Committee and co-ordinators of EU projects. UK funders are formally represented in this group. The Group has undertaken to review the state of TSE research in the EU, encourage the exchange of information, and identify areas where further research could be undertaken. In addition, individual UK scientists have sat on various advisory committees such as the EU Scientific Steering Committee, where UK science has made a significant input. The EU is currently restructuring its committees following the establishment of the new European Food Safety Authority and the UK will need to consider how it can best support this new system.

1.3.5 Scientific consultation

1.3.5.1 Funders use a number of independent scientific advisory groups to assist them in the interpretation of the latest scientific findings to allow informed policymaking. Most notable in this context are:

- The Spongiform Encephalopathy Advisory Committee (SEAC) and its subgroups and working groups. Details can be found on the SEAC website.
- The Advisory Committee on Dangerous Pathogens (ACDP) and its subgroups and working groups.
• The Committee on the Microbial Safety of Blood and Tissues for Transplantation (MSBT).
• The National CJD Surveillance Unit Steering Group.

1.3.5.2 Funders also use a number of independent scientific advisory groups to assist them in defining their research strategy. They include:

- DH/MRC TSE Research Advisory Group
- Defra TSE Research Advisory Group
- BBSRC BSEP Working Party
- DH TSE Tissue Management Group
- Defra Independent Archive Advisory Group (IAAG)

Terms of reference of these groups can be found in Annex 2

1.3.5.3 In addition, the UK blood services have a number of groups that advise them on CJD-related issues, to which DH is invited to send an observer. The most important of these is the vCJD subgroup of the Standing Advisory Committee for Transfusion Transmitted Infections (SACTTI).

1.3.6 Co-ordination of TSE research

1.3.6.1 The BSE epidemic in 1986 and the appearance of variant CJD in 1996 in the UK changed the way that TSEs were viewed, because of the increased risk to the health of both humans and animals. As a result, the boundaries between animal and human health management have been lowered to acknowledge the interdisciplinary nature of the problem and this has required greater co-operation and co-ordination between government agencies.

1.3.6.2 The combining of MAFF and DETR, which led to the formation of Defra and the establishment of the FSA, has helped to improve communication, co-ordination and public openness in TSEs, within Defra and across other parts of government.

1.3.6.3 The TSE R & D Funders Co-ordination Group is the main body overseeing TSE research. It consists of funding agency staff and meets 4-5 times per year. It provides a forum to ensure that TSE research is addressing priority issues of national interest and is following a coherent strategy. Joint activities have become a frequent occurrence. Combined calls for scientific proposals, workshops, the sharing of expertise at scientific reviews, and cross-representation on advisory groups are examples of the types of activity undertaken. These activities are supported by a searchable database that includes all UK publicly funded TSE research.

1.3.6.4 The High Level Progress Chasing Committee on Research and Development in TSEs: Butler Committee is chaired by the government’s Chief Scientific Adviser and includes senior colleagues from the funding agencies. Its role is to ensure that the appropriate expertise and mechanisms are in place to deliver a UK research strategy on TSEs and where necessary to make recommendations to resolve potential barriers to progress. As TSE research in the UK has become established the committee has reduced its meetings.

1.3.6.5 Successful biological research requires good quality reagents and tissues. UK funders have worked closely with the scientific community to establish a number of resources that are available internationally, including:

The NIBSC CJD Resource Centre - The NIBSC CJD Resource Centre has been established with UK Department of Health and World Health Organisation (WHO) support to develop, characterise and produce reference materials for CJD diagnostic assays and...
to facilitate research into CJD. It is a collaboration between the Divisions of Virology and Haematology and is located in a purpose built facility on the NIBSC site. It works closely with the WHO and the working group on international reference materials for diagnosis and study of transmissible spongiform encephalopathies.

**The NCJDSU tissue bank** - A tissue bank of material obtained from patients with sporadic or vCJD has been established at NCJDSU with support from the MRC and DH. This collection contains mainly brain tissue, but samples of other tissues as well as blood and some bodily fluids are also available. This is a valuable but limited source of material for research. A Tissue Management Steering Group oversees access to samples.

**The VLA tissue bank** - The Transmissible Spongiform Encephalopathy (TSE) Archive receives and stores a range of animal tissues and fluids collected ante and post mortem from UK field cases and some experimental programmes. This archived material is available to approved research groups working towards the development and validation of TSE diagnostic tests.

The Archive is the largest and most comprehensive store of TSE material worldwide and operates to a quality management system compliant with ISO9001:2000.

**The IAH TSE Resource Centre** - The TSE Resource Centre, funded by the BBSRC and supported by the MRC, was established in 1998 to collect, store, characterise, produce and distribute a range of reagents, from monoclonal antibodies to infective materials. The primary role of the Centre is to supply a range of specialised research reagents needed for TSE research.

1.3.6.6 Outside the UK, although there is significant research activity across Europe and in the USA, co-ordination tends to be supported only by the EU or is developing on more of an *ad hoc* basis. In many EU member states, fragmented programmes tend to exist, perhaps reflecting their more recent development. This could explain why an EU initiative to establish a European Funders’ Group has struggled to identify lead co-ordinators in many countries. The European Commission is encouraging this work. It has produced a report on activities within the EU and has established links to some national websites.

1.3.6.7 One aspect where EU coordination is proving to be successful is surveillance. A coordinated surveillance system for all forms of CJD was established in 1993 and now includes all member states of the EU, Australia, Canada, Norway, Iceland, Israel and recently the USA. The aim is to identify all cases of CJD including variant CJD in participating countries and to study clinical, epidemiological and molecular aspects of human prion disease.

1.3.6.8 The EU Framework Programmes for research, technology and development have provided a number of ways of funding research into livestock diseases and for collaborative research across the Community – increasingly with its associated states and others such as Switzerland, Hungary, Poland, Australia and the USA. The Community drew up a TSE action plan in 1996 in which some €35 million was set aside under Framework Programme 4 (FP4) to establish projects and networks. That programme was taken forward under Framework Programme 5 with total funding of €85 million, with UK research teams featuring prominently. Framework Programme 6 runs from 2002-2006 with a total budget of more than €16 billion, providing mechanisms for fostering co-ordination through the European Research Area concept.

1.3.6.9 Outside formal mechanisms, *ad hoc* initiatives such as the ‘ring trial’ have evolved. A number of laboratories in Europe and the USA have been collaborating to develop a molecular test that can differentiate between BSE and scrapie in sheep. This is needed so that large scale and rapid testing of sheep samples can be undertaken to determine whether BSE has infected sheep other than under experimental conditions. Lack of suitable control material for all genotypes of sheep and difficulties in interpreting the test results have meant that progress has been slow. The European commission
asked the VLA to establish a ring trial on any material that has a BSE-like signature with any of the tests under development in those laboratories. A review panel has been established to interpret the results. The ring trial involves two French laboratories, the University of California and the VLA. It is using six, and possibly seven, different methods with encouraging early results.

1.3.6.10 Other funder representation provides links to the World Health Organisation, which has a specific interest in the epidemiology and diagnostics of TSEs and the US National Prion Programme, which has reviewed its first round of researcher initiated awards.

1.3.6.11 European Reference Laboratory In response to the BSE epidemic the EU commission has designated the Veterinary Laboratories Agency (VLA) as the EU Community Reference Laboratory (CRL) for TSEs. The main function of the CRL, together with the EU Commission, is to provide support and coordinate the methods employed for diagnosing BSE in each Member State and to contribute to identifying outbreaks of TSE. The CRL also provide training of scientific experts with the objective of harmonising TSE diagnostic techniques throughout the Community.
1.4 Communications

1.4.1 All UK public funders of TSE research rely increasingly on electronic communication and use websites as the main medium through which they present information and consult the public and other stakeholder groups. Funders use their websites to highlight opportunities for funding, to outline their particular research interests, to present their research portfolio, to publish results of their research, and to seek opinions from the public. Some of their content may be TSE specific, while some is of interest to the broader research community, for example, guidelines on the use of animals in medical research. In addition, funders may use their websites to relay information of public interest such as recommendations for good dietary habits.

1.4.2 A brief resume of relevant funders’ web pages can be found at Annex 1. The complete UK funders’ portfolio of current and completed projects since 1996 can be found on the MRC website. This site includes a searchable database allowing searching of project abstracts, interim and final reports, as available.

1.4.3 Funders also use more traditional methods of communication such as newsletters, magazines, annual reports and press releases. New research funding opportunities are often advertised in scientific journals and announcements of especially newsworthy results may be made through press briefings and press releases, frequently co-ordinated to allow simultaneous announcements from other funders.

1.4.4 With regard to public dissemination, government departments have looked to other ways of making public health information available. DH for example uses NHS Direct and experimental interactive television programmes.
TSE science

2.1 TSEs: background

2.1.1 TSEs have long incubation periods and at present are untreatable. The appearance of their symptoms leads inevitably to death.

2.1.2 Scrapie in sheep was documented in 1732, but it was another two centuries before a human TSE, Creutzfeldt-Jakob disease (CJD), was first described. It can arise spontaneously (sporadic CJD), be inherited (familial CJD) or be transmitted by medical procedures (iatrogenic CJD). Other forms of inherited human TSEs are Gerstmann-Straussler-Scheinker syndrome (GSS) and fatal familial insomnia (FFI). Kuru is another human TSE, first described in the scientific literature in 1957, transmitted between the Fore people in Papua New Guinea during cannibalistic feasts that were part of funeral rituals. In other species, transmissible mink encephalopathy (TME) is a rare disease of farmed mink, first described in 1947, while chronic wasting disease (CWD) was first described in captive mule deer in Colorado in 1967, although it was not recognised as a TSE for over a decade. CWD also infects other species of deer and elk and its prevalence and distribution in free ranging elk, mule deer and white-tailed deer is increasing in several States of North America.

2.1.3 Bovine spongiform encephalopathy (BSE) was first recognised as a new disease in UK cattle in 1986, although it is now known that the first clinical case presented in 1985 and it is likely that undiagnosed BSE infections had existed since the late 1970s. Although it is clear that, once initiated, the epidemic of BSE was driven by the recycling of infectivity through the inclusion in animal feed of meat and bone meal (MBM) derived from BSE-infected carcasses, it is not known how BSE first infected cattle. The favoured explanation is that it arose from a strain of scrapie that survived the rendering of sheep carcasses used to make MBM that was fed to young calves.

2.1.4 The recognition of BSE in cattle was followed closely by TSEs in exotic ruminants (for instance nyala and kudu) in zoos. 1990 saw the start of a small epidemic of feline spongiform encephalopathy (FSE) and throughout the 1990’s several species of large cats (e.g. cheetah, tiger and lion) in UK zoos succumbed to new TSE infections. A new form of CJD, variant CJD (vCJD), was reported in 1996, although the first clinical cases had occurred a year or two earlier. vCJD was quickly shown to be linked to BSE.

2.1.5 Unlike infectious diseases caused by bacteria or viruses, TSEs do not trigger a conventional protective immune response, although the immune system is actively involved in the replication and transport of the infectious agent in many TSEs. In addition, treatment with antibodies (the product of an immune system) carrying specificity for the prion protein has been shown to offer some protection against an infection (see Section 2.14). The agent can be widely distributed throughout the body, but the degenerative effects of TSE infection appear to be restricted to the nervous system.

2.2 The prion and other hypotheses

2.2.1 An animal with a TSE infection commonly exhibits an abnormal form (PrPSc or prion) of the cellular prion protein (PrPC). Deposits of PrPSc can take many forms ranging from diffuse widespread distribution to local concentrations of highly ordered aggregates, called amyloid plaques. Deposits of PrPSc can be identified under the microscope in sections of body tissue by selective staining and can be separated from PrPC because PrPSc is insoluble and relatively resistant to protease enzymes used to digest proteins.

2.2.2 The correlation between TSE infection and PrPSc led to the prion hypothesis, that the abnormal form of prion protein is the infectious agent. Although accepted by many in
the field today, it was highly contentious at first because it went against the then-accepted view that all inherited biological information must be encoded in nucleic acid, DNA or RNA. Alternative hypotheses for the infectious agent include a conventional virus of unknown type\textsuperscript{11} or a “virino”- an informational molecule, expected to be a small piece of nucleic acid, which is not encoded in the host and which codes for its own replication and binds to the prion protein\textsuperscript{12}.

2.2.3 To date neither a virus nor a virino nucleic acid has been found and most research has focussed on the PrP molecule. Although progress has been made in understanding the structure, function and role in disease of various forms of PrP, the molecular basis of its suggested infectiousness and how it is propagated have not been described. Nor has normal PrP been converted into an infectious form in a test tube in a laboratory, which would provide a formal proof of the hypothesis.

2.3 The structure of PrP

2.3.1 PrP\textsuperscript{C} is synthesised as a continuous chain of about 255 amino acids, the exact size of which varies from species to species. The first 22 (the signal peptide at the start, or N-terminus) are removed shortly after synthesis and another 23 are removed from the other end of the molecule (the C-terminus). This happens when a lipid tail, the GPI (for glycoposphatidylinositol) anchor, is added to embed the PrP\textsuperscript{C} molecule in the cell’s membrane. The molecule is also glycosylated by having sugar residues added at two specific sites. Mature PrP\textsuperscript{C} is about 210 amino acids long.

2.3.2 To solve the structure of PrP, a lot of very pure soluble protein is required. Natural PrP\textsuperscript{C} has been purified from brains, but recombinant PrP (PrP\textsuperscript{Rec}) can be produced in much larger quantities by bacteria or cell cultures genetically modified to express PrP. PrP\textsuperscript{Rec} made in bacteria is not processed, modified and folded in the same way as PrP\textsuperscript{C} made naturally in an animal or human cell. It must be refolded to study its structure, leaving some uncertainty about the relationship of any PrP\textsuperscript{Rec}-derived structure to that of natural PrP\textsuperscript{C}.

2.3.3 The structures of dissolved PrP\textsuperscript{Rec} for several species, for instance, human\textsuperscript{13}, cow\textsuperscript{14} and mouse\textsuperscript{15}, have been determined using nuclear magnetic resonance (NMR) spectroscopy. They have similar structures in which the N-terminal half contains five copies of an eight amino-acid sequence (octapeptide) arranged one after the other or tandemly. Overall, the N-terminal region appears to be disordered so that its structure cannot be determined by NMR, but within the region the octapeptide repeats are structured and may act as a site for pH-dependent folding and aggregation\textsuperscript{16}. The C-terminal half of PrP is globular with a well-defined structure containing three alpha-helices (1, 2, and 3), which are composed of amino acids arranged in spirals. Helix 1 is preceded by a short section of beta-sheet 1, an ordered flat rigid structure in contrast to the spiral helices and the loops, and is separated from helix 2 by another short section of beta-sheet 2 followed by a disordered loop. In the cell, helices 2 and 3 are likely to be linked chemically since both contain a cysteine amino acid, which, under the right oxidising conditions, pair together via a di-sulphide bridge.

2.3.4 Crystals of PrP are formed following incubation of highly concentrated solutions of PrP for long periods of time. X-ray crystallography of human PrP\textsuperscript{Rec} reveals a paired (dimeric) structure in which the two helix 3s are “swapped” between the PrP partners\textsuperscript{17}. The dimer will be stabilised by the disulphide bridges formed between helix 2 of one PrP molecule and helix 3 of the other. Such intermolecular disulphide bonds, formation of which may be promoted by the presence of added sugars at the nearby asparagine residue, may be important in stabilising PrP\textsuperscript{C} against PrP\textsuperscript{Sc} formation\textsuperscript{18} or, following reducing conditions which favour the breaking of the disulphide bond, enabling formation of PrP\textsuperscript{Sc}-like structural forms\textsuperscript{19}. Amino acids that are changed by mutations linked to inherited forms of CJD are concentrated in the swapped helix 3 and the neighbouring helix.
2 against which helix 3 sits, suggesting that they cause disease by affecting the ability of PrP to form stable dimers.

2.3.5 If the prion hypothesis is correct, and TSE infectivity is encapsulated in the change in shape of PrP\textsubscript{C} to PrP\textsubscript{Sc}, the structure of PrP\textsubscript{Sc} needs to be determined to see how it differs from PrP\textsubscript{C}. This might show how the PrP molecule becomes “infectious”. However, PrP\textsubscript{Sc} in aggregates and fibrils is insoluble and cannot be studied by NMR, nor can it form crystals. Insufficient quantities of a PrP\textsubscript{Sc}-like molecule been made by refolding PrP\textsubscript{Rec} in a test tube for its structure to be determined by NMR or X-ray crystallography as has been done with PrP\textsubscript{C}. Treating PrP\textsubscript{Sc} with an enzyme which degrades proteins, Proteinase K (PK), cleaves the full length molecule at a position (depending on the host species and strain of TSE) between amino acid residues 74 and 102\textsuperscript{20}, removing the disordered N terminal section and leaving a PK-resistant fragment called PrP\textsubscript{27-30} (on account of its molecular size of, generally, 27 to 30kDa)\textsuperscript{21}.

2.3.6 PrP\textsubscript{27-30} can be induced to form ordered two-dimensional crystals that can be seen in an electron microscope. During its formation from PrP\textsubscript{C}, PrP\textsubscript{Sc} is known to be depleted of alpha-helix and enriched in beta-sheet. On the basis of the electron microscope studies it is postulated that the basic subunit of PrP\textsubscript{Sc} is an association of either three molecules (trimer) or three pairs of molecules (a trimer of dimers) in which the beta-sheet regions of molecules associate together on the inside and the remaining alpha-helices with attached sugars are arranged around the outside\textsuperscript{22}. The electron microscope study focussed on the structure of PrP\textsubscript{27-30} which represents the end product of the transition from PrP\textsubscript{C} to PrP\textsubscript{Sc}, but an alternative way to investigate the nature of PrP\textsubscript{Sc} is to try to simulate its spontaneous or “seeded” formation \textit{in vitro} (see next Section 2.4). Experiments of this kind have indicated that an association of eight PrP molecules may be the critical first step in the transition to a PrP\textsubscript{Sc}-like molecule\textsuperscript{23}. A detailed structure of PrP\textsubscript{Sc} is required to understand the process by which PrP\textsubscript{C} is converted into PrP\textsubscript{Sc} and enable it to be replicated \textit{in vitro} using PrP\textsubscript{Rec}. It is also needed to explain the molecular basis of the many different strains of TSEs (see Section 2.5).

2.3.7 Insights into structurally important parts of the PrP molecule, especially those involved in the conversion of PrP\textsubscript{C} to PrP\textsubscript{Sc}, have been gained from a number of indirect studies. Analysis of the consequences of naturally occurring mutations in the gene that codes for PrP (Prnp) will be considered later. But studies with transgenic mice\textsuperscript{24} and antibodies which bind to specific regions of PrP\textsubscript{C}\textsuperscript{25} have identified helix 1 and the adjacent disordered loop as being important (although not completely essential) for PrP\textsubscript{C}-PrP\textsubscript{Sc} binding, an essential first step for PrP\textsubscript{Sc} formation. Consistent with this, a region at the end of helix 1 and in beta-sheet 2 has a greater tendency to unfold when exposed to acidic conditions, similar to those present in some cellular compartments, indicating that it may provide a starting point for unfolding as a prelude to the PrP\textsubscript{C} to PrP\textsubscript{Sc} conformational shift\textsuperscript{26}.

2.4 The \textit{in vitro} conversion of PrP\textsubscript{C} or recombinant PrP to a protease-resistant form

2.4.1 A powerful way to analyse the formation of PrP\textsubscript{Sc} from PrP\textsubscript{C} is to convert PrP\textsubscript{C} or PrP\textsubscript{Rec} in a test tube into a form that resembles PrP\textsubscript{Sc} in that it is relatively resistant to digestion with the PK. This is referred to as PrP\textsubscript{Res}. PrP\textsubscript{C} and PrP\textsubscript{Rec} are both called PrP\textsubscript{Sen} in these experiments because they are sensitive to PK. Like the build-up of PrP\textsubscript{Sc} in disease, the PrP\textsubscript{Sc}-seeded conversion of PrP\textsubscript{Sen} to PrP\textsubscript{Res} is not rapid, typically taking several days. In the initial experiments, conversion was inefficient, requiring partial denaturing conditions and large excesses of PrP\textsubscript{Sc} to drive the formation of PrP\textsubscript{Res}. Subsequently, various modifications have enabled the formation of PrP\textsubscript{Res} under physiological conditions\textsuperscript{27}, provided greater efficiency, for example, by repeated rounds of sonication and incubation\textsuperscript{28} or the addition of RNA\textsuperscript{29}, and resulted in the formation of PrP\textsubscript{Res} in the absence of a seed of PrP\textsubscript{Sc} (19, 30, 31).
2.4.2 PrPRec is made in genetically manipulated bacteria, so it is relatively easy to modify through mutation and observe the effects on PrPres formation. It is also straightforward to add reagents that might enhance or interfere with the PrPSen to PrPres conversion reaction. Rapid identification of molecules that interfere with the conversion process provides a screen for molecules with potential therapeutic properties (see Section 2.14).

2.4.3 Crucially, although PrPres has many of the properties of PrPSc, it has not yet proved possible to make it infectious, thus failing to fulfil the key prediction of the prion hypothesis. This may be because the conditions have not been right to transfer the essential infectious feature of PrPSc, perhaps because some other molecule required for infectiousness is missing or that a chaperone, which is essential to fold PrP in the hypothetically infectious state, is absent. The production of infectious PrPres in vitro, and finding out what other molecules may be required, are key challenges for the future.

2.4.4 The in vitro conversion system has been used to model the species barrier, which is the relative difficulty in transmitting a TSE between species (see Section 2.12). The relative conversion efficiencies in vitro under physiological conditions correlate well with the transmissibilities of various strains of TSE agent in different species27. Experiments using mutant PrPRec molecules also identify helix 1 and the adjacent disordered loop as key determinants of conversion efficiency that mimic the species barrier32. Even changing a specific single amino acid in this region can block conversion33.

