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Transmissible Spongiform Encephalopathies

The European Union's Research Response to a Major Public and Animal Health Challenge

Directorate-General for Research
2007 Biotechnologies, Agriculture and Food

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Introduction

The Bovine Spongiform Encephalopathy (BSE) epidemic has had a dramatic impact on Europe, with the loss of life due to variant Creutzfeldt Jakob disease (vCJD) a tragic consequence. Economic damage to the food sector has been extensive and the shockwaves from the epidemic contributed to an erosion of public trust in farming, science and in the food that we eat.

In addition to the immediate changes that were imposed in order to limit the spread of the disease and protect public health, the epidemic also led to more widespread changes in the assurance of food safety and, concomitantly, in food safety research. Consumer protection has become much more prominent as a driver for research and there have been moves to separate the management and assessment of food safety risks in Member States as well as at the European level[1].

The purpose of this booklet is to highlight the research response of the European Commission to the spectrum of diseases known as the Transmissible Spongiform Encephalopathies (TSEs), which include BSE (primarily a disease of cattle); Scrapie in sheep and goats (small ruminants) and variant and classical or sporadic CJD in man. It also provides information on three of the larger research programmes carried out in Member States of the European Union. The booklet focuses on key projects funded by the Directorate-General for Research of the European Commission from the early 1990s to the present day and, in doing so, aims to give an overview on important work carried out in the past as well as on current research priorities.

Research funded by the European Commission addresses not only particular needs – the need to know more about epidemiology and diagnostics, fundamental research on the nature of the infectious agent and work on therapeutics, for example – but also aims at filling gaps and exploiting the benefits of the enlarged scale and scope which only trans-national co-operation can provide. All projects described in this booklet are, therefore, characterised not only by the excellence of their participants – assured by a stringent peer review process – but also by their multi-partner, multi-national approach.

Research results from these projects have provided important input for the risk assessment and management of the BSE crisis with the objectives of protecting public and animal health and assuring the quality and safety of our food. They have been fed into the deliberations of various expert groups and public authorities at EU and Member State levels. This booklet provides contact details of project co-ordinators who, in addition to Member State Programme managers, are thanked for providing summaries of their projects.

Further information may also be requested at the following website:
http://ec.europa.eu/research/enquiries

[1] More information on the risk assessment and management of the epidemic at the EU level can be obtained from the relevant pages of the Directorate General for Health and Consumer Affairs and European Food Safety Authority websites (http://www.ec.europa.eu/dgs/health_consumer/ and www.efsa.europa.eu/ respectively).

Full title Prevention, control and management of prion diseases
Duration 01/09/2003 – 31/12/2008
EC contribution €14.4 million

Introduction

Transmissible Spongiform Encephalopathies (TSEs) are a class of neurodegenerative diseases that affect sheep, cows, deer and other animals as well as humans. They are transmitted by proteinaceous infectious particles called prions that are remarkably resilient. Once they invade an organism, the prions attack the central nervous system. The resulting cellular damage leaves the brain looking like a sponge, hence the term spongiform, killing the host usually within less than a year.

Protecting Europe's human and animal inhabitants against TSEs requires a coordinated response. The diseases and the prions that cause them are not yet well understood. Our knowledge regarding detection of prion diseases, their prevention, treatment of infected individuals and risk assessment of new threats must be improved. A Network of Excellence of prion researchers, entitled NeuroPrion, is leading this charge.

Recruiting an army of prion researchers

Networks of Excellence (NoEs) are a new type of project launched under the Sixth Framework Programme of the European Union. NoEs are based on the principle that Europe's talented scientists can achieve more when working together rather than separately, moving towards the creation of a durably integrated organisation. NoEs also aim to optimise the use of the limited public funding available by preventing repetition and reducing overlap.

The NeuroPrion NoE comprises over 300 experts from nearly 100 teams at 52 different research institutes. It has drawn in prion researchers from all the major regions of Europe: Scandinavia and Iceland to the north, the British Isles, continental Europe, the Mediterranean to the south as well as Central and Eastern Europe. Over 20 countries in all are represented.

Opening up the lines of communication

The NeuroPrion coordinator, the Commissariat à l'Énergie Atomique (CEA), aspired to cultivate a culture of collaboration. Central to this objective was resolving issues of protection of Intellectual Property Rights (IPR) and encouraging responsible exchange of information.

One of the best ways to share information is to gather the world's top prion researchers together at a conference. That is exactly what CEA did at NeuroPrion 2005, with over 800 participants presenting 58 papers and 282 posters. In fact, Stanley Prusiner, the winner of a Nobel prize for discovering prions, called it "the biggest ever gathering of prion researchers". The NeuroPrion 2005 conference established a global precedent that CEA has sustained in the years since with annual conferences to discuss and deliberate state of the art in prion research.

Virtual Research Centre

Due to the substantial geographical distance between the NeuroPrion partners, it was necessary to exploit the power of the Internet to bring them closer together, at least virtually. An advanced web site (http://www.neuroprion.com) was constructed to provide the public and the media with extensive information. The key, however, was the creation of the NeuroPrion intranet, including the necessary technological backbone to facilitate restricted access for each of its 300+ users. It enabled the genesis of the NeuroPrion Virtual Research Centre (VRC).

The VRC contains over 50 different workspaces where users can upload and download documents, share experimental data and other media (e.g. photographs), and learn about the latest internal and external funding opportunities, all in a secure environment. A special document management tool helps partners jointly compose and edit papers, research proposals and other paperwork electronically. The VRC also promotes researcher mobility with advertisements for job and training opportunities. The website has since established itself as the top Internet portal for prion disease research. Future improvements target the provision of continuous video streaming capabilities.

Four pillars of protection

Despite the recent decline in the number of new cases of new variant Creutzfeldt-Jakob Disease (nvCJD), the new form of TSE that appeared in humans following the Bovine Spongiform Encephalopathy (BSE, aka Mad Cow Disease) epidemic in Great Britain, nvCJD has been detected in several new countries in the past few years. Aware of the very real threat of future TSE epidemics, the NeuroPrion NoE set up 24 task groups and assigned them to four basic pillars of prion research: prevention, treatment, control and risk.

The primary challenge facing the task groups allocated to prevention is the need to develop standardised diagnostics for reliable identification of TSE in both humans and animals. To that end, the NeuroPrion experts are pursuing diverse technologies, from proteomics to Surface-Enhanced Laser Desorption Ionisation (SELDI). Once identified, it is also necessary to differentiate among the different strains of TSE in humans (CJD), sheep (scrapie), cows (BSE) and deer (Chronic Wasting Disease or CWD). Other aspects that require attention are: disease transmission via blood transfusions, decontamination of TSE-infected animals and food processing controls to safeguard the food supply from TSE.

Unfortunately, due to the low level of incidence of CJD in humans, approximately one in one million, there is little financial incentive for drug companies to invest the funds necessary to drive treatment research. Furthermore, access to public research funding is increasingly competitive. The NeuroPrion participants are nevertheless pressing forward with the development of new treatment strategies believing it is critical that the remaining uncertainty surrounding prion replication and pathogenesis is resolved. The task groups are also involved in the outlining guidelines for clinical trials if and when new drugs make it to this stage.

The issues of control and risk assessment are vital for properly managing any further TSE outbreak. Ties were established between NeuroPrion and the national surveillance centres as well as international monitoring groups (e.g. the OIE – the World Organisation for Animal Health). Task groups are in charge of recording all new instances of the various animal and human forms of TSE and performing the requisite geographical and other analyses. An important tool the NeuroPrion NoE is striving to introduce is a virtual tissue bank, which hosts information about the tissue banks maintained by each of the NeuroPrion partners.

Communication amongst NeuroPrion's 24 task groups is crucial for the NoE's research objectives to be met. Close collaboration with the Research Directorate-General (DG), the Health and Consumer Protection Directorate-General, the European Food Safety Authority (EFSA) and other European agencies is also essential. Additional benefits are being realised by cooperating with related projects funded by the Sixth Framework Programme (e.g. the BrainNet Europe NoE).

Taking the message to the masses

CEA and its NeuroPrion partners realise that in addition to high quality science they are also obliged to explain their research to the wider public. To this end, NeuroPrion invested in several grassroots measures aimed at showing the European public how science is working for them. The actions include holding special workshops to foster dialogue between scientists and the public, explaining the complex science behind TSEs in layman's terms and creating an open house at some of the world's top research laboratories. The NeuroPrion website is also an important vehicle for communication with the general public.

The creation of the NeuroPrion Network of Excellence will help safeguard Europe against future health and economic crises. A network of excellence holds the belief that the sum is greater than its parts and NeuroPrion is evidence that Europe's scientists can indeed accomplish more together than they can separately as individuals. NeuroPrion has also helped cement Europe's status as the global leader in prion research.

Full title Prevention, control and management of prion diseases

Duration 01/09/2003 – 31/12/2008

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Title	Co-ordination of national surveillance programmes for CJD in the European Union	CJD: Epidemiology, Risk Factors and Diagnostic Tests	Creutzfeldt-Jakob disease in the European Union-incidence and risk factors	CJD: Epidemiology, Risk Factors , Diagnostic Tests and Genetics	Surveillance for vCJD in Central and Eastern Europe and China; Risk Assessment, Tranmsmission and Surveillance
Acronym	NEUROCJD	EUROCJD		NEUROCJD	SEEC-CJD
EC contribution	€535 000	€386 000	€440 000	€1 million	€800 000
Start Date	01/04/1998	01/10/2000	01/05/1997	01/10/2001	01/08/2001
End Date	30/09/2001	30/09/2003	30/04/2000	01/10/2005	30/11/2004

Tracking a rare disease

A series of projects coordinated research between national surveillance centres, initially in seven European countries: the United Kingdom, France, Italy, Germany, the Netherlands, Slovakia and Spain, before going on to include other countries later as described below. These projects were led by Professor Robert Will of the Department of Clinical Neurosciences at the University of Edinburgh, accompanied by his team at the Western General Hospital. Well over 90% of the recorded cases of BSE occurred in the United Kingdom and the first cases of vCJD appeared on British soil.

Statistically speaking, the non variant forms of CJD are very rare diseases, with an incidence of approximately one in a million. vCJD is also a rare disease, having been confirmed in fewer than 160 individuals at the time of writing. It became imperative, therefore, to gather as much information as possible about the few confirmed cases of CJD, whether in its original form, sometimes referred to as classic or sporadic CJD (sCJD), or the newly discovered vCJD.

In addition to the usual battery of tests, data were collected regarding dietary habits, visits to regions where cattle had been affected by BSE, and so on. A significant effort was made to harmonise data collection and validation methods among the partners. The organisation of regular meetings of surveillance centre staff as well as international conferences contributed to efficient information dissemination, and to reaching a consensus on the best way forward.

A centralised database was created to house the results of this exercise; it currently contains data for approximately 4000 cases of CJD, including nearly all known instances of vCJD that occurred both inside and outside Europe's boundaries. It served as an invaluable tool to the researchers in their analysis of the patient data, especially in the identification of risk factors associated with contracting CJD. Furthermore, it aided their efforts to establish a more explicit link between BSE and vCJD.