2.4.5 Individual animals of several species vary in susceptibility to TSEs, largely, but not entirely, because of differences between the genes that code for PrPC (see Section 2.6). The in vitro conversion characteristics of common variants of sheep PrPSc correlate with scrapie susceptibility and their survival times associated with genes that code for them34, adding to the potential use of in vitro conversion as a predictive tool for estimating the transmissibility of TSEs to humans35.

2.4.6 There are two distinct PrP sequence-specific stages to the seeded conversion of PrPSen to PrPres; the initial binding of PrPSen to PrPSc and the conversion of PrPSen to PrPres. Binding can be inhibited by an antibody which specifically recognises the amino acids at the C-terminal end of PrP, but which probably acts by blocking another site in the PrP molecule36. PrPSen can bind to heterologous PrPSc with little conversion to the PrPres state and, in doing so, can block the conversion of homologous PrPSen to PrPres. The interference is primarily due to inhibition of conversion rather than binding, indicating that closer amino acid identity is required for conversion to PrPres than for the initial binding step37.

2.4.7 In an infected animal or human both the PrPSc that would be converted to PrPSc and the PrPSc with which it needs to interact are anchored at the cell surface by the insertion of their GPI tails in specialised rafts of lipid on the cell membrane (see Section 2.8). Such association of both molecules within rafts appears to be required for the conversion to take place38, 39. Attempts have been made to model the effects of membrane anchorage on the in vitro conversion of PrPSc by incorporating PrPSen in artificial raft-like structures that are added to membrane-bound PrPres isolated from scrapie-infected brain. These experiments have shown that the raft carrying the PrPSen must be physically fused with the membrane that carries the PrPSc or that the PrPSen must be released from the raft to enable it to associate directly with the membranes carrying PrPres before it can be converted into PrPres (40, 41). This suggests that close contact within a membrane is optimal for conversion to take place.

2.5 The challenge of TSE strains

2.5.1 TSE agents are not uniformly infectious and they each cause disease with different, predictable and specific characteristics.
2.5.2 The existence of distinct strains was recognised when sources of sheep scrapie were serially transmitted to mice. A single inbred line of mice, in which all individuals make the same PrP^C, can propagate several different strains of scrapie, each with its own distinctive incubation period, pattern of damage in the brain and PrP^Sc properties^{42, 43}. Strain variation also occurs in natural scrapie, and the recent discoveries of previously unidentified strains, such as 221C^{44} and Nor98^{15}, suggests that the spectrum of different strains may change with time. Since different strains can have very different patterns of pathology and distribution of PrP^Sc, their presence may be missed by the application of standard sampling and testing protocols used for surveillance^{45}. Although a single strain of BSE appears to be responsible for the vast majority of cases around the world^{46, 47}, evidence is emerging that suggests that there may be other strains which have different properties when transmitted to mice^{48} or result in different pathology or properties of PrP^Sc in infected cattle^{49, 50}.

2.5.3 Since different strains can be replicated in an identical PrP^C background the proteinaceous component of PrP^Sc produced by each strain must be the same. Thus, if PrP is the only component of the infectious agent, the distinguishing features of strains must be enciphered in hypothetically different shapes adopted by PrP^Sc. At least one of the properties of strains, the size of the PrP fragment remaining after protease digestion, can be transmitted to PrP^C during conversion to PrP^Res in vitro, which shows that PrP^Sc can pass on elements of its distinctive 3-D structure to PrP^C^{51}. Whether this structure determines the properties of a strain, or is a consequence of it, can not be resolved in this system because it has not yet proved possible to produce newly formed PrP^Res that is infectious.

PrP^Sc isolated from brains of hamsters infected with various strains of TSEs have different degrees of resistance to unfolding and, by implication, different structures^{52}. This is consistent with the idea that the shape of PrP^Sc is unique to a strain, but at present there is no molecular model of what these different conformations might be or how they could have such diverse biological effects.

2.5.4 Many strains of TSEs, even those that have been biologically cloned by repeated passage within a species, can change their characteristics when transmitted from one species to another. This is perhaps not surprising, if the prion hypothesis is correct, given that different species have PrP^C s with different amino acid sequences. BSE has been transmitted to a number of different species either “naturally”, through contaminated food, as with cats, exotic ruminants, and humans, or experimentally, through injection or feeding, for example sheep, goats and pigs. Each of these species has a different PrP^C, but where it has been tested, the strain characteristics of their BSE-derived TSEs are the same as for BSE from cattle. This “stability” of the BSE strain in different PrP backgrounds challenges the notion that shape of PrP in PrP^Sc encodes the properties of a strain. The shape that, according to the prion hypothesis, defines the BSE strain must be independent of differences, often large, between the amino acid sequence in PrP^C. Alternatively, there may be one or more other molecules (yet to be identified) that associate with PrP to determine the strain properties. Knowing the molecular basis of TSE strains is, therefore, at the heart of understanding the nature of the TSE agent.

2.6 The role of the host’s genes in TSEs

2.6.1 A gene, Sip in sheep and Sinc in mice, that controls the incubation period of TSEs, has been known for several decades, long before PrP was discovered. The recognition of the important role that the prion protein plays in scrapie, its purification and, later, the isolation of the gene (Prnp) that encodes it led to the idea that that Prnp and Sinc or Sip may be one and the same.

2.6.2 There are two forms of Sinc, s7 which results in mice having a short incubation period after infection with ME7 scrapie, and p7 with which mice have a prolonged incubation time. Similarly, mice have two forms, “a” and “b”, of Prnp, which differ only by their amino acids at coding positions 108 and 189. Sinc s7 mice always have Prnp^a, whereas Sinc p7 mice have Prnp^b. This could mean that Sinc and Prnp are one and the
same or that they are separate, but closely linked. They were shown to be identical by making a transgenic mouse in which only the codons for the two amino acids of Prnp at 108 and 189 were changed to those of Prnp. The mouse had also changed and become Sinc p7-like.

2.6.3 Sheep also have several forms (alleles) of Prnp, some of which are strongly associated with the incidence of natural scrapie or susceptibility to experimental scrapie or BSE. Until 1997 it was a matter of debate whether scrapie was a genetic disease, arising as a direct consequence of a sheep carrying a particular form of Prnp, even though it may not have been exposed to any “external” infection. Sheep in Australia and New Zealand, where there are no reported cases of scrapie, contain the same scrapie-associated forms of Prnp as are found in sheep in the UK, where there is a high incidence of scrapie, showing that scrapie is not a genetic disease, but a genetic susceptibility to infection by an external scrapie agent.

2.6.4 Sheep that carry two copies of the Prnp allele that codes for PrP with amino acids valine (V) at position 136, arginine (R) at position 154 and glutamine (Q) at position 171 are called homozygous VRQ and are the most susceptible to scrapie. Sheep with two alleles encoding amino acids alanine (A) at position 136 and arginine (R) at positions 154 and 171 (homozygous ARR) are the most resistant. With the exception of an unconfirmed case in Japan, clinical signs of scrapie have not been reported to occur naturally in ARR homozygous sheep. However, as a result of active scrapie surveillance in Europe, based on the post mortem application of rapid diagnostic tests, two cases of scrapie in homozygous ARR sheep have been diagnosed. Furthermore, scrapie surveillance of sheep in Great Britain identified seven homozygous ARR sheep that tested positive for scrapie by the Bio-Rad Platelia ELISA test, but these could not be confirmed as scrapie-positive by immunohistochemistry and thus remain unclassified. Detection of scrapie infections in asymptomatic ARR homozygous sheep, which rarely, if ever, show clinical signs of scrapie, could indicate that ARR homozygous sheep may be able to be sub-clinical carriers of TSE infections (see Section 2.12).

2.6.5 Homozygous ARR sheep are resistant to experimental peripheral challenge with scrapie or BSE - that is by a route that does not go directly into the central nervous system or brain, for example, via the skin, a vein, orally or via the peritoneum - but do succumb to an injection of BSE directly into their brain, after a very long incubation period. Animals with one copy of each of the VRQ and ARR alleles (heterozygous VRQ/ARR) or one or two copies of the other Prnp alleles have various levels of intermediate resistance. The relative susceptibility of VRQ and ARQ alleles versus the relative resistance of the ARR allele is also found in the in vitro conversion of PrPSen to PrPRec as well as the relative infectability in culture of Rov cells expressing the different allelic forms of PrP.

2.6.6 The sheep Prnp alleles that affect scrapie (and BSE) susceptibility have been ranked and assigned to risk groups for scrapie. It is this that forms the basis of the National Scrapie Plan, which seeks to reduce and eventually eliminate Prnp alleles associated with susceptibility to TSEs in sheep. Over 500,000 sheep have been genotyped as part of the NSP and, while the vast majority fit into the known “two allele” genotypes, a small, but significant, number (0.08 per cent) appear to carry three or more PrP alleles. It is not clear what the genetic basis, origin and significance of these complex PrP genotypes might be.

2.6.7 In contrast to sheep, very few Prnp polymorphisms have been found in cattle, and those that have been found, including the common allele that codes for an extra copy of the octapeptide repeat, are not associated with differential susceptibility to BSE. Polymorphic microsatellites have also been identified within the bovine (and ovine) Prnp, but there is no evidence that any of these are linked to differential susceptibility to BSE. Humans, on the other hand, have one common polymorphism (alternative amino acids methionine or valine at coding position 129), about 50 rare point mutations in Prnp, of which about half are associated with various inherited forms of human prion disease, and
many insertions or deletions in the octapeptide repeat region, several of which are linked with inherited disease.

2.6.8 The methionine\textsubscript{129}/valine\textsubscript{129} polymorphism in humans acts in a similar way to polymorphisms in sheep and mice. Having both alleles the same (homozygous) increases the risk of both sporadic\textsuperscript{65,66} and iatrogenic\textsuperscript{67} CJD. So far all investigated cases of vCJD are homozygous for methionine\textsubscript{129}, indicating that individuals with this genotype are the most susceptible to vCJD\textsuperscript{68}. But this does not necessarily mean that individuals who carry one or two copies of the valine\textsubscript{129} allele will not also be susceptible to vCJD, albeit with longer incubation periods.

2.6.9 In addition to its effects on TSE susceptibility the human methionine\textsubscript{129}/valine\textsubscript{129} polymorphism causes two distinct disease outcomes of a single pathogenic mutation at coding position 178; FFI if PrP\textsuperscript{C} contains methionine\textsubscript{129} or familial CJD if it contains valine\textsubscript{129} \textsuperscript{69}. It would be interesting to model this common human polymorphism in transgenic mice to understand the mechanism of its action.

2.6.10 It is possible that the methionine\textsubscript{129} homozygous individuals who have so far succumbed to vCJD have in common other, non-\textit{Prnp}, genes that contribute to the shortening of the incubation period. Genetic studies in mice are revealing several non-\textit{Prnp} genes that influence incubation period\textsuperscript{70-72}. As yet their identities or how they operate are not known, but they are likely to code for some of the many host proteins involved in the replication, transport and effects of the infectious agent. It is important that these mouse genes be identified and characterised so that their equivalents can be assessed for pathogenic effects in humans and other species.

2.6.11 Susceptibility-linked mutations in \textit{Prnp} can be mapped to the molecular structure of PrP to give pointers to the pathogenic roles played by different parts of the molecule. The mutation that is associated with human GSS changes the amino acid proline to leucine at position 102, but cannot be mapped to a structure because it is in the disordered N-terminal tail. This mutation has been studied in transgenic mice with mixed results. Transgenic mice that over-expressed the mutant PrP succumbed to a spontaneous neurodegenerative disease and, although little PrP\textsubscript{Sc} was present in the brains of the affected mice, the disease was transmissible to other transgenic mice expressing low amounts of the same mutant PrP, and to hamsters\textsuperscript{73}. At the time this was an exciting result, because it seemed to provide experimental evidence in direct support of the basic tenet of the prion hypothesis – the spontaneous creation of an infectious form of the prion protein. Recent attempts to repeat this result using “gene replacement” transgenic mice have not resulted in mice which go down with a spontaneous spongiform encephalopathy or which have infectious material in their brains\textsuperscript{74}. Compared to their non-transgenic littermates, however, they have dramatically altered response to a number of TSE strains\textsuperscript{75}, and the mutation that they carry extends the incubation periods of four different strains of murine scrapie, although the extension varies considerably between the different strains\textsuperscript{76}. It is not known how this important mutation exerts its many effects, but it does show the importance of the unstructured N-terminal tail of PrP in affecting the behaviour of PrP and disease outcome. As noted above, there are many other mutations associated with inherited forms of human TSEs. The ability to study them individually in transgenic mice or cell culture, and map them on the structures of PrP, provides an opportunity to understand how they affect the behaviour of PrP\textsuperscript{C} and result in disease.

2.6.12 The N-terminal tail is an important part of PrP\textsuperscript{C} in its normal function and in terms of the pathological consequences of mutations within it. It is not essential for the infectiousness of PrP\textsuperscript{Sc}, once PrP\textsuperscript{Sc} has been formed, since it can be removed without the loss of infectivity. Nor is most of it essential for the formation of PrP\textsuperscript{Sc}, although its presence does accelerate the accumulation of PrP\textsuperscript{Sc} (\textsuperscript{77}). Normally the N-terminal region contains five tandemly arranged copies of the octapeptide repeat. Changes in the number of repeats above or below the normal five copies are often associated with inherited forms of human prion disease\textsuperscript{78}. In these cases disease probably results from mutant PrP\textsuperscript{C} taking
the wrong path through the cell and being accumulated as an incorrectly folded form resembling PrP<sub>Sc</sub>, ultimately triggering the death of nerve cells in the brain<sup>79</sup>.

2.6.13 Genes have extensive non-coding regions, parts of which control their timing and level of expression. Mutations in control regions will affect how a gene is expressed. In general, the more PrP<sup>C</sup> that is made, the shorter will be the incubation period for a particular strain of TSE<sup>80-83</sup>, so a mutation in the control regions of Prnp, which alters the expression of PrP<sup>C</sup>, could affect the susceptibility of the animal that carries it. Several polymorphisms, representing such mutations, have been identified in the control regions of the human Prnp. Some have been associated with the occurrence of spCJD (although not vCJD or iatrogenic CJD), indicating that altered levels of PrP<sup>C</sup> expression may be a risk factor for the disease<sup>84, 85</sup>. It is important to understand how the cell controls PrP<sup>C</sup> expression and how mutations or other treatments may affect it, since shutting down PrP<sup>C</sup> expression may have potential as a strategy for treatment (see Section 2.14).

### 2.7 Inactivation of TSE infectivity

2.7.1 TSE agents are notoriously difficult to inactivate and are largely resistant to the conditions normally used to kill viruses and bacteria. Of all TSE strains, BSE is amongst the most resistant, a characteristic perhaps selected by its past need to have survived multiple rounds of rendering in the production of meat and bone meal.

2.7.2 Much of the research into TSE inactivation has been pragmatic, exposing infected material to harsh physical (heat, pressure) or chemical (acid, alkali, hypochlorite) conditions and measuring the amount of infectivity that survives. From this have developed several effective methods<sup>86</sup>, but understanding why TSEs are resistant to inactivation should also give insight to the nature of the causative agent. Different TSE strains vary enormously in their resistance to inactivation, which is consistent with the idea that strains adopt different PrP conformations. The relative resistance of a strain to heat is unaffected by the amino acid sequence of the PrP of the infectious material<sup>87</sup>. Thus if heat resistance is determined by PrP structure, it must, like the other properties of the BSE strain in different species, be independent of the sequence of PrP in PrP<sub>Sc</sub>. There are three phases to the heat inactivation of a TSE agent in wet conditions. In the first there is no loss of infectivity as the temperature rises to a threshold, which varies widely between strains, but is characteristic for a strain. The second phase follows this threshold and involves rapid loss of some but not all infectivity. The third phase is prolonged with little further inactivation<sup>88</sup>.

2.7.3 The survival of this “resistant” fraction presents the biggest challenge to ensuring the safe disposal of TSE infectivity. The amount of surviving TSE infectivity can be greatly influenced by prior conditions, for example drying, that may “fix” the infectious agent in a dehydrated structure that is stable to disruptive physical or chemical conditions<sup>89</sup>. This retention of an infectious state through dehydration is reminiscent of the survival of infectivity with unaltered strain properties in material chemically fixed by formaldehyde for the preservation of fine biological structure for analysis under the microscope<sup>89</sup> (see also Section 2.8). The ultimate example of a resistant core is the reported survival of a small fraction of infectivity following exposure to dry heat at 600°C<sup>91</sup>.

2.7.4 The notion that fixed or dehydrated material can be infectious suggests that, under some conditions, a “dead” or “dried” template can initiate an infection. If so, complete inactivation of a TSE will only be achieved under conditions that allow the inactivating agent access to the core of the infectious agent. It is important to determine what the resistant material is, both to develop effective methods of inactivation as well as to understand the chemical and physical forms of this infectious material.
2.8 Production and processing of PrP in cells

2.8.1 To understand how PrP<sub>Sc</sub> is formed from PrP<sub>C</sub> it is necessary to know the normal life history of a PrP<sub>C</sub> molecule. It would then be possible to see how exposure to pre-existing PrP<sub>Sc</sub> or the presence of a mutant amino acid in PrP<sub>C</sub> can result in the diversion of PrP<sub>C</sub> to unusual pathways or cellular environments in which it adopts a conformation characteristic of PrP<sub>Sc</sub>.

2.8.2 Many proteins are synthesised on ribosomes which form part of a membranous structure called the endoplasmic reticulum (ER). A new PrP molecule is processed along a complex pathway that starts in the ER and proceeds to the Golgi apparatus, a cell structure which processes proteins generated in the ER. It undergoes several modifications before it reaches the cell’s surface. In the ER it associates with other proteins called chaperones, which help it to fold and be processed correctly. Some of these chaperones associate with a wide range of proteins, others are specific to those that have di-sulphide bonds, like PrP, or require sugar molecules adding at glycosylation sites, such as the asparagine amino acids at positions 180 and 196 in mouse PrP. Still others, yet to be described, may be specific for PrP. On this journey it has N- and C-terminal signal sequences removed, the latter being replaced with a fatty tail (the GPI tail) which anchors it in the membrane, and is folded into its normal structure. Chemicals which inhibit the correct functioning of any one of the many chaperones have the potential to result in misfolded PrP. The chaperones also monitor the correctness of folding and processing and if anything goes wrong with the PrP molecule during its time in the ER it is shunted into a pathway normally designed to end with its degradation. This is one of the cell’s mechanisms for getting rid of “mistakes”. It is called endoplasmic reticulum-associated degradation (ERAD). But if the final degradative steps in the proteasome are blocked the “waste” proteins accumulate in the cell with potentially harmful effects.

2.8.3 A PrP<sub>C</sub> molecule that passes all the quality checks arrives at the cell surface, anchored in specialised lipid regions called rafts, or caveolae-like domains, by its GPI tail, but it stays for only a short time before it is taken back into the cell in small vesicles called endosomes by a process called “endocytosis”. Endocytosis appears to be driven by PrP<sub>C</sub>’s N-terminal region since it is blocked by mutation or deletion of this region<sup>92, 93</sup>. The N-terminal region is known to bind sulphated proteoglycans<sup>94, 95</sup> which can stimulate endocytosis of PrP<sub>C</sub><sup>96</sup> and which have also been shown to inhibit accumulation of PrP<sub>Sc</sub><sup>97</sup>. Once in an endosome the majority of PrP<sub>C</sub> molecules is recycled back to the cell membrane, perhaps with removal of the N-terminal region<sup>98, 99</sup>, but a small proportion is degraded in each cycle. The raft-containing endocytic pathway for PrP<sub>C</sub> may not be typical for GPI-anchored proteins and, as such, may provide a special subcellular process in which conversion of PrP<sub>C</sub> to PrP<sub>Sc</sub> can occur<sup>100</sup>. PrP<sub>C</sub> may also be cleaved from the cell surface to enter the intercellular space.

2.8.4 Interesting, and perhaps important, alternative forms of membrane-attached PrP, CtmPrP and NtmPrP, have been identified and it has been suggested that CtmPrP plays a role in the disease<sup>101</sup>. Whereas the majority of PrP<sub>C</sub> molecules sits wholly on the outside of the membrane attached at the C-terminus via the GPI tail, CtmPrP and NtmPrP are anchored in the membrane at a transmembrane domain near the centre of the molecule, so that they span the membrane with their C-terminus or N-terminus, respectively, on the outside. CtmPrP, which unusually retains its N-terminal signal sequence as well as its C-terminal GPI anchor, is therefore attached to the membrane at two points, but, in cultured cells at least, is retained in the ER<sup>102</sup>. CtmPrP does not increase in amount in scrapie-infected cells, indicating that it is unlikely to play an essential role in triggering cell death<sup>103</sup>. Although disease-associated mutations within the transmembrane domain enhance the formation of CtmPrP, pathogenic mutations in other parts of the PrP molecule have no effect on the amounts of CtmPrP and NtmPrP<sup>104</sup>. Thus, the significance of these transmembrane forms of PrP in normal function or in disease is not yet understood.