In search of vCJD

While most fatalities associated with vCJD occurred in the United Kingdom, some cases have been found in other European countries and also in Canada and the USA. This was most likely to be the result of exposure to BSE-contaminated products exported from the UK, prior to the discovery of BSE and the subsequent control measures put in place to prevent its further spread, or to residence in the UK during the risk period.

It soon became clear that the original scope of surveillance had to be expanded. In 1998 additional funding was secured from the BIOMED2 Programme to set up and incorporate national surveillance centres from the remaining EU Member States, as well as from Switzerland, Iceland, Israel, Norway, Canada and Australia. This trend continued under the aegis of the Fifth Framework Programme. Three complementary projects, EUROCJD, NEUROCJD and SEEC-CJD, all supported by the Quality of Life Programme, brought in new partners from eastern and central Europe as well as China. Training and knowledge transfer were essential in getting the new centres up and running as quickly as possible.

Diagnosing the disease

One of the main obstacles encountered by the health service community in dealing with CJD in all its forms is the issue of proper diagnosis. Led by the University of Edinburgh, research was carried out that culminated in the definition of specific diagnostic criteria for each type of CJD. Lists of symptoms allow straightforward differentiation between sCJD and vCJD. The criteria are further enriched with genetic markers for the culprit prions, protein tests (most notably CSF 14-3-3), brain scans and other techniques.

In addition to websites for the national surveillance centres, a combined website **(http://www.eurocjd.ed.ac.uk/)** was established in the framework of the EUROCJD project and later expanded to include the NEUROCJD project. Although these projects have since been completed, the website continues to be updated regularly with the latest information.

Staying on guard

In the process of responding to the challenge of BSE and vCJD, Europe's scientists are at the forefront of research in these areas. The University of Edinburgh and its partners accumulated significant experience and expertise in both the animal and human forms of TSE. The research to establish reliable diagnostic criteria forms an integral part of an extensive set of safeguards put in place to protect public health.

The number of new cases of vCJD in the UK has dropped significantly in the past few years. However, Europe cannot afford to let its guard down. There is still much to be learned about CJD. For instance, little is known about how incubation time. The time between exposure to the infectious prion and the onset of the disease, varies between persons and populations. Furthermore, it is not clear how many people were exposed to BSE-infected beef and beef by-products prior to the identification and virtual eradication of BSE.

The threat of future outbreaks still exists and it is imperative that the national surveillance centres continue their mission, working with scientists both in Europe and worldwide.

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Title	Human transmissible spongiform encephalopathies (prion diseases): neuropathology and phenotypic variation	European centralised facility for human transmissible spongiform encephalopathies (prion diseases).	TSE Lab Human TSEs: The European Diagnostic Laboratory
Acronym	Prionet I	Prionet	TSE Lab
EC contribution	€360 000	€1.12 million	€1.73 million
Start Date	01/06/1997	01/08/1998	01/09/2002
End Date	31/05/2000	31/07/2001	31/08/2005

Understanding the disease

The linkage of the Bovine Spongiform Encephalopathy (BSE) epidemic of the 1980s and 1990s to a new type of Creutzfeldt-Jakob disease (CJD) in humans caused great public concern.

Variant (v)CJD differed from classical forms of the disease in that it affected much younger persons and seemed to be transmitted by exposure to contaminated products.

Professor Stanley Prusiner was awarded a Nobel Prize in 1997 for his work on prions – proteinaceous infectious particles – the abnormal forms of which he hypothesised were the transmissible agents of TSEs (transmissible spongiform encephalopathies) – the family of diseases including BSE, the various forms of CJD, Scrapie in sheep and other neurodegenerative disorders in other animals.

The development of rapid, preclinical diagnostic methods for vCJD was a key objective in the work of these projects, which relied upon large, pan-European, standardised data and tissue banks, for the investigation of human TSEs.

Developing diagnostics

Coordinated by Professor Herbert Budka, a series of projects involving 86 European laboratories spearheaded diagnostics research. Central to their programme was the expansion of a European database containing definitive human TSE diagnoses. The project team aimed to gain further insight into the development of the disease in these patients. Their objective was to define human TSEs, using improved diagnostic tools for detailed neuropathological assessment, according to the patients' genetic make-up. This enabled diagnostic and classification criteria to be redefined. The project members also investigated the link between abnormal prion (PrPSc) deposition and accumulation and tissue damage, as well as other neurodegenerative conditions suspected to be caused by these devastating rogue proteins.

The team members used established diagnostic tools and refined technologically advanced methods during the project to find out more about the disease. Magnetic resonance imaging (MRI) studies confirmed abnormalities in the area of the brain connected with attention and decision-making, indicative of damage to neurons and supporting tissues. Similar abnormalities were observed in the thalamus area of the brain, one example of the development of a criterion for the diagnosis of vCJD. Cerebrospinal fluid samples from 1000 patients were analysed using a prion protein test from which it was concluded that oxidative stress to neurons is important in the development of disease. In addition, the rogue protein was found not only in the nervous system of patients, but also in the areas of the body connected with the immune system, i.e. the tonsils, lymph nodes and spleen.

This early work also confirmed the transmissibility of TSEs from humans to other animals as well as providing strong evidence supporting the hypothesis that exposure to BSE could cause vCJD in the UK and beyond.

Breaking more boundaries: TSELAB

In the subsequent TSELAB project, also coordinated by

Professor Herbert Budka, work focused on refining and increasing the sensitivity of diagnostic procedures. Detection of rogue prions in blood was achieved, albeit in extremely small concentrations and was therefore not readily detectable by standard procedures. Detection of prions in blood may represent a way to discover preclinical or subclinical infections – which may reveal a subset of the population, who will not become infected but who may be capable of transmitting the infection to others by blood transfusion. The project team used the revolutionary 'protein misfolding cyclic amplification' (PMCA) technique to make normal detection methods more effective. This method used sound waves to bombard a sample, causing the small amounts of aggregated abnormal prion protein to break up and catalyse the transformation of non-infectious prion into the abnormal form. This multiplication caused abnormal prions to reach levels detectable by standard diagnostic tests.

The importance of the development of such techniques was thrown into sharp focus when the project discovered vCJD infection in three blood transfusion recipients. The potential dangers of blood transfusion can be reduced by refining tests for these contaminating proteins. Questions also arise concerning procedures that involve sterilisation of surgical implements because standard techniques are rendered inadequate in the face of prion properties. This means that irradiation and autoclaving, common hospital sterilization procedures are ineffective against these infective agents.

Fluorescence correlation spectroscopy (FCS) and Scanning for intensely fluoroscent targets (SIFT) were two more modes of diagnosis that revealed promising results.
FCS is a method used to detect extremely low concentrations of biomolecules. The method hinges on the fact that each type of molecule has a signature depending on the energy it emits as fluorescent light, detectable by a spectroscope. Because it is non-invasive and non-damaging it is an ideal choice for the study of molecular dynamics inside living cells. SIFT is used to scan the resulting photographs for the target molecules that are fluorescing because of their energy output. Further work is underway, so as to combine dyes with the prion molecules, to make their detection even more accurate and sensitive.

Social and economic implications

Overall, the implications of the presence of TSEs in animal and human populations are cause for serious considerations that need to be addressed. Public health and healthcare codes are one of the most important issues. Furthermore, the phenomenon of transmission from species to species, especially through the food chain, is of importance. Animal husbandry techniques, including feeding regimes, have been revised and this reviewing process will continue in the light of new research.

The importance of accurate health tests for animals that enter the food chain and eventually end up on our supermarket shelves is obvious. It has to be pointed out that this disease only affects one in a million people each year worldwide but, as we have witnessed, epidemics on a small scale have occurred. For the sufferers and their families, TSEs exact a high cost in human terms. Research in this regard therefore aims to safeguard the integrity of food chains.

Further information can be obtained from: **http://www.kin.at**
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Full title European network for surveillance and control of TSE in small ruminants: with emphasis on epidemiology, pathology and diagnostic tests

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Scrapie is a TSE of sheep and goats (small ruminants), a fatal progressive neurological disorder, which is non-transmissible to humans and has been recognised for over two hundred years. The purpose of the SRTSENETWORK project has been to develop a sustainable long-term network to facilitate better understanding and control of small ruminant TSE. This has resulted in the creation and dissemination of knowledge plus the improved integration of research efforts at the European level.

Taking a wide-ranging approach

A multidisciplinary network has been created that extends across the European Union and beyond and has consisted of both formal meetings and collaborative work. A sustainable means of information exchange was developed and its continuance facilitated by the relationships developed and the distribution of a comprehensive contact directory. The project was concluded in September 2006 but there has been cooperation throughout its duration to integrate activities within the Framework 6 Network of Excellence Neuroprion especially with regard to the database of tissue samples.

The SRTSENETWORK project has created and shared new knowledge that has improved the understanding and control of TSEs in small ruminants. A whole range of small ruminant TSE scientific endeavours were employed to ensure that a holistic and integrated approach was taken. The network has been sustainable over the long term, facilitating the exchange of information and maximising advances made in the understanding and control of TSEs in sheep and goats.

The following areas were the focus of this Concerted Action:

1) epidemio-surveillance
2) transmission and maintenance of infection
3) pathogenesis and development of diagnostic tests
4) integration of risk analysis into control and surveillance activities
5) rational development of control methodologies, taking into account new knowledge and circumstances
6) advanced epidemiological methods (e.g. mathematical modelling and risk analysis) were incorporated into work package structure to ensure the effective application of these techniques into research, surveillance and control methods.

Spreading the message

The project circulated information on the above subjects to all interested stakeholders within the EU and beyond, including governmental and research bodies and the general public. Interested parties have been able to use the information to improve their understanding of the latest small ruminant TSE research and provide a basis for their own work. There has been a great willingness to share information, which has helped improve the integration and prevent the duplication of activities between different project partners. This in turn has enhanced the development of participants' own research infrastructure.

A wide range of methods was employed while coordinating the research effort including meetings every six months, which acted as a forum for the gathering and sharing of knowledge. Many colleagues who were not formally part of the network attended the meetings and this was a valuable way of building a more extensive network. Particularly useful was the attendance of scientists from what were at that time new Member States who had not had the opportunity to become formal members of the network.

Shared databases were set up, a common website www.srtse.net was established and a newsletter produced to keep researchers up to date with the latest developments. The website was developed to supply emerging needs of those working on small ruminant TSEs both in the EU, the Associated States and beyond. It contained information on TSEs and gave access to presentations following the meetings.

Exchange visits were also undertaken allowing scientists working in similar fields to work closely together, collaborating in

research when it was appropriate. Following the end of the SRTSENETWORK project, the informal network of experts that has been developed continues to operate, sharing and creating knowledge.

Additional benefits

The Concerted Action has resulted in many less tangible benefits, the main ones being improved coordination of research efforts throughout the European Research Area, productive collaborative relationships between participants and effective communication within and beyond the EU after the end of the Concerted Action. These were achieved through increased awareness of what all European research groups were doing plus more frequent and productive personal contacts between researchers. The project was also successful in reaching out to other networks, especially Neuroprion, as well as the publication and distribution of the contact directory. Further benefits have included collaborative proposals for new work and sharing knowledge of participants' expertise and resources. The project team was also able to make recommendations for best-practice with regard to establishing effective scientific networks.