2.8.5 When proteasome degradation of waste proteins is blocked in uninfected cells, misfolded PrP accumulates around the nucleus, in some circumstances in specific
structures called aggresomes\textsuperscript{105}, in an aggregated form. Like PrP\textsuperscript{Sc}, this aggregated PrP is insoluble in detergent and partially resistant to digestion with PK. Following removal of the proteasome block, accumulation of PrP\textsuperscript{Sc}-like PrP continues for some time, but it is not known whether this PrP\textsuperscript{Sc}-like PrP is able to convert more PrP\textsuperscript{C} molecules to its own PrP\textsuperscript{Sc}-like state\textsuperscript{106}. It is estimated that in these \textit{in vitro} cell systems normally about 10 percent of all new PrP\textsuperscript{C} molecules are diverted for degradation by the ERAD pathway\textsuperscript{107}, but the presence of a mutation, which in humans is associated with inherited and transmissible forms of the disease, results in more PrP\textsuperscript{Sc}-like PrP than in cells expressing normal PrP\textsuperscript{Sc}\textsuperscript{(106, 108)}. This suggests that one way in which sporadic or inherited TSEs may arise is through the accumulation of misfolded, protease-resistant forms in the ER, which are diverted to the cytoplasm and accumulate there with cytotoxic effects. On the other hand, while PrP molecules carrying disease-associated mutations are delayed early in their biosynthetic passage to the cell surface, with a proportion never making it through the ER, the accumulation of PK-resistant forms in proteasome-inhibited cells may result from the experimental systems used and not be a general characteristic of normal cells\textsuperscript{109}. This is consistent with the observation that accumulated PrP destined for ERAD in primary neurons is not converted into a form that resembles PrP\textsuperscript{Sc} and is not toxic, but instead may protect neurons from endogenously triggered cell death\textsuperscript{110}. Given the central roles that the cellular synthesis of PrP\textsuperscript{C} and formation of PrP\textsuperscript{Sc} play in TSEs it needs to be established what the mechanisms are for generating PrP\textsuperscript{Sc} and which chaperones help in the process.

2.8.6 PrP\textsuperscript{C} must be anchored in cholesterol-rich rafts at the cell surface for it to be converted to PrP\textsuperscript{Sc}\textsuperscript{(111, 112)} and prevention of its arrival\textsuperscript{113} at or its removal\textsuperscript{114} from the surface membrane can result in a cure of an infected cell. Anything that prolongs the presence of PrP\textsuperscript{C} at the cell surface may increase the risk of PrP\textsuperscript{C} associating with PrP\textsuperscript{Sc} if it is there. Mutations in the N-terminal region of PrP\textsuperscript{C}, such as deletion, addition or mutation of the octapeptide repeats, or even shortening of the entire region, inhibit turnover by endocytosis, and result in PrP\textsuperscript{C} remaining longer on the cell surface\textsuperscript{115, 116}. A PrP\textsuperscript{C} molecule present on the surface of one cell can be transferred to another cell if their membranes are in close contact\textsuperscript{117} providing a potential method by which infection may spread from cell to cell.

2.8.7 In addition to allowing PrP\textsuperscript{C} to transfer between cells, close contact of cell membranes enhances the transmission of TSE infection. At the individual molecule level, bringing PrP\textsuperscript{C} and PrP\textsuperscript{Sc} close together by fusion into the same membrane greatly increases the conversion of PrP\textsuperscript{C} to PrP\textsuperscript{Sc}\textsuperscript{(40)}. At the cellular level the presentation of PrP\textsuperscript{Sc} within the context of an intact membrane increases by 1,000-fold the efficiency of infection of a cell\textsuperscript{118, 119}. Furthermore, cells can be infected, as measured by the presence of PrP\textsuperscript{Sc}, by co-culture with aldehyde-fixed (and therefore dead) infected cells\textsuperscript{115} or following contact with washed PrP\textsuperscript{Sc}-coated wires\textsuperscript{120}. This suggests that, so long as PrP\textsuperscript{Sc} can be brought into close contact with PrP\textsuperscript{Sc}, actual physical transfer of PrP\textsuperscript{Sc} may not be necessary to seed a new infection. Transmissions of infection to a cell and between cells are critical processes in the development of a TSE which need to be understood and the mechanisms may vary with the type of cells involved.

2.8.8 PrP\textsuperscript{Sc} formed by different strains of TSEs has different and characteristic glycosylation patterns, which suggests that the glycosylation state might play some role in the conversion of PrP\textsuperscript{C} to PrP\textsuperscript{Sc}. It is important, therefore, to understand the pathways by which the sugars are added to PrP\textsuperscript{C}, even though unglycosylated PrP\textsuperscript{C} can be converted into PrP\textsuperscript{Sc} more efficiently than glycosylated PrP\textsuperscript{121, 122}. Glycosylation involves the addition of a complex set of sugars at each site\textsuperscript{123}, is dependent on the distance between the glycosylation site and the C-terminus\textsuperscript{124} and is affected by the presence of the disulphide bridge between the two cysteine amino acids in helices 2 and 3\textsuperscript{125}. A single cell type is able to replicate the different glycosylation patterns of two strains of scrapie, showing that it is the strain, and not the host cell, that exerts the main control over the selection of glycosylated forms of PrP\textsuperscript{C} for conversion into PrP\textsuperscript{Sc}\textsuperscript{(127)}.

2.8.9 Research on the cell biology of PrP has been hampered by the lack of good cell systems in which to study the synthesis and processing of PrP\textsuperscript{C} in normal cells and the
formation of PrP<sub>Sc</sub> in infected cells. New developments in this area have included highly susceptible clones of mouse N2a cells, N2a cells which over-express PrP<sup>Sc</sup> or express PrP "on demand", a Schwann cell line, a rabbit cell line that over-expresses sheep PrP<sup>Sc</sup>, and infected and cured lines of SMB cells.

2.9 The Function of normal PrP

2.9.1 It was anticipated that the ability to knock out Prnp would give an insight into the normal function of PrP<sup>C</sup> by allowing observation of what happened to mice that lacked the gene. It was surprising that the first two PrP “knock out” mice (Prnp<sup>−/−</sup>) showed no obvious physiological effects and lived for a normal life expectancy. This suggested that, whatever function PrP<sup>C</sup> might have, its loss could be compensated for by other proteins. A closer look at these mice, however, did show some behavioural changes, for example to their circadian rhythms, but gave little insight into the underlying function of PrP<sup>C</sup>. The mice were also found to be more sensitive to oxidative stress, consistent with PrP<sup>C</sup> having a role in the cellular response to oxidative stress, perhaps through its proposed superoxide dismutase (SOD) activity (see below). Absence of PrP altered the numbers and morphology of mitochondria, which might also be a consequence of cellular stress. Transgenic mice that are born making PrP<sup>C</sup>, but which can be induced to lose their PrP genes well after birth and which may then be unable to compensate for the sudden loss of PrP<sup>C</sup>, also remain healthy with no evidence of brain damage for up to 15 months after PrP<sup>C</sup> loss. However, in these mice there are significant effects on nerve cell function in the hippocampus of the brain, suggesting that PrP<sup>C</sup> may be involved in modulating nerve cell excitability.

2.9.2 Two further lines of Prnp<sup>−/−</sup> mice did show pronounced clinical signs of ataxia late in life with accompanying loss of Purkinje cells in their brains, suggesting that a lack of PrP<sup>C</sup> might have some functional consequences after all. Further examination of these mice showed that the methods used to remove the PrP gene had resulted in abnormal expression of the gene next to Prnp, and it was this that probably caused the disease. The gene, which was called “doppel” or Prnd, turns out to have many features in common with Prnp, but lacks the flexible N-terminal region. Expression of N-terminally truncated, but otherwise normal, PrP in the Purkinje cells of the original Prnp<sup>−/−</sup> mice also leads to loss of Purkinje cells, suggesting that there must be some common mechanism of Purkinje cell death related to the absence of the N-terminal regions of doppel or PrP<sup>C</sup>. Even less is known of the normal function of doppel than is known for PrP, but unlike PrP, it is not required for prion disease progression or PrP<sub>Sc</sub> formation, nor does its over-expression appear to alter the development of a TSE.

2.9.3 PrP<sup>C</sup> binds copper ions through the histidine amino acids in the octapeptide repeat region and in so doing acquires superoxide dismutase (SOD) activity, which helps to protect cells against oxidative stress. Copper binding must occur during the folding of newly synthesised prion protein, since PrP<sup>C</sup> has no SOD activity if the copper is added after folding. Absence of the octapeptide repeats, as well as copper, results in loss of SOD activity. PrP<sup>C</sup> has a high affinity for copper at near neutral pH but far less under acid conditions, suggesting that PrP<sup>C</sup> might play a role in the transport of copper into a cell, taking up copper ions under the near-neutral conditions at the cell surface and releasing the copper following internalisation via endosomes where the conditions are much more acid. A similar role has been proposed for PrP in the maintenance of zinc in neurons by acting as a transporter of zinc into the cell or as a zinc sensor. While many of these PrP functional studies have used PrP<sup>Kcs</sup> it appears that PrP<sup>C</sup> performs similar functions in vivo. Infection of cells in culture with scrapie reduces copper binding and impairs the cell’s ability to respond to oxidative stress, which are consistent with PrP<sup>C</sup> playing a role in copper homeostasis and having SOD activity as part of the cellular response to oxidative stress, respectively. In addition to its SOD-like activity, PrP with bound copper may be involved in the regulation of the anti-oxidant system in neurons. It is also suggested that PrP<sup>C</sup> may bind glycosaminoglycans (GAGs), through copper ions bound to the
2.9.4 PrP has been shown to have other binding activities with potential functional implications. It interacts tightly with the laminin receptor on the surface of cells\textsuperscript{150}, which may explain its ability to bind laminin\textsuperscript{151}. It also has nucleic acid binding properties similar to those of the nucleocapsid protein of retroviruses, such as the human and feline immunodeficiency viruses (HIV and FIV); PrP\textsubscript{C} can promote the joining of two RNA molecules and the synthesis of viral DNA\textsuperscript{152, 153}. Sequence-specific binding of DNA to PrP\textsubscript{Rec} converts PrP\textsubscript{Rec} from the normal refolded alpha-helix rich structure characteristic of PrP\textsubscript{C} to a soluble beta-sheet rich form similar to that found in PrP\textsubscript{Sc}. The characteristics of the binding reaction suggest that it is a dimer of PrP\textsubscript{Rec} that binds the DNA\textsuperscript{154}. Human PrP\textsubscript{Rec} has also been shown to bind to small highly structured RNAs (shsRNA), leading to PrP aggregation, the conversion of PrP\textsubscript{Sen} to PrP\textsubscript{Res} and the protection of the bound shsRNA from hydrolysis by a ribonuclease\textsuperscript{31}. Furthermore, addition of mammalian (as opposed to invertebrate, fungal or bacterial) RNA to an \textit{in vitro} PrP\textsubscript{C}-to-PrP\textsubscript{Res} conversion system specifically enhanced the production of PrP\textsubscript{Res}, suggesting that host encoded RNA molecules may play a role in the production of PrP\textsubscript{Sc} \textit{in vivo}\textsuperscript{29}. Given the various associations of PrP\textsubscript{C} it will be necessary to establish which ones have physiological relevance in normal circumstances, which ones play a role in pathogenesis and even whether absence of functional PrP\textsubscript{C} triggers development of disease.

2.10 Studies of prions in fungi

2.10.1 The discovery of the prion protein in the early 1980’s, and the proposal that it might form the basis of the scrapie infectious agent, prompted the search for similar “infectious” proteins (“prions”) in other organisms. The concept of self-replicating “prions” was invoked to explain the properties of two yeast elements called [URE3] and [PSI]\textsuperscript{155}. These are the yeast equivalent of PrP\textsubscript{Sc} and are the “prion” form of normal yeast proteins Ure2 and Sup35, respectively, but unlike the pathogenic PrP\textsubscript{Sc}, their presence does not result in the death of the yeast and may indeed have selective advantages\textsuperscript{156}. Further “prions” have been found in yeast as well as other fungi, and at least one of these, the [Het-s] protein in \textit{Podospora anserine}\textsuperscript{157}, has been shown to have a normal function in controlling incompatibility between the hyphae of different strains.

2.10.2 Despite the vast evolutionary distance between fungi and mammals, fungal “prions” have many features in common with PrP\textsubscript{Sc} and provide far simpler systems in which to study them. Just as PrP\textsubscript{C} is a soluble protease-susceptible protein and PrP\textsubscript{Sc} is an insoluble protease-resistant protein, Ure2 and Sup35 are soluble and digestible with proteases whereas [URE3] and [PSI] form insoluble aggregates and are resistant to proteases. Aggregates isolated from [PSI] can seed the conversion of soluble Sup35 protein into amyloid-like fibres in a manner analogous to the way in which PrP\textsubscript{Sc} seeds the \textit{in vitro} conversion of PrP\textsubscript{C} to PrP\textsubscript{Res}. Amyloid aggregates made \textit{in vitro} from recombinant HET-s are, like PrP\textsubscript{Res}, resistant to PK digestion, but unlike PrP\textsubscript{Res}, are “infectious” and, like PrP\textsubscript{Sc}, the PK-resistant core retains infectivity\textsuperscript{158}.

2.10.3 Over-expression of PrP\textsubscript{C} increases susceptibility to infection, while over-expression of Ure2 and Sup35 leads to the \textit{de novo} formation of [URE3] and [PSI]. The yeast Sup35 protein has five copies of a nine amino-acid repeat and the introduction of extra repeats induces new [PSI] “prions”, which has close parallels with the disease related consequences of extra copies of the octapeptide repeat in PrP\textsubscript{C}.

2.10.4 Studies of yeast have shown that one “prion” can induce the formation of others\textsuperscript{159} and have highlighted the important role of other proteins in their formation and propagation. For example, the protein Mks1p is required for the generation of [URE3], but not its replication\textsuperscript{160}, whereas protein chaperone Hsp104 is required for replication of [PSI], but not for its formation\textsuperscript{161, 162}. Yeast can be cured of [PSI] by treatment with
2.11 Pathogenesis of TSEs

2.11.1 A TSE begins when an infectious agent gains entry to the body, although for sporadic and familial forms of CJD it may be that the infectious agent is formed spontaneously within the body. The most likely routes of natural infection are through the mouth or gut following consumption of contaminated food or water (oral) or through the skin if cuts or abrasions are exposed to infectious material (cutaneous). Spread through medical interventions (iatrogenic) has also happened, though rarely, in both animals\(^{164, 165}\) and humans\(^{166}\).

2.11.2 Unlike most other TSEs, scrapie is maintained in flocks naturally and must therefore be transmitted horizontally and, perhaps, vertically, but it is still not understood how this occurs. PrP\(^{Sc}\) is detected in about 80 per cent of placentas formed in scrapie-infected sheep\(^{167}\), but is restricted to only the maternally derived caruncular endometrium and the foetally derived cotyledonary chorioallantois. This suggests that the developing foetus may never be exposed to infection in the uterus because it is separated from the PrP\(^{Sc}\)-positive allantois and choroioallantois by the PrP\(^{Sc}\)-negative amnion\(^{168}\). The accumulation of PrP\(^{Sc}\) in the placentas of scrapie-infected ewes is controlled by the Prnp genotype of the foetus and is only detected in the placentas of fully (homozygous) susceptible foetuses\(^{169, 170}\). However, the pattern of accumulation of PrP\(^{Sc}\) in tissues of fully susceptible lambs after birth is the same for those born to scrapie-infected ewes as it is for those born to uninfected ewes, strongly indicating that infection of lambs with scrapie is a post-birth event\(^{170}\). This suggests that a placenta becomes infected with scrapie when a ewe is infected and its lamb is of a susceptible genotype, and contaminates the lamb at birth. Any other susceptible lamb in the flock exposed to this risk could also become infected, but clearly that risk would be higher on farms using group lambing and the same location each year (see Section 2.15.2). There is, however, much still to learn about how sheep become infected in scrapie-affected flocks and, if contamination of the environment plays a role, how long the agent persists.

2.11.3 Once inside the body, by whatever route, the infectious agent must find a site to replicate and then spread to other tissues and organs. The cells of the immune system (lymphoreticular system, LRS) are intimately involved in this. The infection must then gain access to the central nervous system (CNS), travel along nerve cells to reach regions deep in the brain and, in the process, trigger the events that result in failure of nerve cell function and eventually cell death. Particular clinical signs result from this targeted destruction of nerve cells within specific regions in the brain. The clinical signs are the final events in what is usually a long incubation process. In some cases, the incubation period may be so long that clinical signs never appear and the animal or human carries the infection until they die of old age.

2.11.4 The incubation period is not solely determined by genes (see Section 2.6). It is influenced greatly by the initiating dose of infectivity. Lower doses result in longer and more variable incubation periods and very low doses may not initiate an infection at all\(^{171}\), but the question of whether there is a threshold dose of a TSE, below which an infection can not be initiated, remains open. The important question of whether several successive very small doses, such as those that humans may have been exposed to in relation to BSE, can accumulate in the body to form a larger and more dangerous dose also remains unanswered. Experiments with scrapie in hamsters indicate that successive oral doses can be retained over short periods of time and accumulate in the body, although not to the same level as if they had they been combined and administered as a single dose\(^{172}\). Thus the risk of infection increases with repeated doses, but to a lesser degree than would be expected if exposures combine independently or in a cumulative manner, and the resulting incubation periods are longer than would be expected if all challenges acted independently\(^{173}\). There must, therefore, be an interfering interaction between the...
infectious agent in successive challenges, perhaps in a way similar to that seen with different strains\textsuperscript{174}, or a mechanism for slowly clearing infectivity from the body, but more research needs to be done to understand this key but complex issue.

2.11.5 The gut is well supplied with lymphoid tissue and both the LRS and gut are supplied with nerves which ultimately connect with the brain and spinal cord. In young sheep specialised lymphoid tissues called the Peyer’s patches are an important site of uptake and production of PrP\textsuperscript{Sc} and replication of scrapie infectivity. They also provide close contact between nerve fibres and lymphoid cells shown to contain abundant PrP\textsuperscript{Sc}, including the follicular dendritic cells (FDCs), and from them infectivity spreads to other gut-associated lymphoid tissue (GALT), eventually finding its way into the network of nerve fibres that supply the digestive tract\textsuperscript{175-177}. The transfer of sufficient infectivity from FDCs to nerve fibres necessary to initiate a normal infection is likely to occur within a few days, since “loss” of functional FDCs upon treatment with lymphotoxin $\beta$ receptor-immunoglobulin fusion protein (LT$\beta$R-Ig, see below) 14 days after oral challenge has no effect on susceptibility or incubation period\textsuperscript{178}.

2.11.6 Studies in rodents have shown that PrP\textsuperscript{Sc} can be taken up directly into the cytoplasm of enterocytes lining the duodenum and jejunum of neonatal mice\textsuperscript{179}. Specialised M cells, which reside in the lining of the intestine and whose normal function is to assist in uptake of molecules from the interior surface of the gut (lumen), are also likely to be involved in the uptake of infectivity\textsuperscript{180}. Dendritic cells in GALT, which have been shown to take up PrP\textsuperscript{Sc} \textit{in vitro}\textsuperscript{181}, migrate \textit{in vivo} and may take up and transport PrP\textsuperscript{Sc} from the lumen of the gut, through the lymphatic system, to lymphoid tissue. The uptake of infectivity, unlike its subsequent replication, appears to be unaffected by the difference in amino acid homology between the infecting PrP\textsuperscript{Sc} and the host’s PrP\textsuperscript{C}, nor the presence in the host of Prnp alleles linked to scrapie resistance. Some of the cells likely to be involved in uptake of infectivity or PrP\textsuperscript{Sc} from the gut may have been identified (but not yet for the skin), but little is known about how this key step in the infection is achieved.

2.11.7 The lymph nodes and spleen of the LRS have germinal centres, in which reside the long-lived non-dividing FDCs. The normal function of FDCs is to trap immune complexes for presentation to B lymphocytes, but they also play a central role in the replication of TSE infectivity\textsuperscript{182}. The normal function of FDCs in trapping immune complexes may be involved in the initial uptake of PrP\textsuperscript{Sc} and infectivity by FDCs. Treatments which deplete or remove elements of the complement system, which assist in the normal trapping of immune complexes, delay the onset of scrapie following peripheral infection and reduce the accumulation of PrP\textsuperscript{Sc} in the spleen\textsuperscript{183, 184}. The development and maintenance of FDCs are crucially dependent on the presence of other cells of the immune system, for example B and T lymphocytes, which may explain in part the need for differentiated B lymphocytes for neuroinvasion by scrapie\textsuperscript{185}. Conditions that result in the loss of FDCs or that impair their function also affect the course of a TSE infection. Transgenic mice lacking FDCs are relatively resistant to scrapie after peripheral injection and no infectivity or PrP\textsuperscript{Sc} is found in the spleen\textsuperscript{186}. FDCs disappear temporarily following treatment of mice with LT$\beta$R-Ig. If induced at or around the time of exposure to scrapie infectivity, this disappearance of FDCs is accompanied by a reduction in susceptibility and delays in the accumulation of infectivity in the immune system and neuroinvasion\textsuperscript{187-189}.

2.11.8 The involvement of the LRS in the uptake and replication of the scrapie agent explains the widespread peripheral distribution of PrP\textsuperscript{Sc} and infectivity in many breeds and Prnp genotypes of scrapie-infected sheep\textsuperscript{190}. Since BSE infection is also widely distributed in the peripheral organs and blood of orally dosed sheep\textsuperscript{191-193} it is surprising that little infectivity or PrP\textsuperscript{Sc} have been found in the peripheral organs of BSE-infected cattle, either in clinical cases from the field or at all stages of an experimental infection\textsuperscript{194}. Peripheral infectivity in orally dosed BSE-infected cattle is largely restricted to the distal ileum, in keeping with an initial uptake of infectivity from the gut by the Peyer’s patch. PrP\textsuperscript{Sc} has also been detected in some of the follicles of the Peyer’s patch in experimentally exposed animals, but not in those of naturally occurring clinical cases of BSE, and, sparsely, in the neurons associated with the distal ileum\textsuperscript{195}. A low level of infectivity has also been
detected in the tonsil, another lymphoid organ associated with the alimentary tract, 10 months after experimental oral infection. This suggests that in cattle the infection travels directly from the lymphoid tissue within the Peyer’s patch (and perhaps tonsil) to the CNS via peripheral nerves rather than spreading further within the LRS. It may therefore be difficult to find a non-CNS based test for BSE in cattle, but, if at all possible, a suitable accessible target tissue should be sought to make large scale surveillance on live animals a reality. Fundamental to this is the question of what determines whether a strain of TSE will replicate widely in the LRS or restrict itself to the nervous system. This remains a mystery that needs to be solved. The assumption that TSE infection only results in loss of function from damage in the CNS should also be questioned with, for example, studies to establish whether TSEs affect the functioning of an infected immune system in which they reside and replicate.