Developing a surveillance system

SRTSENETWORK was pre-dated by, and linked with, FAIR5-CT98-07021 (co-ordinated by Mark Rogers, University College Dublin, Ireland), a project which developed a surveillance system that could test for the presence of TSE in European flocks of goats and sheep and herds of cattle. This involved the participation of 14 European countries and was carried out with the aim of restoring consumer confidence in meat and meat products, and helping international trade in meat and livestock.

The project investigated three main areas: epidemiology, immunodiagnosis and clinical signs. During the epidemiological study, different mathematical models were developed to examine different aspects of BSE. Models for investigating scrapie had already been developed by other projects. The clinical signs study produced guidelines which could be used for the training of veterinarians in all aspects of the clinical diagnosis of neurological disease in cattle and sheep. The project also made recommendations for diagnostic tests based on an evaluation of the protocols available at the time.

Full title European network for surveillance and control of TSE in small ruminants: with emphasis on epidemiology, pathology and diagnostic tests

Duration 01/02/2003 – 31/01/2008

EC contribution €1.5 million

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Full title European project to study BSE strain in sheep
Duration 01/01/2003 – 31/12/2007
EC contribution €2.66 million

Introduction

Scrapie, a TSE strain found in sheep, was first recorded over 200 years ago, but is not thought to represent a risk to human health and is not associated with any variant Creutzfeldt-Jakob (vCJD) like disease. The possibility exists, however, that sheep could have been infected with BSE during the cattle epidemic as sheep can be experimentally infected with BSE and were exposed to the same infectious agent as cattle, via infectious meat and bone meal (MBM). Adding the fact that this infection is more widely distributed throughout the animal's tissues than is the case with cattle, the potential health and economic consequences of a BSE epidemic in sheep were judged considerable.

The possibility of BSE in sheep

The BSE IN SHEEP researchers carried out research and developed techniques that allowed them to carry out a reliable risk assessment for the possibility of BSE in sheep, on the basis of which rational control measures may be designed. Scientists investigated the risk of BSE, and other TSE strains, being transmitted to sheep from cattle or cattle products, as well as between infected and healthy sheep. The possibility of transmission between species, and the risk to humans from a BSE strain adapted to sheep, was also assessed. The ability to distinguish BSE from natural scrapie in sheep was also studied. The scientific tools currently available for detecting BSE were also evaluated.

Placentae from scrapie affected sheep represent a significant source of contamination in the environment, and hence a potential mechanism for horizontal transmission of the disease within flocks. This project investigated whether a control strategy which relies on the introduction of scrapie resistant alleles as part of a controlled breeding strategy could reduce the level of infectivity in placentae and hence reduce this horizontal transmission route. By selectively mating resistant and susceptible ewes with resistant and susceptible rams, a combination of resistant and susceptible lambs could be produced, with corresponding placentae. In some cases, a single ewe carried both resistant and susceptible lambs at the same time. In the cases where ewes were scrapie infected, only the placentae of susceptible lambs were found to be infectious. This implies that a breeding strategy based on the introduction of resistant alleles into a population can reduce the amount of environmental contamination and hence disease transmission due to placentae.

The researchers were also interested in determining the minimum oral infectious dose of BSE for sheep, which was found to be lower than 0.05 grams of BSE cattle brain for the genetically susceptible sheep being studied. This information can now be used to help calculate the contamination risk of sheep exposed to the infectious agent. The results for animals not genetically predisposed to the disease have indicated very low rates of infection. It is important to note that the hypothetical 'normal' route of infection would not be, as in the laboratory, through ingesting BSE infected cattle brain, but through the less effective route of environmental contamination. Historical infection would, if occurring, have taken place via the oral route through contaminated feed.

The problem of false positives

The project findings showed some transmission of BSE between adult ewes (i.e. those more than a year old) when infected animals were mingled with uninfected animals in a non-infectious environment (one in which infection could not derive from environmental contamination). This indicated the potential for additional within-flock transmission. Experiments were also carried out on uninfected ewes being housed with their contaminated lambs, over a three-month period. Some of the adult animals developed clinical signs of BSE, also indicating a horizontal transmission in adult ewes, more than 12-months old, placed in a contaminated environment.

Comparisons were made between sheep contaminated with BSE and those contaminated with scrapie, which indicated that the clinical signature and PrPsc distribution profile in brain may not yet be sufficiently refined to discriminate BSE in sheep from classical scrapie.

When the brains of infected animals were studied, scientists were unable to discriminate between sheep that had suffered from BSE and those that had suffered from classical scrapie. These results mean that some cases of classical scrapie could be incorrectly identified as potential cases of BSE, which causes problems, as it results in the production of false positive results when flocks are tested for BSE. This is of major importance, since the identification of BSE in a greater number of sheep than previously thought will have far graver consequences than for scrapie. This needs to be taken into consideration by those authorities responsible for reporting BSE cases in sheep, and taking decisions regarding flocks suspected to be infectious.

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Full title Studies on the alimentary pathogenesis of BSE agent and natural scrapie in sheep and mice. Implications for diagnosis and control

Duration 01/12/2001 – 01/12/2006

EC contribution €1.37 million

Introduction

Transmissible spongiform encephalopathies (TSEs) or prion diseases are considered a threat to food safety and to the health of both animals and humans. It is crucial to diagnose and detect these fatal diseases as early as possible if they are to be effectively controlled. The misfolded proteins of prion disease are infectious, and their appearance in the food chain has had serious consequences for both the European economy and society. The main pool of prion disease within the EU is that of scrapie in sheep, which takes many forms depending on the strain of the infectious agent and the genetic makeup of the host. Whilst not demonstrably infectious to human beings, scrapie requires study both in its own right as a livestock disease, and also as a cousin of BSE. The SC-GUT project has addressed the key issues related to the development of Scrapie and potential BSE in sheep, and developed tools for their early detection.