2.11.9 As with scrapie and experimental BSE infections in sheep, infectivity and PrP\textsuperscript{Sc} appear to be widely distributed in peripheral tissues and CNS in vCJD patients, whereas they were thought to be restricted to the CNS in cases of spCJD. However, the application of detection methods with greater sensitivity has identified the presence of PrP\textsuperscript{Sc} in the spleen and skeletal muscle of about one third of a group of patients who died of spCJD in Switzerland between 1996 and 2002. Patients with peripheral PrP\textsuperscript{Sc} also had longer duration of disease. In view of the potential for ante mortem diagnosis, it will be important to establish, given sufficient sensitivity of test, whether peripheral PrP\textsuperscript{Sc} can be detected in patients with all forms of CJD.

2.11.10 Exactly how a TSE agent enters the CNS from the LRS is also not understood, and there may be several routes. One is likely to be through the nerves that innervate the lymphoid organs, either through direct contact with FDCs or indirectly through other lymphoid cells. Chemical or immunological removal of the sympathetic nervous system delays or prevents scrapie infection, whereas an over-supply of sympathetic nerves to lymphoid organs shortens incubation periods, suggesting that the supply of nerves to lymphoid organs can be a bottleneck to an infection gaining entry to the CNS. Furthermore, the distance between FDCs and splenic nerves controls the rate at which neuroinvasion occurs, being faster the closer the two cell types are together.

2.11.11 Another candidate cell for delivery of infection to a peripheral nerve might be a different form of dendritic cell, CD11c\textsuperscript{+}, which, when collected from the spleen of a scrapie-infected normal mouse, can transmit scrapie to RAG-1\textsuperscript{0/0} mice without any PrP\textsuperscript{Sc} accumulating in the spleen of the recipient RAG-1\textsuperscript{0/0} mice. These mice, like SCID mice, lack FDCs and resist TSE infection by a peripheral route, suggesting that the CD11c\textsuperscript{+} dendritic cells can carry scrapie infection directly to nerves without any other LRS involvement. Mice with disabled CD11c\textsuperscript{+} cells and lacking FDC can be infected by peripheral high-dose inoculations of scrapie, suggesting that there must be yet other routes of entry to the CNS.

2.11.12 PrP\textsuperscript{Sc} is detected in GALT and nerves of the enteric and autonomic nervous systems about one third of the way through the incubation period in hamsters orally infected with 263K, and can be followed in its path to the CNS in material prepared from successively later stages in the incubation period. This suggests that the infectious agent primarily uses the vagus and splanchnic nerves to invade directly initial target sites in the brain and spinal cord separately, with subsequent spread within the CNS to other parts of the brain and spinal cord. Injection of TME into a hamster’s tongue is 10,000 times more efficient at infecting an animal than oral ingestion, and PrP\textsuperscript{Sc} is found associated with individual fibres of the hypoglossal nerve after only seven days. Nearby lymph nodes also have deposits of PrP\textsuperscript{Sc}, but the initial appearance of TME infection in brain regions associated with the hypoglossal nerve indicates that it moved to the brain directly via the hypoglossal nerve, which is faster than going via the LRS. Movement of molecules up and down long nerve fibres is achieved by processes known as “axoplasmic transport”. The rate of movement of infectivity along a peripheral nerve towards the CNS is estimated to be about 1mm per day, which is significantly slower than normal axoplasmic transport. Unlike the movement of normal nerve cell components it is a replicative process.
process, generating more infectivity as it moves along the nerve. The introduction of prions into nerves of transgenic mice that are defective in fast axonal transport (one of the processes of axoplasmic transport) resulted in normal progression of neuroinvasion, further suggesting that the mechanism for moving a TSE infection along nerve fibres is different from those of normal axoplasmic transport. But what this mechanism might be remains an important unanswered question.

2.11.13 Different models of TSEs differ in the sequence of events that occur in the brain following initial infection, which makes it difficult to deduce what are “causes” and what are “effects”. For example, in one model of ME7 murine scrapie, consistent with PrPSc having some causative role, PrPSc is deposited in neurons first, shortly followed by a loss of synapses (connections between nerve fibres), nerve endings and spines on dendrites (branching finger-like processes of nerve cells). This effectively disconnects these cells from their neighbours and probably triggers their death and loss. On the other hand, in an 87V model of murine scrapie, PrPSc is first detected almost 100 days after the network of dendrites becomes constricted and deformed, with some signs of nerve cell death. In the Sc237 model of hamster scrapie, there is an increase in the branching of dendrites but no detectable loss of cells or dendritic spines despite the presence of PrPSc.

2.11.14 Some of the neuron loss that occurs in TSEs appears to be mediated by microglia that have been activated by changes in neurons following exposure to PrPSc. Nevertheless the inflammatory response to damage in a TSE-infected brain is unusual because the normal pro-inflammatory cytokines are not activated; instead upregulation of transforming growth factor β1 may play a role in maintaining the microglial profile. It is likely, however, that in those TSEs where substantial nerve cell losses occur, the selective loss of specific subsets of nerve cells (targeted by the properties of the particular strain of TSE) will be the underlying basis for the clinical signs that appear. PrPSc is not only necessary for replication of the infectious agent, its presence in cells is also essential for the spread of infection along a nerve. PrPSc must be expressed by a cell or neuron for the pathological consequences of infection to be seen, even when PrPSc is present in neighbouring cells. This indicates that PrPSc is not directly neurotoxic, but that neurotoxicity results from aberrant PrP metabolism in the neuron that is making PrPSc or that PrPSc is a necessary receptor for the molecule that triggers nerve cell death. For scrapie in mice, it has been known for a long time that the pattern of pathology and deposition of PrPSc in the brain can vary enormously, depending on the strain of mouse infected and the strain of scrapie with which it is infected. The same has been shown for different breeds and Prnp genotypes of sheep infected with experimental or natural sources of scrapie. The distribution of PrPSc is affected by the strain of scrapie and the host’s Prnp genotype.

2.12 The species barrier and the “carrier” state

2.12.1 The “species barrier” refers to the observation that it is usually more difficult to transmit a TSE between two species than it is within a species. Transmission to a new species can result in a low proportion of animals succumbing to disease, often after long incubation periods. Other features of the species barrier include a shortening of the incubation period for successive transmissions within the new species, the selection of variant strains and altered pathogenesis. Cattle BSE transmits readily to mice, which allowed them to be used for measuring BSE infectivity, but there is a significant species barrier because mice are approximately 500-fold less susceptible to BSE than cattle and there is significant shortening of the incubation period of BSE between primary and secondary transmission in mice. This species barrier appears to be removed by the expression of high levels of bovine PrP in transgenic mice, although no direct comparison of susceptibility with cattle has been reported using the same preparation of infectious material, as was the case for the species barrier between cattle and wild type mice. The basis of the cattle-to-mouse species barrier may reside in the difficulty cattle BSE has in initiating an infection directly in the mouse’s brain, needing instead to be processed through the lymphoid system. Unlike mouse-to-mouse transmissions,
intracerebral inoculation of mice with cattle BSE results in longer incubation periods than does intraperitoneal injection and SCID mice, which are defective in peripheral replication of TSEs, are relatively resistant to cattle BSE, even after intracerebral injection.

2.12.2 One of the favoured experimental models of an extreme species barrier has been the inability to produce clinical signs of TSE disease in mice following infection with the 263K or Sc237 strains of hamster scrapie, and it had been assumed by many that this represented a complete block to the infection of mice. However, an absence of clinical signs does not necessarily mean that the infectious agent has not replicated and spread within the host, even causing recognisable lesions within the brain. For example, transmission of six different sources of sporadic CJD to four strains of mice produced no clinical signs of disease and no significant differences in survival relative to uninfected controls, but did result in characteristic TSE-related brain pathology in the majority of such mice surviving beyond 500 days.

2.12.3 The hamster/mouse species barrier and the nature of the resultant carrier state have recently been revisited. As expected, transmission of hamster scrapie to mice produced no overt disease and no evidence of TSE replication for about a year, after which there was active replication and adaptation of new strains capable of causing disease in mice, with limited evidence of PrP accumulation except after about 600 days. During the first pass in mice, strains retained their virulence for hamsters, but after three or four successive sub-passages in mice, mouse-trophic strains emerged.

2.12.4 Subclinical infection indicative of a carrier state can also be established after within-species transmissions. Serially diluted, orally administered, 263K hamster scrapie resulted in the detection of PrP in some healthy animals which survived to be culled at the end of the experiment, some 239 days after the last clinical case. However, the small difference between the calculated LD (the "lethal" dose which results in 50 percent of the animals dying of clinical scrapie) and the ID (the dose which results in 50 percent of the animals showing some sign of infection, which includes both scrapie deaths and PrP-positive animals showing no clinical signs) indicates that the number of carrier animals would be unlikely to exceed greatly the number of clinical cases.

2.12.5 The potential existence of asymptomatic infected "carrier" animals or humans is of great relevance to the effectiveness of surveillance and control programmes for BSE, scrapie and vCJD and needs to be pursued. Lack of detectable PrP would preclude detection by current diagnostic tests and such "carrier" animals or humans would represent hidden reservoirs of infectivity, implying a risk of onward infection to others through natural (scrapie) or iatrogenic (human) transmission.

2.13 Diagnosis of TSEs

2.13.1 Currently, the diagnosis of a TSE infection in a live animal or human relies on the recognition of characteristic clinical signs, with confirmatory diagnostic tests being applied to samples of live tissues (biopsies) or post mortem material. Most sheep infected with scrapie exhibit behavioural changes, pruritus (itching indicated by rubbing or nibbling of body parts), ataxia (inability to coordinate movement) and weight loss, whereas those infected experimentally with BSE are more likely to present with sudden-onset ataxia in the absence of pruritis. However, because of the variability of clinical signs, it may be impossible to distinguish a sheep infected with BSE from those infected with one of the many forms of scrapie in the field. An important clinical sign for the diagnosis of BSE in live cattle is the presence of over-reactivity to external stimuli, even in the very early clinical phase.

2.13.2 In humans, accumulated clinical experience over many years has enabled standardised clinical diagnostic criteria to be agreed for sporadic, iatrogenic and inherited forms of CJD, but such experience has not existed long for vCJD. Emerging diagnostic criteria for vCJD are early psychiatric symptoms, followed within about six months by
ataxia, involuntary movements and cognitive impairment\textsuperscript{232}. Patients with vCJD do not show distinguishable features in electroencephalograms (EEG), nor consistently the presence of protein 14-3-3 in cerebrospinal fluid (CSF), often associated with spCJD\textsuperscript{233}, but they do show a characteristic signal in magnetic resonance images (MRI) of the brain\textsuperscript{234, 235} and PrP\textsuperscript{Sc} can be detected in tonsil biopsies\textsuperscript{199}.

2.13.3 The development of diagnostic tests to identify TSE infections requires a specific marker of infection and reagents with which to identify it, as well as detailed knowledge of the pathogenesis of the disease (for example, identifying in which tissues the infection can be detected and at what stage in the incubation period). Viral or bacterial infections are diagnosed by the presence of their proteins or of nucleic acids that are distinct from those of the host, or by the presence of antibodies that the host has produced in response to the infecting pathogen. No such agent-specific proteins or nucleic acids have been found for TSEs and a TSE infection does not appear to elicit the production of detectable amounts of infection-specific antibodies. Thus, to date, all diagnostic tests for TSEs are based on the detection of the only established marker for infection, PrP\textsuperscript{Sc}. This has the inherent difficulty that even uninfected animals and humans have significant amounts of PrP\textsuperscript{C}. A diagnostic test must therefore be able to distinguish disease-specific PrP\textsuperscript{Sc} from all non-infection related forms of PrP, including normal PrP\textsuperscript{C}.

2.13.4 For the future, the dramatic and specific loss of the messenger RNA that codes for a protein involved in the differentiation of red blood cells (EDRF) late in TSE disease\textsuperscript{236} raises the prospect of a non-PrP based diagnostic test on blood, an easily accessible material. Elevated levels of fatty acid binding protein (FABP) in CSF, and its heart form (H-FABP) in blood plasma, of CJD patients may also provide a basis for a blood based screening test\textsuperscript{237}. It would be useful to have more non-PrP based markers of TSE infection to assess for diagnostic potential. One way that these could be looked for is by microarray analysis of the different genes expressed following TSE infection but for now diagnosis depends on finding PrP\textsuperscript{Sc}.

2.13.5 The presence of PrP is detected by binding an antibody, but as yet there are no antibodies or reagents that recognise only PrP\textsuperscript{Sc} and not PrP\textsuperscript{C} with proven diagnostic usefulness. Antibodies have recently been described which bind only to PrP\textsuperscript{Sc}\textsuperscript{238} and RNA aptamers have been selected with more than ten-fold higher affinity for PrP\textsuperscript{Sc} than PrP\textsuperscript{C}\textsuperscript{239}, giving the potential to form the basis of a validated TSE-specific test. Currently the distinction between PrP\textsuperscript{C} and PrP\textsuperscript{Sc} has to be made on the basis of some additional biochemical treatment, such as solubility\textsuperscript{240}, protease sensitivity\textsuperscript{241} or conformational stability\textsuperscript{52, 242}, or the recognition of specific patterns of PrP\textsuperscript{Sc} deposition in antibody-stained histological sections.

2.13.6 Using these approaches to detect PrP\textsuperscript{Sc} in tonsil biopsies, scrapie has been diagnosed in live susceptible sheep as early as one third of the way through an incubation period and over one and a half years before the onset of clinical signs\textsuperscript{243}. The lymphoid tissue that is present in the accessible “third eyelid” of sheep also contains PrP\textsuperscript{Sc} in scrapie infected sheep, which can be used as a diagnostic test in scrapie surveillance\textsuperscript{244}. Lymphoid tissues from vCJD patients, but not those with sporadic or inherited forms of CJD, were found to contain PrP\textsuperscript{Sc}\textsuperscript{199} and tonsil biopsy is now used as an aid to positive diagnosis of vCJD. Recently, small amounts of PrP\textsuperscript{Sc} have been found in lymphoid tissues and muscle suitable for biopsy in about one third of patients in Switzerland with spCJD\textsuperscript{200} and deposited in the neuroepithelium on the inner surface of the nose\textsuperscript{245}. These may eventually provide accessible tissues for confirmatory ante mortem diagnosis of spCJD.

2.13.7 The detection of PrP\textsuperscript{Sc} in tonsil biopsies provides an important step towards a practical method of ante mortem diagnosis, but a test based on blood or urine would be more acceptable and more applicable to large-scale surveillance. The transmission of experimental BSE and natural scrapie between sheep by transfusion of blood taken half way through the incubation period indicates that blood can carry at least low levels of the infectious agent\textsuperscript{193}, which should be detectable if a test is sensitive enough. Perhaps this is what the immunocapillary electrophoresis (ICE) test\textsuperscript{246, 247} is reportedly measuring.
However, application of the original ICE method failed to distinguish between extracts of leucocytes from the blood of healthy and CJD-infected chimpanzees or humans\textsuperscript{248}, indicating either that the method is not sensitive enough for, or that there is no infectivity in, the blood of these species. Proteinase K-resistant PrP has been identified in the urine of TSE-infected cattle, humans and clinical and preclinical hamsters\textsuperscript{249}, but it is not infectious like PrP\textsuperscript{Sc}. Nevertheless, it presents another potential approach to non-penetrative sampling for TSE diagnosis.

2.13.8 At the time of writing there are five tests based on PrP\textsuperscript{Sc} approved by the European Commission for the \textit{post mortem} diagnosis of BSE in cattle\textsuperscript{241, 250} and there are plans to evaluate further tests. All validated tests are 100 per cent specific for BSE-infected brain material and have met defined standards of sensitivity which equate to the best mouse bioassay systems. For the future, the ability to detect very small quantities of PrP\textsuperscript{Sc} may be enhanced by the cyclic amplification of protein misfolding of PrP\textsuperscript{Sc} by the presence of PrP\textsuperscript{Sc} (28). While it is desirable to have sensitive tests capable of detecting very small quantities of PrP\textsuperscript{Sc}, the ability of a test to detect early infection in an animal or human also depends on whether PrP\textsuperscript{Sc} is present in the target tissue at early stages in the incubation period. For example, the current EC-validated PrP\textsuperscript{Sc}-based tests probably detect BSE-infection in the brain stems of cattle no earlier than about 6 months before clinical signs appear, while cattle may have initially been infected 3 or more years earlier. Evidence is accumulating about the tissue distribution of PrP\textsuperscript{Sc} and infectivity in clinically affected humans, cattle and sheep\textsuperscript{251}, but relatively little is known about their presence or tissue distribution at earlier stages in the incubation period. It is important, therefore, to gather more information on this to know which tissues should be targeted for early diagnosis and ensure that the test is able to work on these.

2.13.9 Tests based on surrogate markers of infection allow the positive identification of an infected animal or human at or after the point during the incubation period when the marker is first detectable, but do not allow detection and quantitative measurement of infectivity. For example, while PrP\textsuperscript{Sc} is thought to be a good marker for infection, not all PK-resistant PrP is infectious and large differences have been found in the specific infectivity of various PrP\textsuperscript{Sc} preparations\textsuperscript{252-254}. Infectivity must be measured by bioassay. In the past mice have generally been used, which, for all TSEs of importance, has involved crossing a species barrier, with variable consequences for sensitivity. The use of transgenic mice expressing high levels of human\textsuperscript{255}, bovine\textsuperscript{222, 256} and ovine\textsuperscript{257} PrP is one approach to overcoming the species barrier and providing rapid, sensitive assay systems for CJD, BSE and scrapie, respectively. Ideally, sensitive infectivity assays based on cell culture systems need to be developed to give quick results and to reduce the use of animals in the test process. Sublines of N2a neuroblastoma cells that are highly susceptible to mouse RML scrapie infectivity have been isolated\textsuperscript{258}, laying the basis for a quantitative cell based assay of infectivity. However, a way will have to be found to broaden the range of strains to which the assay cells are susceptible if the need for a large collection of species- and strain-specific cell lines is to be avoided.

2.13.10 In addition to knowing whether an animal or human is infected with a TSE it is desirable to know the strain of TSE, and particularly to distinguish between the different human TSEs and between scrapie and BSE in sheep. Strains are classically identified on the basis of their transmission properties to inbred lines of mice\textsuperscript{224}, and while this is still an important way to define strains, it is a long and expensive process. Strains of TSEs also differ in the biochemical and biophysical properties of the PrP\textsuperscript{Sc} they produce. The PrP\textsuperscript{Sc}s produced by different strains differ in the relative proportions of PrP molecules which have both glycosylation sites filled (diglycosyl), one or other of the glycosylation sites filled (monoglycosyl) or no glycosylation sites filled (aglycosyl). The position at which PK cuts the PrP\textsuperscript{Sc} molecule can also vary between strains, resulting in slightly different sized PK-resistant fragments.

2.13.11 These properties were first used to distinguish vCJD from other types of CJD while linking it with BSE. The similarity between the PrP\textsuperscript{Sc} profiles of vCJD and BSE-related TSEs indicates that these properties of BSE, like those of transmission to mice, are maintained.
upon passage in several different species\textsuperscript{259}. PrP\textsubscript{Sc} profiles of several different strains of mouse scrapie also appear to be stable to multiple passage in the same species\textsuperscript{260}. Although there is still some debate as to the precise classification, PrP\textsubscript{Sc} profiles, together with PrP genotype information, form the basis of a system for typing sporadic CJD\textsuperscript{261, 262} and distinguishing them from vCJD. Detecting unusual protease-resistant fragments of PrP may also become useful in the diagnosis of specific forms of CJD, for example the presence of an 11-12 kDa C-terminal fragment may be characteristic of certain iatrogenic and familial forms of CJD\textsuperscript{263}. In applying PrP\textsubscript{Sc} profiling it is necessary to analyse the same tissue – most often a defined region of the brain – because the pattern can vary between different tissues from the same individual\textsuperscript{199}.

2.13.12 PrP\textsubscript{Sc} profiling is able to distinguish BSE from scrapie in sheep on the basis of the ratios of the different glycosylated forms and the smaller size of the aglycosyl fragment in sheep experimentally infected with BSE. Using the monoclonal antibody P4 to detect the different fragments of PK-digested PrP\textsubscript{Sc} enhances the ability to distinguish BSE from scrapie, as it only gives a strong signal with PK-resistant fragments from scrapie sources\textsuperscript{264}, presumably because the enzyme cuts within and destroys the P4 antibody recognition site in PrP\textsubscript{Sc} from BSE infections. Sources of sheep scrapie and experimental BSE can also be distinguished on the basis of the immunohistochemical staining patterns of brain sections using a panel of anti-PrP antibodies. The different patterns probably represent the selective targeting of brain regions by strains and differential processing and \textit{in vivo} fragmentation of PrP\textsuperscript{265, 266}. While the various forms of PrP profiling look promising as potential screening tests for BSE in sheep, some caution needs to be exercised. Little is known about what the BSE PrP profiles would look like in all genotypes and breeds of sheep, especially after a number of passages. It is also important that the PrP profiles of the many natural strains of scrapie be determined and assessed for differentiation from BSE, since one source of scrapie (strain CH1641) is known to have a very similar profile to that of BSE\textsuperscript{264, 267}.

### 2.14 Prospects for treatment

2.14.1 Treatment for a TSE once clinical signs have appeared seems unrealistic at present, because it would involve understanding how to initiate and control nerve regeneration to rebuild damaged parts of the CNS. While transplantation of foetal nerve cells can prolong the survival of specialised brain cells normally lost during scrapie infection of mice\textsuperscript{268}, it does not appear to reverse or halt the progress of clinical disease. Thus, for the present, the development of treatments will probably focus on intervention at earlier stages in the infection, before irreparable damage to the CNS has occurred. This presupposes that the infection can be diagnosed early enough for treatment to be effective. So the development of diagnostic methods for use on live asymptomatic humans (or animals) must go hand-in-hand with any emerging treatments. Approaches to the identification of molecules that might provide effective treatment have been guided by basic research at the level of the PrP molecule and pathogenesis\textsuperscript{269} and this needs to continue to inform research on therapies.

2.14.2 Since the length of the incubation period is inversely related to the level of expression of PrP\textsuperscript{C} it follows that one approach to treatment might be to devise methods to reduce the amount of PrP\textsuperscript{C} in an infected animal or human. Removing PrP\textsuperscript{C} from the surface of cells in culture protects them from infection and cures already infected cells\textsuperscript{114}. That such a concept might be applicable \textit{in vivo} is suggested by the protection from scrapie disease of transgenic mice by switching off the Prnp gene just before infection\textsuperscript{270}. Furthermore, turning off the synthesis of PrP\textsuperscript{C} in neurons by the neuron-specific removal of their PrP genes after the initiation of a scrapie infection can reverse early spongiform change and prevent further neuronal loss in the brain, as well as halt progression to clinical disease\textsuperscript{217}. However, the experimental genetic trick that was used to achieve this would not be applicable in a clinical setting, but importantly it shows clearly that reduction or cessation of PrP\textsuperscript{C} production in neurons is a potential treatment for TSEs. For such an approach to lead to a practical treatment, however, ways must be found to switch off the
PrP gene or prevent translation of its RNA message in normal individuals. One potential approach to this may be through small double-stranded interfering RNA (RNAi), which, when expressed in cultured cells, specifically reduces the amount of PrP<sub>C</sub><sup>(271)</sup>, but a way would need to be found to get it into target cells in vivo in sufficient quantities.

2.14.3 Another general approach has been to search for drugs that interfere with the binding and conversion of PrP<sub>C</sub> to PrP<sub>Sc</sub>. Rapid in vitro conversion<sup>(272)</sup> and cell based<sup>(273)</sup> screening methods have been developed to assist with this. Several potential compounds have been identified, but much would need to be done to develop them to the point of clinical use. Candidate compounds include analogues of congo red<sup>(274)</sup>, heparan sulphate mimetics<sup>(275)</sup>, bis-acridines<sup>(276)</sup> and cyclic tetrapyrroles<sup>(277)</sup>. However, efficacy in in vitro tests does not necessarily lead to effective in vivo treatments<sup>(278)</sup>. The presence in vivo of a novel soluble dimeric form of PrP<sub>C</sub> delays PrP<sub>Sc</sub> accumulation, agent replication and the onset of clinical disease, indicating that certain forms of PrP itself may be able to block the formation of PrP<sub>Sc</sub><sup>(279)</sup>.

2.14.4 The related drugs quinacrine (used to prevent malaria) and chlorpromazine (used as an antipsychotic drug) both inhibit PrP<sub>Sc</sub> formation in scrapie infected ScN2a cells, but quinacrine can only transiently “cure” scrapie infected ScGT1 cells after lengthy treatment<sup>(280, 281)</sup>. Furthermore, quinacrine and chlorpromazine fail to inhibit accumulation of PrP<sub>Sc</sub> or increase the survival time in a murine model of BSE<sup>(281)</sup>. Pentosan polysulphate (used for the treatment of interstitial cystitis) cured SMB cells of scrapie infection<sup>(27)</sup> and significantly increased the incubation periods of mice infected with various strains of scrapie, when administered at or around the time of infection<sup>(282)</sup>. Quinacrine<sup>(283)</sup> and pentosan polysulphate have been given to CJD patients on compassionate grounds but their clinical value remains to be established.

2.14.5 Antibodies that specifically bind to PrP<sub>C</sub> on the cell surface prevent infection of N2a cells with scrapie and inhibit PrP<sub>Sc</sub> formation in scrapie-infected ScN2a cells, eventually curing them<sup>(25, 114)</sup>. The rate of loss of PrP<sub>Sc</sub> in antibody-treated cells is greater than would be expected from dilution through cell division, so there must be some natural active loss of PrP<sub>Sc</sub>, which could be exploited in PrP<sub>Sc</sub> targeted treatment. Anti-PrP antibodies have also been shown to be effective in preventing TSE disease in vivo. Continuous expression of an anti-PrP antibody in a transgenic mouse prevents disease following injection of RML scrapie<sup>(284)</sup> and treatment of RML scrapie-infected mice twice weekly with an anti-PrP antibody results in much longer survival times than for untreated mice<sup>(285)</sup>. Unfortunately, passive transfer of antibodies has no effect if administered late in the incubation period (i.e. after the infection had reached the CNS) or if scrapie is injected directly into the brain. This might be expected since it is unlikely that the large antibody molecules would pass the blood-brain barrier.

2.14.6 The development of therapeutic strategies for TSEs has hardly begun. Solving the structures of PrP<sub>C</sub> and PrP<sub>Sc</sub> and knowing how they interact should lead to the design of small molecules that can block the formation of PrP<sub>Sc</sub>. To be fully effective they would need to be able to gain access to, and be active in, the brain. Studies on pathogenesis and the mechanism of neurodegeneration should show the key steps and cell types to target and lead to the design of new drugs.

2.15 Epidemiology of TSEs:

2.15.1 BSE

2.15.1.1 BSE was first recognised in cattle in November 1986, although undetected earlier cases must have existed. It was not long before epidemiological studies had identified contaminated feed as the most likely cause of the emerging epidemic<sup>(4)</sup>. This led in July 1988 to the ban on the feeding of ruminant-derived meat and bone meal (MBM) to ruminants, which, after a long delay representing the extended incubation period, resulted in a sharp reduction in the incidence of BSE in the UK. However, significant numbers of
animals born after the introduction of the MBM ban began to emerge (born after the ban animals or BABs), probably as a result of cross contamination in feed mills that also prepared permitted MBM-containing rations for pigs and poultry\textsuperscript{286}. The ban was extended to cover the feeding of any mammalian MBM to any farmed animal, including fish, in 1996, shortly after the first cases of vCJD were reported. This eventually reduced the incidence of disease in animals born after 1996 further, but BSE is still arising in some animals born after the extended ban (born after the “reinforced” ban animals or BARBs)\textsuperscript{287}.

2.15.1.2 Analysis of the epidemic in 1996 indicated that the average incubation period for BSE in cattle “in the field” was about five years and that most animals were infected in the first two years of life, probably between 6 and 18 months of age\textsuperscript{288}. Considered against the husbandry and slaughter practices of the time, this indicated that there must have been about four times as many infected animals entering the food chain as were diagnosed with clinical BSE\textsuperscript{289}.

2.15.1.3 Although it is accepted that contaminated feed has been the major route of infection for cattle, epidemiological studies have looked for evidence of vertical (dam to calf) or non-feed horizontal transmission. Horizontal transmission, if it occurs at all, appears to be rare and unable to maintain an epidemic within cattle\textsuperscript{288}. A long-term cohort study, in which the incidence of BSE was compared in the offspring of BSE-affected and unaffected cows, indicated a 10 per cent additional risk of BSE infection in the progeny of BSE infected dams, the added risk being greatest if the birth was close to the time of clinical signs in the dam\textsuperscript{290}. A similar small increase in risk of infection in calves born to BSE infected dams was identified in a study of the dams of confirmed BABs\textsuperscript{291}. Since it is now known that some level of BSE contamination was present in cattle feed at this time, this risk could be due to true maternal transmission or might indicate differential genetic susceptibility in the progeny\textsuperscript{292} or a combination of both. Increased genetic susceptibility in the calf to contaminated feed is unlikely to be the sole explanation since the risk of maternal transmission increased towards the end of the incubation period in the dam. From what is known about the pathogenesis of BSE in cattle and the lack of detectable levels of infectivity in most peripheral tissues it is difficult to see what mechanism could enable BSE to be passed directly from dam to calf, although it is possible that a small proportion (e.g. 10 percent) of BSE-infected cattle have a different pathogenesis and that this has not been represented in those animals studied so far. The demonstration that embryos are unlikely to carry BSE infectivity\textsuperscript{293}, even if collected from the dam at the end-stage of the disease when the risk of transmission is thought to be highest, indicates that maternal transmission, if it occurs, must be a late gestation event. Another explanation for the increased risk could be that the progeny of BSE-affected dams, for some reason such as low birth weight, consumed more feed, which, if contaminated, would increase their risk of becoming infected. The results of the cohort study suggest that this explanation may also be incorrect since it has been estimated that the progeny of a BSE-affected dam would need to have consumed three times as much BSE-contaminated food as the progeny of a non BSE-affected dam.

2.15.1.4 If horizontal or vertical transmission of BSE occurs in cattle, it should become apparent if cases of BSE continue to arise in animals born after all possibility of infection via feed has been removed, in other words, supposedly in the BARBs. Up to 6 October 2003 fifty nine BARB cases of BSE had been confirmed\textsuperscript{294}. This marks a significant reduction in the risk of BSE infection in cattle born after July 1996 compared to those born earlier. The geographical distribution of BARB cases is much more uniform across the country than for BABs, indicating an ongoing widespread, but very low, risk. The evidence does not support maternal or horizontal transmission as the source of infection in the majority of cases, nor is it consistent with a genetic based origin, such as increased genetic susceptibility or a mutation resulting in spontaneous disease. On the basis of the first 16 BARB cases\textsuperscript{295} it was proposed that animals were infected by an exogenous feedborne source that was potentially contaminated with mammalian MBM, which was legally traded across continental Europe in Member States of the European Union until the Europe-wide ban on 1 January 2001. This remains the most plausible mechanism for infection of the 59 BARB cases\textsuperscript{294}.
2.15.1.5 The introduction of active surveillance using post mortem tests based on PrPSc detection is providing useful information on the true prevalence of BSE infection in the national herd and indicates that there has been between a 2 to 4 fold under-ascertainment of clinical cases over the course of the BSE epidemic. The results of this ongoing surveillance will provide useful data for epidemiological studies.

2.15.2 Scrapie

2.15.2.1 Epidemiological research is dependent on having data on the true incidence of disease, and work on scrapie has been hampered by significant and ongoing suspected under-reporting. In an attempt to overcome this problem a postal survey of 11,554 farms was conducted in November 1998 to try to get more representative information on the incidence of, and risk factors for, scrapie in the UK. A second postal survey of 12,800 farms was carried out in 2002. The surveys produced 48.6 and 53.1 per cent, respectively, useful replies and indicated that only 13 and 38 per cent, respectively, of farmers who suspect they have scrapie in their flock report it. The 1996 survey estimated that 2.7 per cent of responding farms had experienced a case of scrapie in the preceding 12 months, but this dropped to 1 per cent in the 2002 survey. Whether or not this truly represents a decline in the incidence of scrapie remains to be established, since, at the time of writing, a full analysis of the results of the 2002 survey was not available. The 1996 survey showed that there was regional variation in scrapie incidence, that larger farms and those with purebred sheep were at higher risk, that farms differed in their sheep purchasing policies and that on the majority of farms the first case of scrapie appeared in a purchased animal. Farms that used the same location every year for lambing or those that lambed sheep in groups were more likely to get scrapie than those that allowed sheep to lamb unconfined at pasture or which lambed sheep in individual pens. Farms with scrapie had more sheep that were found dead and more elderly ewes.

2.15.2.2 Other estimates of the true incidence of scrapie are provided by active surveillance of animals culled in abattoirs and animals that die on farms (fallen stock). This 2002-03 abattoir survey, which tested sheep over 18 months of age, gave a crude measured prevalence of infection of 0.13 per cent, which, after correction for several factors related to the nature of the sample, corresponds to an estimated true prevalence of infection in the whole GB flock of 0.33 per cent. This compares to an estimated prevalence of 0.22 per cent in an abattoir survey in 1997-98. The results of the fallen stock survey suggest that a minimum of 0.39 per cent of flocks in Great Britain are infected with scrapie.

2.15.2.3 There is concern that sheep may have become infected with BSE since they are susceptible to experimental challenge with it and they were exposed to the same contaminated MBM that infected cattle. However, the 1996 postal survey showed no evidence for a peak in the number of farms newly infected with scrapie before, during or after the BSE outbreak in cattle, making it unlikely that there was a substantial epidemic of BSE mis-diagnosed as scrapie. This is consistent with theoretical estimates of between 10 and 1500 cases in the peak of a hypothetical epidemic of BSE in sheep. In this model the estimated current prevalence would be very low, although, if horizontal transmission of BSE in sheep were to occur, the epidemic could become large over time. Therefore, should BSE ever be found in sheep, it will be important to have established whether, like scrapie, it can be transmitted horizontally.

2.15.2.4 Prnp genetic profiling of two scrapie-affected flocks and two scrapie-free flocks showed that they have similar Prnp genotypes, but that affected flocks had a small excess of susceptible genotypes, particularly in younger animals, despite the losses of susceptible genotypes through scrapie. A similar excess of scrapie-susceptible, and deficit of scrapie-resistant, genotypes in comparison to the general sheep population was found in a study of 15 scrapie affected flocks. In the former study, the age structure of the affected flocks suggested that there are far greater losses directly or indirectly from scrapie than are recognised by farmers and that significant losses occur in sheep of even moderate age.
susceptibility. The existence of a class of such losses in sheep is consistent with the higher prevalence of BSE infection in cattle and scrapie infection in sheep that are casualty and fallen stock and the larger numbers of sheep found dead on farms with scrapie.

2.15.2.5 Mathematical modelling of a scrapie infection in a flock demonstrates that, left unchecked, an outbreak would probably last several decades. It would lead to a reduction, but not the elimination, of susceptible genotypes.

2.15.3 Human disease

2.15.3.1 With the first reported cases of vCJD following some 10 years after the start of the BSE epidemic, and the recognition that the infectious agents of BSE and vCJD are indistinguishable, it is generally assumed, although not yet proved, that BSE reached humans via contaminated meat or meat products. The ongoing case control study has not yet identified any major risk factors for vCJD, such as consumption of particular meat products, but may in the future be important for identifying potential iatrogenic cases of vCJD. Perhaps the closest link so far between vCJD and contaminated meat is in the so-called Leicester cluster, five geographically associated cases of vCJD in Leicestershire. Four of the five may have been exposed through eating beef purchased from a butcher’s shop where meat could have been contaminated with brain tissue. It is unlikely, however, that this scenario applies to the majority of vCJD patients. But if the hypothesis is correct, it points to an incubation period in these four cases of between 10 and 16 years. Leicester remains the only statistically significant cluster of cases of vCJD in the UK. The incidence of vCJD is about twice as high in the North of Great Britain as it is in the South, but, so far, it has not been possible to link this definitively to dietary differences.

2.15.3.2 It is difficult to ensure that all cases of vCJD are correctly diagnosed and reported and there is always the worry that some could be missed. Variant CJD occurs in a much younger age group than sporadic CJD and it is a concern that cases are perhaps being missed in children and older adults. A survey of over 1,000 children with progressive intellectual and neurological deterioration (PIND), a group that would include vCJD patients, found only previously reported cases of vCJD, indicating that vCJD in children is not being missed. Although vCJD has been diagnosed in a 74 year old patient, the possibility remains that cases of vCJD are being missed amongst the elderly. One attempt to estimate the prevalence of infection, as opposed to the incidence of clinical vCJD, is based on a search for the characteristic presence of PrPSc in infected lymphoid tissue in archived specimens of tonsils and appendices removed from patients aged 10 to 50 between 1995 and 1999. The interim findings of this study reported one PrPSc-positive appendix amongst 8318 samples examined, which gives an estimated detectable prevalence of PrPSc accumulation in 120 per million of the population. However, the finding of a further two PrPSc-positive samples, giving a total of three positive samples in 12 674 samples examined, confirm the wisdom of screening the larger number of samples being collected as part of the National Tonsil Archive.

2.15.3.3 As with scrapie, but not BSE, the Prnp genotype determines the incidence of vCJD. In the UK about 37 per cent of the population are homozygous for methionine, 12 per cent are homozygous for valine, and the remaining 51 per cent are heterozygous. So far, all cases of vCJD that have been genotyped have been homozygous for methionine at coding position 129. A similar, relatively greater, susceptibility of homozygous methionine carriers is found in patients diagnosed with CJD following growth hormone treatment and in Fore people succumbing to Kuru, but it can not be assumed that valine carriers will not also be susceptible to vCJD, albeit with longer incubation periods.

2.15.3.4 There have been several predictions of the future course of the vCJD epidemic, based on mathematical models using the limited number of cases at the time. They have in common a high level of uncertainty, with the predicted number of infections
ranging from a few hundred to over a million. A recent study indicates that, although the level of uncertainty and the predicted maximum size of the epidemic have decreased significantly, the estimated number of future cases for the present epidemic ranges from 10 to 7000. The study also confirms significant age-related susceptibility and/or exposure to infectivity, with those aged between 10 and 20 years of age being at the highest risk of infection. To accommodate the predictions of large numbers of infections, vCJD must have a very long average incubation period, beyond that of the current normal life expectancy of humans, so that only a few thousand would ever present with clinical disease.

2.15.3.5 The onsets of, and deaths from, vCJD are analysed statistically on a regular basis to see whether overall trends can be detected. By 2000, onsets and deaths were increasing year-on-year by 23 per cent and 33 per cent, respectively, indicating that the epidemic was still increasing exponentially. A similar analysis in 2002 of deaths from vCJD suggests that the previously increasing trend may have slowed down with the death rate having peaked in 2000, at least for the post-1969 born methionine homozygotes. This is very encouraging, although the situation could change dramatically if there were to be a further rise in numbers. Indeed, hopes of an early end to the epidemic could be dashed if the cases of vCJD seen to date represent a particularly susceptible group and there are waves of infections in more resistant groups still to come.
UK TSE research strategy

3.0.1 “TSEs have long incubation periods and at present are untreatable. The appearance of their symptoms leads inevitably to death.”

3.0.2 The transmission of prion disease through the food chain was recorded among the Fore people of Papua New Guinea nearly 40 years before vCJD, and its linkage to BSE, was identified in 1996. But vCJD was the first natural example of transmission from animals to man and the first TSE thought to result from food-borne exposure of a large human population. The complexities of food production and of medical and veterinary science in western societies, allied to the mobility of the population and the widespread transport of livestock, have added to the potential scale of the problem. The testimony from the families of the earliest victims stands as a stark reminder of the horror that we are working to avert.

3.0.3 The full extent of human exposure to BSE is not known. All of the vCJD cases tested to date have shared an identical feature in their PrP\textsuperscript{C} genotype (methionine homozygosity in codon 129). It is unclear whether this predisposes individuals to infection, reduces incubation time or both. The prevalence of this genotype in the population is estimated at 39 per cent and therefore additional cases in people with other PrP\textsuperscript{C} genotypes remains a possibility. The total human death toll from vCJD may be unclear for many years.

3.0.4 Whilst the UK shares with the rest of the world an inherent scientific interest in what appear to be truly novel infectious agents, the exposure of our population has brought an added imperative to applied research that addresses the diagnosis, care and treatment of patients, breaking routes of infection, and the eradication of the infectious agent from our food supply. To date the five UK funders of TSE research have spent over £300 million on research in this field and remain committed to securing the highest quality research.

3.1 Understanding the infectious agent

3.1.1 The prion hypothesis

3.1.1.1 The prion hypothesis is widely but not universally accepted. It has yet to explain all the observed differences between TSE strains. In particular, the prion protein has not yet been proven to be the infectious agent of TSE disease. Although progress has been made in the study of related fungal proteins, the conversion of the normal form of PrP to an infectious form has not been demonstrated. However, in the absence of definitive knowledge, the prion hypothesis forms the basis for present day risk assessments, decontamination strategies and the identification of diagnostic and treatment targets. To investigate potential limitations of the prion hypothesis, Defra funds work that is establishing the correlation between abnormal PrP conformers and the presence of TSE infectivity as determined in mouse bioassays. Establishing an appropriate molecular model of the infectious agent remains a priority for the research councils and is of fundamental importance to the research strategies adopted by all the funding bodies.

3.1.1.2 In addition to further investigation of the prion protein, MRC, BBSRC and Defra give serious consideration to research proposals investigating alternative or modified hypotheses for the origin, propagation and possible associated causes of TSEs. Exposure to organophosphates, an imbalance in the uptake of trace elements, exposure to fungal toxin and an auto-immune response to micro-organisms have been suggested as sole or contributory causes for BSE although none have been validated scientifically to date.
3.1.2 The challenge of TSE strains

3.1.2.1 In sheep, where prion disease has been established for many decades, scrapie is found as distinct strains that have different patterns of pathology, different resistance to inactivation and different PrP\textsuperscript{Sc} distribution. We need to understand the significance of different strains of TSE and why some are capable of producing disease in some host species but not in others. Such knowledge will form the basis for molecular models of the infectious agent, its propagation and transmission, and may offer novel therapeutic targets. Understanding animal strains will inform risk assessment and thus help establish proportionate and cost-effective policies.

3.1.2.2 Currently, panels of mice are used to determine strain type, but this is a long process, and in the case of scrapie, transmission on first passage to mice is often variable and can be unsuccessful. To improve this process, Defra is funding work that is applying statistical and modelling methods to analyse mouse bioassay data. The sensitivity of strain typing methods may be improved by using lines of mice that express sheep and bovine PrP alleles as transgenes. As mouse host genetics play an important role in characterising strain types, there is a danger that what is observed in the mice (particularly in subsequent passages) will bear little resemblance to field cases of TSEs. Some research is addressing this issue by investigating whether scrapie strains defined in mice can be distinguished in sheep and whether the strain characteristics are preserved after passaging though sheep. Another research priority for Defra is the development and characterisation of molecular methods that discriminate between TSE strains.