Protecting Europe's flocks

The SC-GUT consortium has employed a range of new experimental techniques, which have resulted in a better understanding of the early development of scrapie and potential BSE in sheep. Researchers are studying scrapie, because whilst it has never been associated with disease in human beings, its presence may obscure other diseases such as BSE in sheep if present. The relationship between the abnormal forms of scrapie, BSE and variant Creutzfeldt-Jakob disease (vCJD) is still not clear. Knowledge of the biological processes responsible for the different forms will help in the development of detection techniques for the diseases in their early stages, protect the safety of our food and improve the welfare of animals. Because of a possible link to BSE, the identification and eradication of TSE from European sheep flocks has become a top priority for the EU.

The work of SC-GUT has focussed on the uptake and accumulation of the prion protein (PrP) and infectivity in gut-associated and peripheral lymphoid tissue, and its role in the infection of nerve endings. Mammalian gut contains large collections of lymphoid nodules known as Peyer's patches, and this gut-associated lymphoid tissue is an important site of entry and consequent accumulation for the scrapie agent, as a result of infection in the sheep by the oral route. The transport of PrP across the lining of the gut and the effect of disease-related PrP on white blood cells, immune cells and nerves was studied in novel systems. These included isolated intestinal loops, surgical micro-infections and transgenic mice.

Surgical inoculation would be expected to be a more efficient route for infection compared to oral inoculation; however, the reverse has been true in these experiments. This could be due to the existence of a specific route of infection after oral inoculation that targets infectivity directly to the enteric nervous system (ENS), the nervous system of the gastrointestinal system, without the need for build-up of infectivity in the Peyer's patches. Such a route could involve direct infection of the ENS after oral inoculation that would not be infected in the case of surgical inoculation into the gut. In the latter case, infectivity is likely to be deposited in the muscle layers.

Prion Theory Questioned

The infectious agent responsible for Scrapie, BSE and vCJD is generally considered to be rogue prion proteins. Although the presence of abnormally formed prions is a feature of these diseases, the possibility remains that they are not the initial infectious agent. This hypothesis is the result of research by the SC-GUT project into how these proteins are absorbed by the guts of sheep. Sheep intestines were inoculated with brain extract containing the deformed prion protein (PrP). To the researchers' surprise, the inoculated material was only detected in the wall of the gut for a brief period and found absorbed at sites different from where prion proteins generated by the disease itself, first accumulated one month after inoculation.

This latest discovery does not necessarily dismiss the fact that prion protein, if absorbed in large

enough quantities, may be able to cause the disease, or that the prions infect the nervous system in some other way. However, the SC-Gut researchers raise another, interesting possibility, namely that the prion theory may be incorrect in explaining TSE infection. With this thought in mind, it is clear that further research into the subject is necessary.

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Full title Improvement of goat TSE discriminative diagnosis and susceptibility based assessment of BSE infectivity in goat milk and meat

Duration 01/12/2006 – 30/11/2010

EC contribution €3.80 million

Following the implementation of active surveillance for TSEs in goats in 2002, the confirmation of the first Bovine Spongiform Encephalopathy (BSE) case in a goat in 2005 gives cause for concern. Goat BSE aims to provide sound scientific information to assess the risk of human exposure to BSE via goat milk, meat and products.

Early detection in goats

The members of this consortium bring to the GoatBSE project their wide experience in small ruminant prion research. The project intends as an overall objective to generate data and materials allowing the quantitative assessment of the risk of human exposure to the BSE agent via goats.

Measures to control BSE (in addition to the prohibition of the feeding of processed animal proteins to farmed animals, upper age limits for animals entering the food chain and testing) include the removal of so called 'specified risk materials' (SRM). These are tissues that have been demonstrated to contain infectivity, such as the spinal cord. Unlike BSE in cattle, where most infectivity is found only within the Central Nervous System (CNS), TSE-infected sheep and goats have a more widespread tissue distribution of infectivity. The aim of these experiments is to determine the tissue distribution of BSE infectivity in goats.

Specified risk materials for cattle and sheep were defined according to a similar set of experiments to those which are proposed in the GoatBSE project. Animals were experimentally infected with BSE and the infectivity of their various tissues measured over time. In this way, a set of rules were established by which any risk which had not been eliminated by the food ban, by age limits and by testing would be further reduced through the removal of those tissues most susceptible to containing infectivity.

The project also aims to investigate the influence of PrP (Prion Protein) genotype on the susceptibility of goats to Transmissible Spongiform Encephalopathies (TSE), particularly BSE. Research into PrP genetics in sheep has revealed their fundamental importance to understanding TSE in sheep. Given the similarities between goat and sheep species it is reasonable to assume that PrP genotype may be equally important in determining goat susceptibility to TSE.

This will entail studies in naturally scrapie-infected herds, and the experimental inoculation of animals with different PrP genotypes with TSE inocula derived from animals with different genotypes. Work will also be carried out on transmissibility and species barriers in different goat populations, and transgenic mice (these are mice in which the normal mouse PrP gene has been replaced with the corresponding goat PrP gene).

The determination of strain types and the geographical mapping of prions in goats, based on the collection of TSE goat cases in Europe, contribute to the assessment of current standard diagnostic methods.

Optimised strain detection and differentiation are also expected results. The possibility of BSE self-maintenance in goat herds through maternal and horizontal transmission, and of new breeding programmes to improve prevention and control strategies for TSE in goats, is to be evaluated as well. With this knowledge, genetic breeding programmes can be planned for the prevention and eradication of TSEs in goats similar to the breeding programmes currently performed for sheep scrapie. Researchers will document European field TSE strain variability in goats by recruiting a large sample of TSE goat isolates from those European countries that have been affected.