3.1.2.3 The relative diversity, in quantitative and qualitative terms, of scrapie strains circulating in the UK and their significance in the expression and incidence of disease is not known. Defra continues to fund research and surveillance work that addresses these issues. For example, atypical patterns of PrP\textsuperscript{Sc} in obex samples taken from sheep with relatively scrapie-resistant genotypes have been detected in surveys of sheep culled at abattoir. Further investigations on these samples to establish the nature and biological significance of these findings are in progress. There is also a concern over whether BSE, if present in sheep, could be distinguished from scrapie when present in a single, or in a mixed, infection and this is also being addressed. Studies are also in progress to determine the effect of agent strain on the distribution of infectivity in tissues by investigating different sheep breeds and genotypes that have been naturally infected with scrapie.

3.1.2.4 Recently atypical forms of BSE have been described in cattle but until the results from transmission studies in mice are known, it is uncertain whether these represent novel strains of BSE. Although the pathology of the BSE strain in cattle is considered to have remained stable over time, ongoing studies are investigating the strain stability of BSE within UK in comparison with an isolate found in Switzerland. Atypical cases of BSE have been reported in other countries (notably Italy and Japan). The Veterinary Laboratories Agency has quality control functions and is both the Community Reference Laboratory (CRL) and the National Reference Laboratory for the UK. The CRL has set up an Expert Group on strain typing, which met for the first time in June 2003. This Group is responsible for putting in place protocols for investigating unusual samples (involving a number of EU Reference Laboratories), as well as defining what constitutes an atypical sample. However, diagnostic uncertainties suggest that confirmation of apparently different strains of BSE in cattle will be dependent upon a better understanding of molecular diagnostic techniques.

3.1.2.5 In humans vCJD, thought to arise from exposure to BSE, is seen with a different presentation from sporadic CJD. The MRC funds a programme of work in its Prion Unit that is undertaking a molecular and phenotypic analysis of human prion strains. As part of this work, it has been shown that infecting two different strains of inbred mice with the same BSE isolate can produce two different types of PrP\textsuperscript{Sc}. Work is now ongoing to see whether these differences will be maintained on passage through the same mouse line.
and thus whether the host genes play a role in strain formation. Both DH and the MRC will continue to support studies in this area.

3.1.3 The structure of PrP and in vitro conversion of PrP<sub>C</sub> or recombinant PrP to a protease-resistant form

3.1.3.1 Elucidating the differences between the structures of the normal and infectious forms of the prion protein - and the mechanism of conversion of one to the other - will be crucial to understanding the nature of the infectious agent of TSEs and the mechanisms of disease transmission. Attempts to identify the structure of PrP<sup>Sc</sup> have been predominantly based on recombinant forms of PrP. These differ from the native form in many ways. Significantly, they are not exposed to post-translational glycosylation systems, lack a GPI anchor and thus, unlike the natural prion protein, are not held in the cell's membrane environment. A better understanding of the structure could offer a greater understanding of the PrP to PrP<sup>Sc</sup> conversion; the identification of possible chaperones; the identification of the component or components that make PrP infectious; and the relationship between strain types. Enhancing knowledge of the structural biology of the different forms of the prion protein remains a high priority for the research councils. Increased knowledge may also offer opportunities for improved targeted diagnostics, and may pave the way for new drugs for animals and humans.

3.1.4 Production and processing of PrP in cells

3.1.4.1 We are beginning to understand how PrP<sup>C</sup> is produced and processed through the cell, including how PrP<sup>Sc</sup> is formed, how its presence may seed PrP to PrP<sup>Sc</sup> conversion, and its association with infectivity. Greater knowledge of the mechanisms involved in the production and processing of the prion protein in normal cells is likely to be essential for understanding its conversion to the infectious form and the subsequent development of disease. Better knowledge of the cell biology of the prion protein - including the development of appropriate systems for studying it in vitro - is a priority for the research councils. Such studies may highlight surrogate markers or chaperones, or lead to the identification of points of possible therapeutic intervention. Good cell systems are needed to study the cell biology of PrP and more cell systems are needed to develop this line of research. The importance of variation in glycosylation between different TSE strains is now increasingly apparent, but more research is needed on its role in regulating PrP activity. Such knowledge would speed the development of diagnostic tests and of treatments to slow the progress of animal and human TSEs. In this respect glycobiology is an important discipline in TSE research where capacity building within the UK is needed.

3.1.5 Studies of prions in fungi

3.1.5.1 Proteins with key features analogous to the mammalian prion protein have been found in yeast and other fungi. These molecules are able to acquire self-perpetuating changes in structure, and associated consequential changes in their biological function. Although not directly comparable with prions seen in their mammalian counterparts, they are valuable as simple systems for studying aspects of prion biology. They can be used for studies of the conversion of “normal” to “abnormal” forms that are relevant to our understanding of TSEs, and useful lessons may be learned from studying their structures, the mechanisms by which they propagate, how chaperones assist in their formation and the extent and nature of their diversity. BBSRC supports some research in this area.
3.1.6 The function of normal PrP

3.1.6.1 The normal function of PrP remains a mystery although some studies have suggested a link to the management of oxidative stress. Mice lacking PrP\textsuperscript{C} showed no clear physiological effects and lived for normal life expectancy. Deeper molecular and/or behavioural analyses of such mice strains may be informative. Some associations of PrP with other molecules have been identified, but it still remains unclear whether these have normal physiological significance, and whether the absence of functional PrP\textsuperscript{C} triggers the development of disease. A deeper understanding of PrP\textsuperscript{C} functionality may help our comprehension of disease states and could highlight possible sites for therapeutic intervention. Improving understanding of the role of PrP in healthy cells continues to be a priority for the research councils.

3.1.7 The role of the host’s genes in TSEs

3.1.7.1 In humans, all patients so far diagnosed with vCJD have shown a consistent clinical and pathological presentation, all are homozygous for methionine at codon 129 in the PRNP gene and most are 14–40 years old. This is in contrast to sporadic disease where significant variation in PrP\textsuperscript{Sc} glycoforms and histopathology can be detected. However, very young children, the elderly and individuals with other genotypes (see epidemiology, below) may present with different pathologies or clinical symptoms. Consequently DH will continue to support the surveillance of disease in these groups. In addition, DH will consider applications to study potential pathologies, which may be expressed in other tissues, such as the lymphoid system, where it is known that PrP\textsuperscript{Sc} is expressed or amplified, or other research to improve disease surveillance.

3.1.7.2 It is clear that mutations in the PRNP gene can lead to inherited forms of prion disease and that polymorphism of the PRPN gene can affect both clinical presentation and susceptibility to developing both sporadic and iatrogenic prion disease. However, there is also much evidence to support the role of other genes in prion pathogenesis. It is important to determine which individuals may be at risk from contracting these diseases. MRC funds two closely linked programmes of work at its Prion Unit; on molecular genetic studies of human prion disease susceptibility and on quantitative trait studies of genes that modify prion incubation time in mice. MRC and DH will continue to fund work in this area.

3.1.7.3 Genetic susceptibility to prion diseases has most clearly been demonstrated in sheep and is exploited in the National Scrapie Plan (NSP) which aims to reduce and eventually eliminate scrapie from the national sheep flock. This will be done by means of breeding programmes that encourage the use of sheep carrying PRNP alleles that are known to confer a high resistance to natural and experimental scrapie infection and to experimental BSE infection. An important research priority for Defra is to ensure that the science underpinning the NSP remains sound. Therefore, several studies that will confirm and extend the current categorisation of sheep PRNP alleles with respect to susceptibility or resistance to scrapie and BSE are supported. Defra supports studies examining whether the selection at PRNP promoted in the NSP affects desirable sheep production traits, the genetic biodiversity of specific sheep flocks and also supports studies on the demography of UK sheep. The data collated in these studies are incorporated into various mathematical models that aim to inform on the progress and potential consequences of the NSP.

3.1.7.4 Knowledge of whether other host genetic factors in both sheep and cattle influence the response to TSEs is also required but such studies are difficult in agricultural animals. It is important to establish whether genetic variation in PRNP, or in other host genes, can affect the level and tissue distribution of PrP\textsuperscript{Sc} expression and the subsequent generation of PrP\textsuperscript{Sc}. This is to ensure that the appropriate tissue is targeted for TSE testing undertaken for surveillance studies and in support of control measures for public health.
3.1.7.5 There is very little evidence to support the existence of an inherited susceptibility to BSE in cattle and, as yet, no polymorphisms in PRNP, or other bovine genes, have been strongly linked with a susceptibility to BSE. Defra funds a study that will re-examine whether polymorphisms at PRNP are associated with susceptibility to BSE in UK cattle. However, the possibility remains that there may be an extremely low rate of sporadic BSE in cattle (and in sheep). Such cases are likely to be represented in the cases of BSE born after the reinforced food ban in the UK and the same study will investigate this possibility.

3.1.7.6 Work on the role of the host’s genes is important to all funders. In addition to the studies mentioned above, the research councils also support a range of underpinning work in this area, for example studies examining susceptibility to prion disease in mice, host factors in transmission and pathology of TSEs, identification and mapping of BSE susceptibility genes in mouse and human, host factors in oral transmission of CJD and phenotypic variation in CJD. The potential importance of this work in transmission, disease progression and in identifying potential targets for therapeutic intervention ensures that it remains a high priority.

3.2 Tackling the spread of infection

3.2.1 The species barrier and the carrier state

3.2.1.1 The possibility of ‘carrier’ states in animals and humans, and our present inability to identify them, pose a potential threat to public and animal health. The susceptibility of humans to BSE infection, and the ability of the disease to remain clinically silent for many years, is of major concern to DH. Although the death of a UK blood donor from vCJD in 1999 three years after making the donation and the subsequent death from vCJD of the recipient in 2003 have not been causally linked, transmission of infection through blood transfusion is the most likely explanation. The case heightens concerns that ‘carriers’ could be transmitting the disease through blood, tissue and organ donation or by contaminating surgical instruments when undergoing surgery. DH will continue to support research to detect infectious prions in human tissue, to investigate the decontamination of surgical instruments and to develop measures to protect blood supplies.

3.2.1.2 Animal models of some TSEs have detected infectivity in blood. Experiments, which have involved transfusing large volumes of blood from infected sheep to healthy recipient sheep, have demonstrated that infectivity can be transmitted by blood transfusion. A central part of DH policy in this area has been the leucodepletion of blood donations and the efficacy of this technology can now be tested in sheep.

3.2.1.3 DH is also working with the National Blood Service to prepare for the introduction of a blood-screening test, should one be developed in the near future. In addition to preparing the logistics for this exercise, consideration is being given to the ethical issues associated with screening blood donations.

3.2.1.4 The possibility of interspecies transmission is a principal concern in animals. Food animals other than cattle including pigs, sheep, poultry, deer and farmed fish have been exposed to BSE infectivity via meat and bone meal. The FSA is funding research to establish whether BSE can be or has been transmitted to other food species and if so, to obtain information on the pathogenesis of the disease. Work funded by Defra is in progress to investigate whether cattle experimentally challenged with scrapie exhibit a disease which resembles BSE. Limited studies have shown that pigs experimentally dosed with scrapie do not succumb to a TSE disease. New research is investigating the experimental transmission of BSE to deer, and future studies may consider its possible transmission to farmed fish. These potential risks, coupled with the knowledge of the recent BSE cases in Canada and the USA, highlight the importance of good surveillance, which remains a priority for government departments.
3.2.1.5 A greater understanding of factors underlying the species barrier is likely to come from progress in the research described in the other sections of this chapter, for example, studies of the structure of PrP in different species, of TSE strains or of the pathogenesis of TSEs. However, since most TSE infectivity assays are conducted in mouse bioassays, it is important to know the relative sensitivity of detection when tissue is titrated across a species barrier. For this reason, studies on comparative titration of infectivity in cattle, sheep and mice (including transgenics) are being funded by Defra. These studies will provide valuable scientific input to models of risk analysis and epidemiological studies.

3.2.1.6 The National Scrapie Plan has proceeded on the understanding that more scientific information about the potential existence of infectious carrier states will be pursued as the Plan proceeds, and on the relationship between PRNP and disease susceptibility and the significance of scrapie strains. A high priority for Defra is to supplement the existing studies that investigate whether sheep carrying PRNP genotypes considered to be resistant to TSEs can act as carriers for infectivity (by searching for infectivity in tissues and bodily fluids) and, if possible, to determine whether such animals can transmit TSEs.

3.2.2 Inactivation of TSE infectivity

3.2.2.1 The safe disposal of specified risk materials (SRM) by using effective methods capable of inactivating the scrapie and BSE agent is a key policy objective for Defra. Alternative methods to rendering have been investigated and research in this area is continuing using a combination of physicochemical methods and thermophilic enzymes from bacteria and using a novel biorefinement process. However, to date there is no technique, including rendering, which can guarantee complete inactivation on a commercial scale, and further work is required in this area. Any means of inactivating TSEs would greatly increase the scope for adding value to the products of the disposal process. As landfill capacity diminishes, novel ways of disposing of animal waste in a manner that will not pose animal or human health risks, will become an increasing priority. Whilst some former practices, such as recycling in animal feed, may never again be deemed to be acceptable, there are strong environmental and commercial reasons for restoring some value to animal products. Defra is keen to find safe alternatives to rendering and incineration which would facilitate this process and bring environmental benefits.

3.2.2.2 Contaminated medical instruments continue to pose significant potential risks to human health. Research on decontamination has shown that autoclaving in the presence of alkali can remove infectious prions without significantly damaging stainless steel instruments. However, further work is needed to establish precise and reproducible conditions. Moreover, delicate surgical instruments may be damaged by such a harsh process and therefore many other novel potential decontamination technologies are being assessed. A DH Decontamination Science and Engineering Advisory Committee (ESAC-PR) has been established to facilitate the transfer of research findings into improved protocols for NHS Sterile Services departments. The ability to measure residual contamination is vital if there is to be confidence in these measures. To this end several research projects are developing novel and highly sensitive methods for detecting residues of infectious prions and other proteins on instrument surfaces. DH will continue to support research in this area and to facilitate the introduction of new technologies by the NHS where appropriate.

3.2.2.3 All legally prepared meat from the UK comes from animals slaughtered in licensed slaughterhouses under the supervision of the Meat Hygiene Service (MHS). The MHS inspect every carcase to check that specified risk material (SRM) has been removed before health marking as fit for human consumption. Because of the resistance of prions and limitations imposed by the requirement for only food grade chemicals to be used in
food production, cleaning operations in slaughterhouses are geared towards preventing cross contamination of fresh meat with SRM. Plants handling SRM have specific procedures for reducing potential cross contamination such as the use of dedicated colour-coded knives for removing SRM and specific cleaning requirements.

3.2.3 Epidemiology of TSEs

3.2.3.1 Research on TSE epidemiology is crucial to the development of the policies and practices needed to protect the public from exposure to these infective agents. This is particularly so for BSE, where the relationship between BSE and vCJD in humans is well established.

3.2.3.2 Although the post mortem diagnosis of vCJD is very reliable, DH is concerned that cases may be missed, especially in the very young and in the very old. Therefore DH will continue to support disease surveillance, mathematical modelling and work designed to assess disease prevalence in all sections of the UK population.

3.2.3.3 As there is currently no diagnostic test for pre-clinical disease, work to estimate the size of the vCJD epidemic will continue by collecting and testing human tissues taken during surgery or for diagnosis. Currently the Health Protection Agency is planning to collect and analyse up to 100,000 tonsils (the National Tonsil Archive) for this purpose. If a sensitive assay, suitable for analysing blood or other bodily fluids, becomes available, DH will consider studies to screen larger sections of the population.

3.2.3.4 The origin of BSE remains a gap in our knowledge, although there are a number of hypotheses. These are documented in the Report of the Horn Committee. It is important to know the potential risk to human health from exposure to TSE agents, via food and/or through direct contact with TSEs. TSEs have occurred in a number of species besides farmed animals (including some exotic zoo species).

3.2.3.5 In the UK, transmission studies have been conducted in cattle, sheep, pigs and poultry to determine their susceptibility to TSEs. These studies include investigating the effect of dose and route of challenge on the transmissibility of TSEs. Evaluations of the minimum oral exposure dose of BSE required to produce disease in cattle and the effect of exposure dose on the incubation period and attack rate for clinical disease are also in progress. This information will assist in refining risk assessments relating to human exposure and BSE transmission. Knowledge of the cumulative effects of doses and of age-related susceptibility to dose may also be important factors in achieving disease eradication, particularly if it becomes evident that environmental persistence is a factor in the cases of BSE born after the feed ban, and in the transmission of scrapie. The FSA is now conducting transmission studies in deer, and work on other animals is underway in the USA and in continental Europe. To date, no reservoir of TSE infection has been identified in wild animals in the UK. Should such an infection be identified, then further work in this area would be required.

3.2.3.6 Epidemiological and surveillance studies, designed to improve ascertainment of TSEs in sheep, will make an important contribution to scrapie eradication and the control programmes for scrapie affected flocks recently established by the EU. It is still not clear how scrapie is transmitted within an endemically infected flock and further understanding of this may permit recommendations for changes in farming practices that will reduce the incidence of disease. Current investigations include monitoring the effects of different farm and husbandry management systems, including veterinary procedures, on scrapie transmission.

3.2.3.7 In addition to experiments involving grazing sheep on potentially “contaminated” pasture, another study is looking at the persistence of TSEs in the soil environment. However, such work is limited, at the moment, by the lack of an efficient method for the extraction of PrPSc from soil although methods have been developed for clay and sand. In
April 2002, Defra convened a workshop on TSEs in the Environment. As a result of this workshop a number of research recommendations were made in order to address gaps in our knowledge.

3.2.3.8 Epidemiological data are used in mathematical studies and risk assessments to provide predictions of past and future exposure to TSEs. For example, models of the decline of the BSE epidemic are used to inform policy decisions on UK legislation banning cattle over 30 months of age from entering the food supply. Similarly, mathematical modelling has been used extensively to attempt to predict the number of human cases and such studies will continue to be supported. Case control studies, designed to identify risk factors associated with disease, will continue. Risk assessments are important in identifying gaps in our knowledge, and it is important that the assumptions made within and between the risk assessments are consistent and scientifically validated wherever possible.

3.2.3.9 In humans, the current statistics for vCJD appear encouraging, with the final figures for 2004 showing a decrease in numbers of both sCJD and vCJD over previous years. While the significance of this is still unclear, it may be hoped that numbers of cases of vCJD in individuals who are MM at codon 129 on the prion gene are decreasing. But this does not preclude a second wave of individuals heterozygous at 129 or homozygous at 129 for valine. It is also uncertain whether these individuals would present the same clinical signs and symptoms as those seen so far, as incubation times are likely to be longer. This might make identifying the condition difficult. Human TSEs are hard to diagnose and whilst the majority of current cases are identified as ‘probable’, brain analysis post mortem remains the standard for diagnosis. If the number of post mortems amongst ‘probable’ cases declined, there would be an increasing risk of failure to identify new cases. Enhanced surveillance and research are needed to ensure that any new wave is spotted at the earliest opportunity.

3.2.3.10 Continued monitoring of the BSE epidemic is a key science priority for Defra. This is being achieved through passive and active surveillance programmes. The surveillance programmes for TSEs in sheep and cattle in turn provide data for predictive models of the future course of the disease. For BSE in cattle, further epidemiological analyses of recent case data are necessary to identify the possible reasons for cases born after the reinforced feed ban. This in turn will inform whether further research is required to investigate other routes of transmission that may affect the rate of decline of the epidemic.

3.3 Pathogenesis, diagnosis and treatment

3.3.1 Pathogenesis of TSEs

3.3.1.1 Pathogenesis studies have been predominantly based on looking for the presence of PrP\textsuperscript{Sc} in tissue samples from animals and humans. Such studies are limited by the sensitivity of the diagnostic reagent used. Results from UK cattle and sheep pathogenesis studies have helped to establish the risk management measures now in place within the UK and elsewhere in Europe by identifying which tissues should be removed and destroyed (as specified risk material – SRM) in bovines and ovines of different ages. The current favoured view for the majority of species is that infected material enters through the gut into the lymphoreticular system before appearing in the central nervous system, although the exact route of transmission remains unknown. Understanding the transmission route is an urgent research priority for the UK. It would allow the identification of possible sites for therapeutic intervention (particularly for humans) and more effective management of the risk of possible secondary infection. Increasing the number of clinicians involved in research in this area is a priority for DH and MRC.
3.3.1.2 Defra continues to fund pathogenesis studies in TSE-infected sheep, of a variety of PrP genotypes, from a range of breeds, and at different stages in the incubation period. Other studies are examining whether the route of challenge, the age at exposure or the dose of infectious agent affect the subsequent pathogenesis of the disease. In these studies, tissues are being tested for evidence of infectivity by mouse bioassay, by detection of PrP$^\text{Sc}$ by Western blotting and by immunohistochemistry.

3.3.1.3 Cattle pathogenesis studies and work to establish the relative infectivity of different tissues from BSE infected animals are now funded by the FSA. Tissues from cattle which have been experimentally dosed with BSE infected brain homogenate are subsequently injected intracerebrally into other cattle to determine whether any infectivity is present in the tissues. Called cattle bioassay, this is the most sensitive bioassay which can be performed, in that there is no species barrier. This work is being expanded to include other species. The information emerging from the cattle studies has been used to develop controls, and contributes to the FSA’s TSE risk assessment work.

3.3.1.4 Understanding the pathogenesis of human diseases, especially the tissue distribution of infectious prions, is crucial to preventing secondary infections. Moreover, a risk assessment of possible CJD transmission during surgery has indicated that the level of infectivity in human tissue and the efficient decontamination of surgical instruments are major factors influencing the risk of transmission. Studies on brain, tonsil, spleen and blood samples from vCJD have already provided assessments of infectivity levels in these tissues. This work is crucial for active risk management and will continue to be supported by DH. It concentrates on analysing ocular and dental material and samples from the organs and tissues most commonly used for transplantation. However, current infectivity assays in experimental animals are too insensitive to allow total confidence that organs and tissues, especially blood, are completely free of infectious material. Therefore DH will also consider supporting work to develop more sensitive assays, either through surrogate markers or the development of improved biological models such as transgenic mice.

3.3.2 Diagnosis of TSEs

3.3.2.1 Besides the need for an in vivo test that can detect disease in pre-symptomatic animals and humans, there is also a requirement for rapid ante mortem and post mortem tests of increased sensitivity. This is a priority for all funders. The most easily managed test would be one that could assess infectivity in an easily accessible tissue or fluid, such as blood or urine, and provide a cost-effective method of screening large numbers of animals. Examination of blood or urine may also allow earlier identification of at-risk individuals who could be treated with a prophylactic before clinical symptoms appear. Most tests focus on the presence of prion protein, but there are limited methods to differentiate between native and disease forms of the protein. Funders have therefore expanded their research portfolios to screen for other disease markers.

3.3.2.2 Defra and the FSA support research to develop improved post mortem and ante mortem tests for BSE in cattle and scrapie in sheep, and the development of tests that are capable of differentiating between experimental BSE and scrapie in sheep. Current EU-approved BSE tests are limited in their sensitivity, are only usable post mortem and at present are only validated for central nervous system material. Within the EU there is a statutorily based testing programme for BSE in cattle and TSEs in sheep. Agreement within the EU on the scientific criteria to differentiate between scrapie and possible BSE in sheep remains a top science priority. The impact, both on the livestock industry and more widely, of discovering BSE in sheep would be large and it would be vital to have confidence in the diagnosis. This can be achieved only through a better understanding of how to interpret test results supported by an improved knowledge of the repertoire of scrapie strains that are endemic in the UK.

3.3.2.3 Ensuring that people are not exposed to potentially TSE-infective material is a concern of the FSA and is the basis of legislation to keep high-risk material out of the
food chain. However, testing every animal that goes into the food chain would be very expensive. An ante mortem test that could detect disease at an early stage in the incubation period would, never the less, provide substantial savings over post mortem tests. Rapid tests currently used in surveillance programmes have been validated on bovine infected brain tissue. Further work is required to define the effect of species type, genotype, incubation period and target tissue on rapid molecular diagnostic techniques.

3.3.2.4 The prion protein has not been unequivocally shown to be the infectious agent of TSE disease. Although some work is investigating the correlation between PrPSc and infectivity, using transgenic mouse models, this remains a fundamental gap in knowledge. The unequivocal identification of the TSE agent would have a profound effect on the development of appropriate diagnostic tests. For this reason, the search for a non-PrPSc marker for disease is important. Some work is ongoing in the search for alternative markers, using approaches such as metabolomics and proteomics. These approaches search for consistent differences between control and infected animals at several stages of the incubation period. However, an alternative marker for the detection of the disease has not yet been identified. Further characterisation of different conformers of PrP after digestion with proteases and the development and application of other conformation-dependent techniques for detecting PrPSc may allow a better understanding of the significance of atypical results observed in large-scale surveillance studies.

3.3.2.5 Besides funding research on the development of diagnostic tests, DH is also considering how to validate human tests as they are developed and is arranging the provision of standard preparations of test materials such as plasma, blood and brain tissue. In addition, the use of a test for routine screening would raise substantial ethical issues if it became available. DH will be encouraging public debate on this issue. DH is also working with the National Blood Service to prepare for the introduction of a blood-screening test, should one be developed in the near future.

3.3.3 Prospects for treatment

3.3.3.1 Current strategies involve screening large pharmaceutical libraries to identify possible therapeutic compounds. However, a better understanding of the structures of prion proteins and their biology could lead to a more targeted approach, allowing the design of small molecules which could block abnormal prion protein formation. Approaches underpinning the development of therapeutics remain a priority for the research councils. The identification of a natural mechanism for clearing infectivity from the body could also be exploited for therapeutic purposes.

3.3.3.2 It seems unlikely that a treatment will become available soon to repair damage to the CNS. The research councils fund work on the generic development of stem cell therapy. However, therapies that seek to repair damaged tissue are far in the future and even then would only be of value once infection had been halted. While several strategies to develop a therapeutic are aimed at inhibiting PrPC to PrPSc conversion, success is likely to be improved by the development of good diagnostics to identify individuals with pre-clinical infection.

3.3.3.3 The development and evaluation of a potential therapy is a detailed process that usually takes many years. In addition to continued support for such work, DH and MRC are establishing the infrastructure for rapid clinical evaluation of any agents that have demonstrated anti-prion activity in animals or in vitro. To this end a clinical trial protocol has been developed in conjunction with families and carers through the Medical Research Council's Prion Unit and Clinical Trials Unit, and building capacity for undertaking such work remains a priority.
3.4 Key goals

3.4.0.1 Food safety is the Food Standards Agency’s top priority. It supports this by funding work that provides consumers with accurate and reliable information to inform decisions when choosing a healthy diet. It recognises that there is scientific uncertainty and that the controls in use are necessarily based on current knowledge. It accepts that better knowledge will allow precautions to be improved, and applied on the basis of better risk assessment. As a result it continuously reviews the food protection controls that are in place to protect the consumer from the risk of TSE infection from food.

3.4.0.2 Public health is the priority for the Department of Health. Its policies are designed to estimate the size of the current epidemic through improving disease surveillance, to advance the care and treatment of patients, and to reduce the risk of secondary disease transmission through contaminated blood and medical devices. Much of this work supports the National Health Service, including research on the decontamination of surgical devices and on the development of diagnostic and therapeutic agents.

3.4.0.3 Protection of animal and human health through minimising the risk of TSE infection via food, environmental contamination, or via other routes of exposure arising directly or indirectly from farm animals and their husbandry is an immediate priority for Defra. Defra’s aims are to eradicate TSEs from farmed and other animals in the UK and to ensure environmentally sound and safe standards of disposal of animal by-products and specified risk materials. Defra works closely with the Food Standards Agency, as much of the scientific information required to achieve these aims is common to both Defra and FSA policy objectives.

3.4.0.4 The MRC has funded basic research into spongiform encephalopathies since the late 1970s. The MRC continues to fund TSE research across the spectrum from basic biological studies, through to applied clinical research, epidemiology and risk assessment. A dedicated MRC Prion Unit is the focus of MRC funded TSE research. The Unit was formally established in 1999 to create an international centre of excellence. It plays a key role in linking basic science to clinical research and focuses principally on human prion diseases. In addition, MRC encourages applications that strengthen our understanding of human TSEs.

3.4.0.5 BBSRC has supported a major programme of research into the biology of spongiform encephalopathies (BSEP) for a number of years. This has built on the existing strengths at the Institute for Animal Health (IAH) to complement and underpin related work supported by Defra, together with research on CJD funded by MRC and the Health Departments. It has sought to enlarge the size of the TSE research community in universities.

3.4.0.6 In particular, BBSRC has a long-standing interest in scrapie, the first TSE, which was originally recorded nearly three hundred years ago. Knowledge of scrapie - both in its natural sheep host and in rodent models - has much to contribute to the understanding and control of other human and animal TSEs that have emerged more recently. Furthermore, the TSEs continue to be of considerable scientific interest in their own right. Much is still unknown about the fundamental biology of these diseases, and many key questions (for example, about the nature of the infectious agent, the basis of strain variation and mechanisms of pathogenesis) have not yet been answered. Although there might be a reduction, in due course, in the requirement for future research directly to address policy concerns about TSEs, there will be an ongoing need for basic studies to investigate further the unresolved scientific issues.

3.4.0.7 The possibility of an infectious carrier state in apparently healthy animals and humans highlights our limited knowledge of TSE diseases. Work on TSEs may also produce results that have significance for the understanding of other groups of diseases.
such as the neurodegenerative changes involved in Alzheimer’s and other amyloid brain diseases. A full understanding of TSE transmission is likely to require advances in the understanding of the immune system and will provide insights into aspects of human and animal genetic variability. The Research Councils (BBSRC and MRC) support this underpinning biomedical science.

3.4.1 The Department of Health has highlighted the following key goals for TSE-related research:

Public health
- The surveillance of human TSE disease
- Epidemiological studies of human disease, especially those in the very young, the elderly and in at-risk populations.
- Research designed to protect the blood supply and the supply of blood products
- Research on the development of sensitive and specific diagnostic technologies

Prevention of infection
- Research to accurately assess levels of infectivity in organs, tissues and body fluids
- Research on novel methods to decontaminate surgical instruments and other medical devices and the introduction of these into NHS practice

Treatment
- The development and assessment of novel therapeutic compounds
- The assessment of these compounds in humans through clinical trials

3.4.2 The MRC has identified the following key areas in which it would like to continue to receive innovative research proposals:

- The biological and epidemiological relationship between CJD and BSE;
- Epidemiological modelling of CJD;
- The analysis, perception and communication of risk in relation to CJD;
- Early disease progression and diagnosis in life including in vivo imaging approaches, with a focus on the development of non-invasive pre-clinical tests for human TSE diseases, particularly vCJD, e.g. a blood test or throat swab;*
- Integrated molecular, epidemiological and clinical approaches to understanding the cause or causes of sporadic CJD and the relationship with atypical dementias;
- Molecular, genetic, cellular and functional approaches to elucidating mechanisms of TSEs transmission, PrP replication, pathogenesis and clinical progression, with a view to improving understanding of how TSEs cause disease, particularly during the very early stages;*
- The biological function of normal prion protein (PrP);*
- The molecular structure of the prion proteins;*
- Development and improvement of animal models and cell culture systems;*
- Development of approaches to reduce secondary (iatrogenic) infection;*
- Rational approaches to developing therapy including vaccine based treatment approaches.*

* Council has approved programmes of work within these areas at the MRC Prion Unit
3.4.3 BBSRC welcomes applications addressing any aspect of TSE research within its remit, but particularly encourages proposals in the following areas:

- The nature of the infectious agent;
- The normal function and structural biology of the prion protein (PrP);
- Molecular methods of strain differentiation;
- Molecular mechanisms and genetics of pathogenesis of both the CNS and PNS;
- Approaches underpinning the development of therapeutics;
- Epidemiology and modelling to underpin scrapie control;
- Scrapie as a natural disease model in the target species;
- Establishing whether PrP$^{Sc}$ is present in sub-clinical animals.

3.4.4 The FSA currently commissions research, following open competition and peer review of proposals, in the following areas:

- The development of rapid detection methods for TSE's in ante and post-mortem food animals.
- The development of detection methods capable of differentiating between TSEs.
- The development of TSE detection methods for animal tissues and animal products (milk and dairy products).
- Determining relative infectivity of edible tissues and animal products from BSE infected cattle, sheep and goats.
- Providing information on the transmissibility of BSE to food animal species (deer).
- Collecting information with regard to TSEs on the routes and consumption patterns of animal products entering the food chain.
- Providing information on the risk of contamination of edible tissues with SRM caused by current stunning, slaughter and carcass dressing techniques and where necessary develop alternative lower risk techniques.
- Carrying out risk analysis to assess the risk to the consumer from TSEs entering the food chain and the effect of any proposed risk reduction measures.
- The development of methods to detect SRM in food products.

3.4.5 Defra funded research includes the following areas in its on-going programme:

**Understanding the transmissible agent**
- Studies on the correlation of PrP$^{Sc}$ detection and infectivity in tissues from infected animals together with relative sensitivity of different species;
- Studies on the source of infection for BSE cases in cattle born after the augmented feed controls in August 1996;

**Detecting and monitoring infection**
- The further development of diagnostic tests, both post and pre-mortem, to allow differentiation between strains of TSE and detection of disease at an earlier stage of the incubation period;
- Monitoring strain stability of BSE in cattle;
- Epidemiological studies of BSE in cattle and TSEs in sheep aimed at ensuring that appropriate control measures are in place, and modelling to provide forecasts of the future course of the diseases;
Tackling the spread of disease
- Determining effective methods for inactivation and decontamination of the infectious agent to ensure safe treatment and disposal of risk materials;
- Studies on the persistence, movement and transfer of infectivity/PrP\textsuperscript{Sc} in soils and the environment;
- The improvement of methods for the detection of species specific compounds in animal feeds to support statutory controls;

Supporting the National Scrapie Plan
- Determining demographic and genotype profiles of UK sheep flock and studies on transmission of TSE infection within and between flocks;
- Investigation of effects of selective breeding programme on sheep production traits, conformation, survival, health and welfare;
- Studies on the atypical forms of scrapie detected in surveillance studies by using improved diagnostic methods;
- Further improvements in methods for the differentiation of BSE and scrapie in sheep.
Assuring safety

4.1 Risk analysis

4.1.1 Throughout the history of the BSE and vCJD epidemics, government has had to take decisions on how to prevent the further spread of these diseases. The long incubation period characteristic of TSEs has affected the speed with which research can be conducted, and also our ability to judge quickly how successful any control measure has been.

4.1.2 Risk assessment models estimate the impact of potential risk-reduction measures, and also enable the effects of new research information to be rapidly evaluated. Risk assessment has therefore been an important tool to inform decision making. Control measures have been built on the two important risk management concepts of proportionality and the precautionary principle.

4.1.3 Since 1990 the Spongiform Encephalopathy Advisory Committee has been the government’s main source of advice on the risks to public and animal health from TSEs. In formulating its advice SEAC has taken into account research findings from the Joint Funders’ programme, and elsewhere in the world, as well as specific risk assessments commissioned by government departments. In the European Union an analogous body, the Scientific Steering Committee, has performed a similar function. Its opinions have underpinned EU-wide legislation on BSE controls.

4.1.4 In April 1998 SEAC held a workshop on risk assessment, reviewing the approaches and methodologies in use, and examining the level of knowledge on the key variables. Scientists from the Royal Society, the Royal Statistical Society and the European Commission's scientific advisory groups took part. An important outcome of this meeting was agreement on the need to consult widely on the models underlying the analysis.

4.1.5 Risk assessments that SEAC has considered in recent years and that have been used to inform risk management policies include:

- Risk to public health from bone marrow tissue and dorsal root ganglia;
- Risks of spreading condensate from rendering plants onto land;
- Risks from BSE via environmental pathways;
- Risks to human and animal health from burning SRM in small incinerators;
- Risks from the disposal of cattle during the foot and mouth disease outbreak of 2001 due to BSE infectiousness;
- Risk assessment of transmission of CJD via surgical instruments;
- Assessment of the risk of exposure to infectivity in blood and blood products;
- Risks from exposure to TSE agents at work.

4.1.6 Such risk assessments are used by the CJD Incidents Panel, to advise Trusts on the management of risks to individual patients who may have been exposed to potentially contaminated surgical instruments, blood products or tissues. Specific projects to inform the Panel’s work have been undertaken or are still under way and include assessments of dental procedures, blood and blood products, and endoscopes. The Panel’s framework document setting out the underlying evidence basis and the ethical principles that have been applied and showing how the Panel proposes to deal with the considerable scientific uncertainties surrounding CJD was published in March 2004.
4.2 Occupational risk

4.2.1 Until more is known about TSE diseases, it is not possible to eliminate all risk of possible exposure. Current strategies for disease management aim to reduce this risk as far as possible through the issuing of operative guidelines and changes in practice.

4.2.2 To date, there have been no cases of sporadic or variant CJD that can be linked to occupational exposure. A small number of cases of sporadic CJD have occurred in healthcare workers (retired laboratory workers and a pathologist), but none could be linked to their occupations. Similarly, although sporadic CJD occurs in dairy workers at higher levels than in the general population, these levels do not differ significantly from levels in dairy workers in European countries that have not experienced a BSE epidemic. If any occupations had an increased risk of people contracting CJD at work, it would be those where individuals handle TSE infected animals or material. Occupations where this may occur include:

- diagnostic laboratory work for the clinical management of patients with known or suspected CJD, and work with animal tissues gathered for surveillance purposes;
- research with infected animals or infected human tissue
- animal workers e.g. vets, farmers, abattoir workers
- refuse processors including incinerator operators, hauliers and landfill site workers
- maintenance workers, for instance at slaughter houses.
- healthcare workers treating CJD patients

4.2.3 For these groups the risk would be considered greatest if they are directly exposed to infected tissues, especially if they sustain a puncture wound or sharps injury, broken skin is contaminated, or mucosal membranes are splashed.

4.3 Occupational intervention

4.3.1 Anyone working with potential sources of TSE agents should take precautions to reduce their likelihood of exposure.

4.3.2 Employers are required to assess the risk of any work with infectious agents and put appropriate control measures in place under the Control of Substances Hazardous to Health (COSHH) Regulations 2002 (see COSHH Approved Code of Practice for further guidance). Most TSE agents are classified in Hazard Group (HG) 3, (except scrapie, which is HG2), and so should be worked with at Containment Level (CL) 3 in the laboratory.

4.3.3 All healthcare workers are issued with guidance by the Department of Health on infection prevention and control. Some key documents can be found on the website. The aim is to ensure that staff are appropriately informed and, following their training, will minimise their risk of exposure to any infection, including that from TSE agents. The responsibility for ensuring that effective arrangements for infection control are in place lies with the hospital or Primary Health Care Trust’s chief executive.

4.3.4 Those working with live animals should also consider the hazards posed by TSEs. Employers should aim to reduce exposure to infected live animals as well as those organs and tissues most likely to carry the infection, including the brain and spinal cord. Employers should ensure that appropriate controls are in place to reduce any risks arising from a work activity involving contact with suspected or known contaminated animals or materials. General guidance for those working with known or suspected TSE sources can be found in the ACDP publications 'Transmissible Spongiform Encephalopathy agents: Safe working and the prevention of infection' and 'BSE: Background and general occupational guidance'.
4.4 Current measures in place to protect public health

4.4.0.1 There have been no cases to date to suggest that CJD can be spread by person-to-person close contact although it is known that sporadic CJD has been contracted through medical intervention (e.g. from the use of infected medical instruments). It is thus considered that specific groups of patients, rather than health workers, are at higher risk of exposure.

4.4.1 Actions taken to prevent medical exposure to prion disease

4.4.1.1 DH has considered a number of measures to reduce medical exposure to TSEs.

4.4.1.2 Safety of blood
The death from vCJD of a UK blood transfusion recipient as well as studies on experimental animals indicate that it is important to take precautions to reduce the risk of transmission of variant CJD through this route. Since October 1999, white blood cells (which may carry the greatest risk of transmitting vCJD) have been removed from all blood used for transfusion. Plasma derivatives such as clotting factors have been prepared from plasma imported from the USA since 1998. Fresh frozen plasma for treating babies has been obtained from the USA since March 2004 and this will be extended to young children as quickly as is practicable. As a further precautionary measure the UK has banned individuals who have received blood transfusions since 1980 from subsequently donating blood.

4.4.1.3 Vaccines
The Committee on Safety of Medicines (CSM), in three separate reviews, concluded that there are no demonstrable TSE-related issues arising from the use of animal material in vaccines now on the UK market or available in the UK since the early 1970s. Following the issue of the first CSM guidelines, vaccine manufacturers have moved away from UK-sourced material.

4.4.1.4 Medicines
Guidelines for the pharmaceutical industry on the use of animal-derived materials have been in place since 1988. They have been amended over the years to reflect emerging scientific knowledge about BSE. The current guidelines address the sourcing and processing of certain animal derived materials used in the manufacture of medicines, and prohibit the use of UK-sourced material.

4.4.1.5 Human-derived materials
The use of human pituitary-derived growth hormone in humans ended in May 1985 when the link with CJD was established. A substitute produced from genetically engineered bacteria is now used. Over 50 CJD cases around the world have resulted from the use of cadaveric dura mater for grafts to patients whose dura mater, the outermost of the brain meninges, had suffered damage from aneurisms or other causes. The use of human dura mater in medicine ended in 1992.

4.4.1.6 Surgical instruments
A risk assessment carried out by the Department of Health and endorsed by SEAC indicated that improving decontamination procedures would provide the greatest reduction in the risk of transmission through surgical instruments. The Department has provided funding to over 100 sites to help meet the cost of decontamination equipment, minor building work and new surgical instruments. Following initial investment to ensure that standards were improved in the areas of greatest need, the Department is now assessing further bids for intermediate investment funding. The intention is to focus on more strategic and long-term solutions. The introduction of single-use instruments is encouraged where this does not compromise the quality of clinical care, but experience with the introduction of a single use set of instruments for carrying out tonsillectomy
indicated that the potential CJD risk must be balanced against other, non-disease related risks, for example, the possibility of injury to the patient or user as a result of cheaper lower quality single use instruments failing.

4.4.1.7 Development of more instruments that can be single use or that are easier to clean
The Department of Health is working with manufacturers of medical devices to encourage the development of single use alternatives and to produce guidance to ensure that decontamination is considered in the design of new instruments.

4.4.1.8 Guidance on safe working and the prevention of infection
Guidance giving advice on work with TSEs in experimental and clinical settings was first published in 1998 and revised in the light of new knowledge in 2003.

4.4.2 Actions taken to protect food from TSE exposure

4.4.2.1 The three key measures that minimise the possibility of BSE entering the food chain are the control on Specified Risk Material (SRM), the ban on feeding meat and bone meal to farm animals and the Over Thirty Months (OTM) rule. The Food Standards Agency has a strong interest in this area.

4.4.2.2 The main BSE control measure is the removal of SRM. It is designed to remove the parts of cattle, sheep and goats that are most likely to contain the BSE agent. The removal of SRM is estimated to remove over 95 per cent of BSE infectivity from cattle with the disease.

4.4.2.3 The feed ban, which became fully effective from August 1996 in the UK, prohibits the feeding of meat and bone meal derived from mammals to all farm animals. This ban has had a significant affect on reducing the spread of BSE in cattle and thus the exposure to humans.