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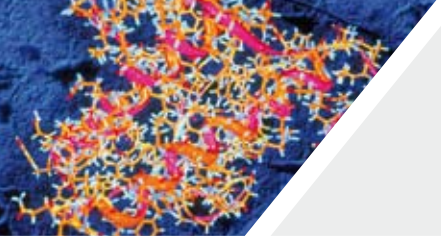

Full title Evaluation of the possible transmission of prions (scrapie and bse) to different fish species

Duration 01/12/2002 – 30/11/2006

EC contribution €1.30 million

Introduction

Transmissible Spongiform Encephalopathies (TSEs) have been detected in sheep (scrapie), cows (Bovine Spongiform Encephalopathy or BSE) and humans (Creutzfeldt-Jakob Disease or CJD). Whilst the origin of the first case in the cattle epidemic of the 1980s and 90s is not known, it is certain that the practice of feeding ruminants with foodstuffs derived in part from processed ruminant offal (meat and bone meal) amplified infection and caused the epidemic. The theory is that exposure to infectivity from scrapie, or from a sporadic BSE case caused BSE in one or more cattle. It is widely known that the subsequent intraspecies recycling of this infectivity caused the BSE epidemic with tragic consequences for human health.

Protecting the food chain

Having established that the disease could be transmitted across species, it became imperative that safeguards were put in place to prevent TSE from further contaminating our food supply. A total EU wide suspension on the use of processed animal protein in feeds for any animals farmed for the production of food, has been in place since 1 January 2001 (in addition to other restrictions previously in place since the emergence of the disease). Processed animal proteins had, however, been used as a source of protein in the rapidly growing aquaculture industry during the early stages of the BSE epidemic. Moreover, little was known about prions in fish.

With this in mind, the consortium assembled a team of experts in marine science, genetics, molecular biology, etc. to address the problem. They embarked on an ambitious programme of research having secured funding from the Quality of Life Programme under FP5.

Fish farmers, like their animal husbandry counterparts, must provide their stocks with nutritious feed comprising up to 50% protein in order to encourage vigorous growth. Animal offal is an inexpensive, protein-rich alternative to more labour-intensive forms of fish feed. The question arises whether fish, whose digestive and other biological systems vary from those of mammals like sheep and cows, could develop TSE after having ingested feed contaminated with mammalian TSE (BSE or scrapie) and whether this TSE could then be transmitted to humans who consumed these fish.

Effects of BSE and scrapie in Fish

The first part of the investigation focused on learning how fish would respond when exposed to BSE and scrapie. The study concentrated on species upon which Europe's dynamic aquaculture industry is based, including sea bass (*Dicentrarchus labrax*), sea bream (*Sparus aurata*) and trout (*Oncorhynchus mykiss*). These species were fed BSE and scrapie samples at regular intervals and observed for several months afterwards for signs of disease. In addition, tissues from the TSE-challenged fish and control groups were subjected to a battery of tests, including immunohistochemical analyses, Western blot, Transmission Electron Microscopy and others, with the goal of detecting Prion-related Protein (PrP) in either mammalian or fish form.

INA-CERTH and its partners discovered that sea bass and sea bream do not appear to be susceptible to TSE transmission from feed contaminated with BSE or scrapie. On-going research has targeted eliminating any possible effects of incubation time by re-dosing the same fish with additional mammalian TSE.

The result, however, was not the same for trout. Nearly all the trout died after being exposed to scrapie, although this result could possibly be due to the fact that trout in general do not respond well to being maintained in captivity and it should not be concluded that the deaths were as a result being dosed with a TSE.

Scientists with the University of Milan decided to dig a little deeper to examine how the TSE could be absorbed during ingestion. Using both in vivo and in vitro experimental set-ups, they are investigating the degree to which scrapie PrP (PrPSc) can successfully cross the fish's intestinal barrier intact.

Studies using mice models

While some fish species appear to be resistant to mammalian forms of TSE, the research group

wanted to be certain that residual BSE or scrapie residing in the tissues of the fish could not infect human consumers further down the food chain. The Consejo Superior de Investigaciones Cientificas (CSIC) inoculated sufficient mice with different tissues obtained from TSE-challenged and control groups of different types of fish. Analyses of tissue samples indicated that some residual infectivity is observed in trout intestine taken one day after oral infection. Some recipient mice inoculated with brain and spleen of turbot resulted positive for scrapie.

Trawling fish genomes for prions

Prion diseases like TSE are known to have a strong genetic component, so to complete the picture of the potential TSE threat from fish, it was necessary to scour the fish genome for sequences encoding prion-like proteins. Similar work on the human and bovine genomes has been carried out, but this marked the first dedicated effort to explore the fish genome. The challenge was taken up by experts at the Norwegian School of Veterinary Science. They started with a fish extremely popular with consumers not just in Europe but worldwide - the Atlantic salmon (Salmo salar). Characterisation of specific segments of the salmon genome revealed a remarkable amount of similarity between its PrP encoding and that of humans. This work was followed up by the University of Milan and TSE and fish project collaborators in Konstanz, using genetic techniques, such as Polymerase Chain Reaction (PCR), to examine specific candidate genes. PCR analysis of carp (*Cyprinus carpio*) and zebrafish (*Danio rerio*) showed that these genes are expressed in brain and other important tissues which may allow the production of antibodies and the identification of markers for neuropathological disorders.

A bright future

Europe has a responsibility to ensure that the food provided to consumers is safe. Properly characterising the risk, if any, posed by historical exposure of fish to TSE will not only protect public health but also support the European aquaculture industry, which is currently valued at several billion euros and makes a significant contribution to job creation, particularly seasonal employment. The work undertaken by INA-CERTH and its colleagues marks an important first step in understanding the interaction between TSE and fish. Armed with this new knowledge, policy makers and other industrial stakeholders will be able to act to ensure the safety of the European aquaculture product and to allow continued growth of the industry.