4.4.2.4 The OTM rule prohibits the sale for human consumption in the UK of meat from cattle aged over thirty months at slaughter. BSE has not been found in the UK in cattle younger than 30 months since 1996. In other EU countries, cattle aged over thirty months are allowed to enter the food chain provided they have tested negative for BSE and SRM has been removed. In 2003 the FSA completed a review of the OTM rule. Following consideration of the review, the Agency’s Board agreed to recommend that it would be acceptable on public health grounds to replace, in two stages, the OTM Rule with BSE testing of cattle older than 30 months. The OTM Risk Assessment Group met again on 2 April 2004 to consider the information that has emerged since the Agency gave its advice to Health Ministers in 2003, and concluded that a little more work needs to be done before any conclusions can be reached. In the meantime the Agency’s advice on OTM rule change remains the same.

4.4.2.5 The Food Standards Agency’s Meat Hygiene Service, local authorities and the State Veterinary Service, part of Defra, ensure that these controls are enforced.

4.4.2.6 At each stage of the slaughter of animals for human consumption, measures are in place to protect public health and prevent meat being contaminated with SRM. The Meat Hygiene Service enforces these measures. Precautions are taken to prevent brain material contaminating meat. Where possible the use of non-invasive stunning using electrical or percussion stunning is encouraged to prevent dispersal of brain material from the cranial cavity. If captive bolt stunning is used the bolt should be wiped clean after each use and the bolt hole should be plugged. The pithing of animals (the insertion of a rod into the cranial cavity after stunning) was banned in 2001 to minimise the risk of brain tissue being transported via the bloodstream to the edible parts of the carcase.
4.5 Current measures in place to protect human and animal health through actions taken on farm animals

4.5.1 Defra has the continuing responsibility for the eradication of BSE in cattle and the control of TSEs in sheep. Policy on BSE and scrapie has been determined largely by research, surveillance, epidemiological analysis of case data, by risk analyses and application of the precautionary principle. In recent years both policy and the requirements for monitoring and surveillance have been driven by EU legislation. Scientific input to the formulation of European policies is achieved largely through veterinary experts representing Member States during discussions at working groups and at the Standing Committee on the Food Chain and Animal Health (SCOFCAH). The TSE/BSE Ad-Hoc Group also prepare papers on the state of scientific knowledge on TSE issues by the EU Standing Scientific Committee (SSC) and, in future, to the European Food Standards Agency. The UK makes a large contribution to European thinking, having more research data and more experience of managing BSE though regulation than any other Member State or third country.

4.5.2 Defra policy for the control of TSEs in sheep is based on the National Scrapie Plan (NSP). The driver for this policy is to remove any (at present theoretical) risk to human health arising from the possible presence of BSE in sheep. The NSP is founded on scientific evidence that certain genotypes of sheep are resistant or refractory to infection with TSEs. The large-scale selective breeding of sheep with resistant PrP genotypes should progressively reduce the incidence of disease to the point where it will be eliminated from the national flock. This policy involves a number of premises e.g. that the carrier state does not occur in resistant genotypes; that there are not strains of scrapie that can persist in resistant genotypes. To ensure the scientific basis of the NSP, Defra has a research programme investigating these issues in parallel with the implementation of the NSP.

4.5.3 The effectiveness of current measures requires constant review. An example is the changing battery of measures that have been developed to reduce hazards associated with animal feed.

4.5.4 In 1987, epidemiological studies of BSE cases identified meat and bone meal as the probable means by which the disease was being spread. In an attempt to prevent further infections a ban on incorporating ruminant protein in ruminant feed was introduced in July 1988. Due to the long incubation period associated with this disease the efficacy of this control measure was not immediately apparent. As time passed it became clear from the number of cases born after the ban that it was not wholly effective.

4.5.5 Epidemiological analysis of these cases showed that a high proportion of them occurred in areas where the pig population was high. This observation, coupled with research data that showed that only a very small dose of the infective material was needed to cause disease in cattle, led to the conclusion that cross-contamination of feed was occurring.

4.5.6 Since 1988, increasingly stringent feed controls have been put in place. Key amongst these have been:

- the ban on the use of specified bovine offal in all animal feed (September 1990);
- the ban on feeding any farmed animal, including horses and fish, with mammalian meat and bone meal. (This began in March 1996 but following this ban there was a recall scheme and the date from which the ban was considered to be fully effective is regarded as being 1 August 1996);
• EU-wide controls on feed which extended the ban to include all processed animal protein, including that derived from birds and fish (implemented in the UK from 1 August 2001).

4.5.7 As illustrated in fig. [ ] these later measures have reduced the spread of BSE. However, they have not been one hundred per cent effective. As at 31st December 2003 there had been 81 cases of BSE in animals born since 1 August 1996 in the UK.

4.5.8 Each of these cases has been investigated in an attempt to identify a possible source of infection. There is no indication that the cases are a result of maternal transmission. None of the dams or siblings of these cases are known to have developed BSE. Environmental contamination also seems unlikely as some cases have occurred on farms on which there had been no previous cases, although this possibility cannot be ruled out on the basis of the information available. A feedborne source still appears to be the most likely explanation. The possible contamination of UK-produced and of imported feed and ingredients is under investigation, but no direct evidence has been found to date.
References


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325. Professor C Bostock, Chapter 2 this volume


Glossary

ACDP
The Advisory Committee on Dangerous Pathogens advises the Health and Safety Commission, the Health and Safety Executive, Health and Agriculture Ministers and their counterparts under devolution in Scotland, Wales and Northern Ireland, as required, on all aspects of hazards and risks to workers and others from exposure to pathogens.

AFRC
The former Agricultural and Food Research Council whose TSE responsibilities were taken over by BBSRC in 1994.

Alzheimer’s disease
A neurodegenerative disease involving presenile dementia, loss of cognitive function and accumulation of amyloid. It can present symptoms similar to those of TSEs in humans.

Amyloid
An insoluble precipitate of protein, generally organised into fibres.

BAB
Born after the Ban: of cattle born after 1988 when MBM was banned as ruminant feed.

BARB
Born after the Reinforced Ban: of cattle born after 1996 when MBM was banned as feed to all farmed species including fish.

BBSRC
Biotechnology and Biological Sciences Research Council: one of the UK funders of TSE research.

BSE
Bovine spongiform encephalopathy.

C terminus (of PrP^C)
The end of the PrP^C molecule which is synthesised last.

Carrier state
Condition in which an animal or person can be infected and carry the infection without showing clinical signs.

CAMR
Centre for Applied Microbiology and Research. Now part of the Health Protection Agency.

Chaperones
Molecules which assist in the correct folding and modification of proteins.
CJD
Creutzfeldt-Jakob disease, a TSE found in humans

CNS
Central nervous system, largely consisting of the brain and spinal cord

codon 129 (of PrP<sup>c</sup>)
The position, counting, from the N-terminus of the PrP<sup>c</sup> molecule, where a human polymorphism leads to differing susceptibility to disease

CWD
Chronic wasting disease, a TSE of deer and elk, currently only described in the United States

Defra
Department for Environment, Food and Rural Affairs: one of the UK funders of TSE research

DH
Department of Health: one of the UK funders of TSE research

DTI
Department of Trade and Industry, the sponsoring ministry for the research councils

dura mater
The outermost and most fibrous of the three membranes surrounding the brain and spinal cord

EFSA
European Food Safety Authority

Endocytosis
One of the processes by which cells take up material from their surrounding environment

ER
Endoplasmic reticulum - A structure where many cellular proteins are synthesized before being passed to the Golgi for modification

ESAC-PR
The DH Decontamination Science and Engineering Advisory Committee which will facilitate the transfer of research findings into improved protocols for NHS Sterile Service Departments

EU
The European Union

FDC
Follicular dendritic cell, a long-lived non-dividing cell found in lymphoid tissues
FFI
Fatal familial insomnia, an inherited (familial) human TSE

Foresight Programme
Run by the Office of Science and Technology at the Department of Trade and Industry, the Foresight Programme promotes collaboration between leading experts across industry, science and government to provide challenging visions of the future

FSA
Food Standards Agency: one of the UK funders of TSE research

FSE
Feline spongiform encephalopathy, a TSE of both large and domestic cats

GALT
Gut-associated lymphoid tissue

Glycosylation
Addition of sugar residues to a protein molecule

Golgi apparatus
A structure which is part of the cell’s protein synthetic and modification machinery and which directs proteins to specialised locations within the cell or to the surface for excretion

GPI tail
Glycophosphatidylinositol tail, which anchors the PrP\textsuperscript{C} molecule to the cell membrane

GSS
Gerstmann-Straussler-Scheinker syndrome, an inherited (familial) human TSE

[Het-s]
A prion-like form of a yeast protein

High Level Progress Chasing Committee on Research and Development in TSEs: Butler Committee
UK Government committee on TSE issues, reporting to the Prime Minister

Horizontal Transmission
Direct or indirect transmission of TSE from animal to animal rather than from mother (or father) to her (or his) offspring

HPA
Health Protection Agency
**Iatrogenic**

A form of an illness caused by medical treatment

**ICE test**

Immuno capillary electrophoresis test. A test that has been developed in sheep to detect prions isolated from body fluids (eg blood)

**Joint Funders’ Group**

A committee comprising representatives of the UK’s five main funding agencies for TSE research: DH, Defra, FSA, MRC and BBSRC

**Knockout**

Having a particular gene disabled: of an experimental animal

**Kuru**

TSE of humans found in New Guinea and associated with cannibalistic consumption of brain material

**Leukodepletion**

Use of filtration technique to remove lymphocytes, a type of white blood cell involved in infection and immune resistance

**LINK**

The Office of Science and Technology’s LINK Programmes support collaborative research between industry and academic researchers

**LRS**

Lymphoreticular system

**MAFF**

The former Ministry of Agriculture, Fisheries and Food which was reorganised to form DEFRA and FSA in 2000

**MBM**

Meat and bone meal

**MCA**

The former Medicines Control Agency which is now part of the MHRA

**Meninges**

The three membranes enclosing the brain and spinal cord

**MHRA**

Medicines and Healthcare products Regulatory Agency
**MM**
Shorthand notation for methionine homozygosity at codon 129 of Prnp, a polymorphism in humans that increases susceptibility to vCJD

**MMR**
Measles, mumps and rubella vaccine

**MRC**
Medical Research Council: one of the UK funders of TSE research

**MRI**
Magnetic resonance imaging: medical imaging using radio waves in the presence of a magnetic field

**MS**
Mass spectrometry: a method for measuring molecular mass and identifying atomic nuclei

**MSBT**
The Committee on the Microbial Safety of Blood and Tissues for Transplantation

**N-terminus** (of PrP<sup>C</sup>)
The “starting” end of the PrP<sup>C</sup> molecule, which is synthesised first

**NBA**
National Blood Authority

**NBS**
National Blood Service, part of the National Health Service

**NCJDSU**
National CJD Surveillance Unit

**NFCI**
National Focus for Chemical Incidents. Now part of the Health Protection Agency

**NIBSC**
National Institute for Biological Standards and Controls

**NMR**
Nuclear magnetic resonance

**NRPB**
National Radiological Protection Board. Now part of the Health Protection Agency
National Scrapie Plan

Plan to remove or minimize scrapie among sheep in the UK by culling those with least genetic resistance to it and breeding from animals with more resistant genotypes

nvCJD

New variant CJD, a synonym for vCJD used when vCJD was first described

OIE

Office International des Epizooties is an intergovernmental organisation created by the International Agreement of 25 January 1924 to guarantee the transparency of animal disease status world-wide and to collect, analyse and disseminate veterinary scientific information

OST

Office of Science and Technology, the part of DTI responsible for the research councils

OTM Rule

Over Thirty Month Rule, bans the UK sale of meat for human consumption from most cattle aged above 30 months at the time of slaughter

Passage

The process of growing infectious material sequentially in a number of host animals or cell lines

PHLS

Public Health Laboratory Service. Now part of the Health Protection Agency

Polymorphism

Variation of genetic, physical or biochemical characteristics between individual members of a species, other than sex differences

Prion

A protein which is abnormally folded, resulting in loss or change of function, and which is able to induce transition to the same abnormal folding in originally normal forms of the protein

Prion hypothesis

Currently the most accepted explanation for TSEs, in which the normal form of the cellular prion protein, PrP^C, is converted to abnormally folded PrP^{Sc} by exposure to pre-existing PrP^{Sc} molecules

Prnp

Gene that codes for PrP^C

Protease

Enzyme that catalyses protein breakdown

PK

Proteinase K - A protease which can degrade PrP^{Sen} but not PrP^{Res}
**PrP**
Prion protein

**PrP**<sub>C</sub>
Normal cellular prion protein

**PrP**<sub>Rec</sub>
Recombinant PrP, the form produced in genetically modified cell cultures or microorganisms

**PrP**<sub>Res</sub>
Form of PrP relatively resistant to protease digestion

**PrP**<sub>Sc</sub>
Abnormal form of prion protein associated with TSE infection

**PrP**<sub>Sen</sub>
Collective term for PrP<sub>C</sub> and PrP<sub>Rec</sub>, sensitive to protease digestion, in contrast to PrP<sub>Res</sub>

**[PSI]**
A yeast protein that behaves like a prion

**SACTTI**
The Standing Advisory Committee for Transfusion Transmitted Infections which advises the UK blood services on CJD-related issues

**Scientific Steering Committee (European Commission)**
EU advisory body on TSEs now subsumed within EFSA

**Scrapie**
TSE of sheep

**SEAC**
Spongiform Encephalopathy Advisory Committee, provider of TSE scientific advice to UK government departments and devolved administrations

**Sinc**
Gene that is the major determinant of TSE incubation period in mice

**Sip**
Gene that is the major determinant of TSE incubation period in sheep

**Species barrier**
The observed difficulty in transmitting an infection from an animal of one species to one of another by comparison with transmission between members of the same species
SRM
Specified Risk Material, in relation to animal tissues with the potential to carry TSE infectivity

TME
Transmissible mink encephalopathy, a rare TSE of mink

TSE
Transmissible spongiform encephalopathy, a family of diseases found in humans and farmed and wild mammals, characterized by prion protein misfolding leading to degenerative loss of brain function

[URE3]
A yeast protein that behaves like a prion

vCJD
A variant form of CJD, first reported in 1996

Vertical transmission
Transmission of TSE from mother (or father) to offspring

Virino
Hypothetical small piece of nucleic acid suggested as the causative agent for TSE infection in an alternative to the prion hypothesis

VLA
Veterinary Laboratories Agency, an executive agency of Defra
## Annex 1 - Useful website references

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Annex 2 – Committees’ terms of reference

Advisory Committee on Dangerous Pathogens

The Advisory Committee on Dangerous Pathogens advises the Health and Safety Commission, the Health and Safety Executive, Health and Agriculture Ministers and their counterparts under devolution in Scotland, Wales and Northern Ireland, as required, on all aspects of hazards and risks to workers and others from exposure to pathogens.

BBSRC Biology of Spongiform Encephalopathies Programme (BSEP) Working Party

• To advise on the overall strategy of the programme;
• To assess applications from BBSRC institutes and higher education institutions for research grants;
• To advise on the allocation of funds;
• To advise on the future direction of the programme.

CJD Incidents Panel

• To assist individual Health Authorities or Health Boards and clinicians to decide on the most appropriate action to take to handle incidents involving potential transmission of Creutzfeldt-Jacob Disease (CJD) and variant CJD (vCJD) between patients through clinical interventions, including via surgical instruments, tissues, organs and blood, and to keep relevant devolved administrations informed.
• To consider what information should be collected on patients who may have been exposed; advise on what studies and follow-up may be needed; advise Directors of Public Health on patient tracing and notification exercises where these are indicated; and advise on whether any other measures are needed to protect the wider public health.
• To make regular reports to the Spongiform Encephalopathy Advisory Committee and Advisory Committee on Dangerous Pathogens Transmissible Spongiform Encephalopathy Joint Working Group (JWG)
• To keep the expert guidance under review and make recommendations to JWG for further guidelines as necessary, in light of experience of incidents and research in progress.
DEFRA TSE Research Advisory Group

1. The TSE Research Advisory Group (the “group”) consists of four members including a Chair. The Chair may request input from, or attendance at meetings of, other scientific experts if judged necessary.

2. The group reviews and advises on the scope and balance of TSE research programme funded by Defra.

3. The group evaluates, and recommends actions to be taken on, concept notes and research proposals referred by the TSE Research Unit (the “Unit”).

4. The group evaluates reports from contractors at the end of a contract, or at significant review points, when referred by the Unit.

5. The group reports directly to a designated member of the TSE Research Unit, who will be responsible for taking forward the group recommendations within Defra and to other stakeholders. The final decision about whether a proposal is funded is made by the appropriate Defra budget holder, taking into account the recommendations of the group, policy needs and financial constraints.

6. All communication about funding decisions and the scope of Defra’s TSE research programme will be the responsibility of Defra and not of members of the Research Advisory Group.

7. The group has been classified as an ad hoc advisory committee by the cabinet office and will operate according to the standards published in Guidelines 2000 for the operation of Government Committees.

DH/MRC TSE Research Advisory Group

a) To advise the DH Director of Research and Development and the Medical Research Council on a scientific strategy and priorities for basic and applied research on the human aspects of spongiform encephalopathies, covering biomedical, epidemiological and health service research in relation to:

- Antecedents of CJD and factors associated with it (including genetic disposition), the links between human spongiform encephalopathies and animal manifestations, transmission studies.
- Epidemiology and modelling.
- Infectivity of human tissues (including iatrogenic transmission) and the human health aspects of relevant animal regulations and of environmental issues.
- Approaches to assessing risk to human health in this area, public and professional perceptions of risk to health.
- Development and implementation of diagnostics and therapeutics.
- Enhancement of understanding of causation and pathogenesis of human TSEs.
- Other research matters considered likely to have a significant bearing on Public Health.

b) To advise DEFRA, BBSRC, FSA and other funders, should there be human health implications for their programmes.
c) To advise on the resources needed ('capacity building') to move the priorities forward in relation to:
   - facilities
   - expertise
   - methodological development (including reagent technology)

d) To take into account:
   - Scientific opportunities
   - Public health concerns
   - The recommendations of SEAC
   - Constraints particular to the field
   - Strategies and programmes of all major funders

e) To assist DH and MRC in developing mechanisms for stimulating new high quality proposals.

f) To assist in the review of research proposals, as the need arises.

**DH TSE Human Tissue Management Group**

- to advise on whether all permanent human material collections relevant to CJD research are managed in accordance with the MRC’s and the Royal College of Pathologists’ guidelines;
- approve guidelines to handle and manage requests for tissues, which could then be used by tissue centres on a day to day basis;
- act as an independent adjudicator if competing requests from research groups are received;
- advise on future requirements for infrastructure to underpin research in this area;
- the Steering Group will report to the Department of Health and the Medical Research Council in a timely manner.

**High Level Committee to Monitor the Progress on Research Relating to TSEs**

- To ensure that a research strategy, which fully addresses UK government’s policy needs in relation to Human and Animal TSEs, is in place and agreed by all funders.
- To ensure that mechanisms are in place to implement the agreed research strategy and that progress to implementation is taking place as quickly as possible.
- To ensure that all relevant sources of expertise are being called upon and the information is being released to them as freely and as quickly as possible.
- To identify any barriers to progress and make recommendations for overcoming them.
- To make regular reports to the Prime Minister
Independent Archive Advisory Group (IAAG)

REMIT OF THE IAAG
To release tissues from the DEFRA TSE tissue archive to TSE researchers, according to a policy which is fair, open and transparent and to advise how the archive can be run to meet this policy.

ROLE OF THE IAAG
The role of the IAAG will be to advise on the release of tissues collected under DEFRA (and some other UK public) funding to scientists wishing to use them in their research into TSEs. To do this, the IAAG will need to advise on:

a) Policy for release of tissues
b) The remit of the archive
c) Tissues to be collected within existing research projects and from field cases
d) Treatments and storage conditions required
e) Quality control
f) The archive database for recording tissue details, storage location and amounts remaining
g) When tissues will be removed and destroyed
h) Whether researchers or other funders should be charged for use of the tissues
i) The location(s) of the archive stores - one or multiple sites
j) The relationship with archives of tissues collected from non-DEFRA funded projects (e.g. FSA and BBSRC)
k) Raising awareness of tissue issues when applying for and assessing research grants
l) The content of the archive website

Advisory Committee on the Microbiological Safety of Blood and Tissues for Transplantation (MSBT)
To advise the Health Departments of the UK on measures to ensure the microbiological safety of blood and tissues for transplantation. In making recommendations in relation to blood, the Committee will bear in mind the need for maintaining an adequate supply of blood of appropriate quality for both immediate use and for plasma processing

Standing Advisory Committee on Transfusion Transmission Infection (SACTTI) vCJD subcommittee
On behalf of the Standing Advisory Committees of the Joint UK BTS/NIBSC Professional Advisory Committee to examine vCJD issues as they affect the UK Blood Services and to build a comprehensive knowledge base from which to provide consistent advice within the Red Book Framework to the UK Blood Transfusion Services.

- To provide a cohesive professional overview and advice on vCJD issues including critical analysis of current and emerging data of relevance.
- To provide input into the Red Book Guidelines for the UK Blood Transfusion Services in collaboration with the relevant SACs if and when necessary.
- To examine the impact of vCJD on the supply of blood and plasma products in the UK.
• Broadly to examine the following issues:

  What is the biology of this agent in the blood?
  What is the likely epidemiology of vCJD in the blood donor population?
  What is the risk of transmission?
  Is there a test?
  Are there other policies which could be developed to contain the risk?

Spongiform Encephalopathy Advisory Committee (SEAC)

To provide scientifically-based advice to DEFRA, the Department of Health, the Food Standards Agency and the Devolved Administrations on matters relating to spongiform encephalopathies, taking account of the remits of other bodies with related interests.

SEAC
1) Uses the output of research
2) Evaluates the information available and assesses risk.
3) Provides advice on basis of that evaluation.
4) Identifies where there are scientific uncertainties and where more research is required.