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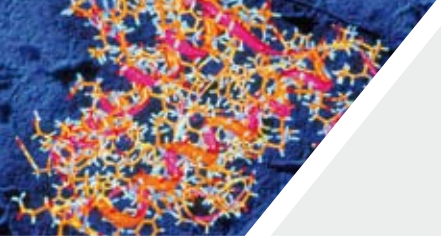

Full title BSE transmission through food and blood products: a study in primates to assess the risk for humans

Duration 01/12/2002 – 30/11/2008

EC contribution €3.93 million

Introduction

The uncertainty regarding the extent of human exposure to BSE, which can lead to variant Creutzfeldt-Jakob disease (vCJD), has been compounded by gaps in our knowledge regarding the efficiency of oral infection and the scale of any cattle-to-human biological barrier to transmission. The BSE TRANSMISSION PRIMATES project was initiated to investigate the oral and blood transmission routes of BSE to the closest available, and hence most accurate, non-human primate species: the cynomolgus macaque (*Macaca fascicularis*).

Determining the minimal infectious dose

Prior to the implementation of fully effective control measures, it was possible that meat contaminated with BSE made its way into food for human consumption. Combining estimates of the total amount of this infectious material with a knowledge of the amount of infectious material required to cause disease in an individual will allow more accurate assessments of the likely number of vCJD cases.

Furthermore, a risk exists that individuals who may have consumed infectious material may also have donated blood, which may in itself represent a further infectious risk. Estimating the risks posed by food and blood transmission is clearly important, but also very difficult.

Because of the importance of these estimates for public health, the decision was taken to use a primate model for this research. Projects involving primates undergo not only the usual scrutiny of peer review but must also undergo a second, ethical review ensuring that participants respect international conventions and declarations, in accordance with the Amsterdam protocol on animal protection and welfare.

The characterisation of BSE material by identifying the particular properties of the disease in other animal models, such as genetically altered mice, is widely used. However, it does not perfectly reflect the natural situation of human exposure to contaminated food. Characterisation of the human vCJD agent in the macaque model is of high significance with regard to risk assessment. The main objective of the study was to determine the minimal infectious dose of BSE contaminated food following oral application. This would allow others to make a more refined prediction of possible future cases of vCJD in humans.

A further objective of the project was the collection of blood and blood product samples from the BSE-infected animals, which was performed during the non-clinical and clinical stages of the trials. This was to ascertain whether the orally induced infection of the animals was transmissible through the transfusion of blood or its products. These quantitative values allow researchers to estimate with greater accuracy the risk of BSE transmission to individuals and the human population as a whole via contaminated food, blood and blood products. The project determined the minimal infective dose of BSE positive cattle brain needed to orally infect non-human primates. The results of the study have provided the basis for estimating the quantitative risk of BSE-transmission to humans by way of contaminated food products.

Carrying out the inoculations

A BSE brain homogenate was prepared that was infectious for cynomolgus macaques after inoculation within the brain. Of the 30 dosed animals 12 went on to develop neurological signs of disease. Diagnosis of a simian vCJD was confirmed in 11 of the 12 cases after death using the techniques of histopathology, immunohistology, Western immunoblot, and PET-blot. Distribution of the pathogenic form of the prion protein (PrPSc) in the brains of infected monkeys demonstrated a vCJD-like disease pattern. Inoculation with 50 mg of BSE brain homogenate resulted in simian vCJD, on average, 985 days after infection and 1837 days following inoculation in animals with a 5 mg dose. Animals that received 0.05 mg and 0.005 mg have not shown any abnormalities. A parallel experiment was carried out with 30 macaques being given an oral dose of the same BSE stock ma-

terial. Animals receiving 16 g of the BSE material had not shown any signs of the disease 1337 days after receiving the dose. A French pilot study gave two macaques a 5g oral dose of homogenised brain from a BSE-infected cow. One of the monkeys developed a vCJD-like neurological disease 5 years after exposure while the other remained free of disease 6 years later.

Determining the safety of blood and blood products

As a result of these findings and results from other studies a preliminary estimate was made regarding the food exposure to humans. This has given added assurance that current public health procedures can prevent the transmission of BSE to man. The BSE TRANSMISSION PRIMATES project also estimated the risk of vCJD infection via blood transfusion from carriers of the disease that did not demonstrate any symptoms. Anti-coagulated blood was taken from BSE blood donors 1 and 3 years after they had been orally dosed. Under conditions similar to blood transfusions in humans 4 recipient macaques had 15% of their whole blood volume replaced. The presence of infectivity in the monkey's cellular blood component was determined during the early stages of the disease in order to clarify whether infection via human-to-human transmission by contaminated blood or blood products is likely to occur. The consortium found that the incubation period of the disease was significantly extended following a tenfold dilution of the BSE material but still affected 100% of the animals. The increased period of incubation in low-dosed animals is an important finding and the observation period for animals inoculated within the brain has been extended to 2009. The determination of the minimal infectious dose using the BSE stock material will help establish an estimated risk for human BSE infection through contaminated cattle-based products.

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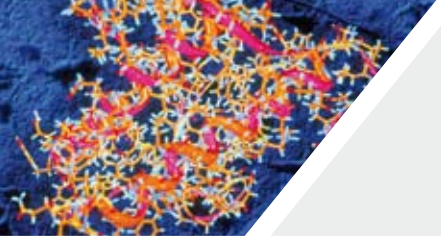


Full title Biotic and abiotic mechanisms of TSE infectivity retention and dissemination in soil

Duration 01/01/2003 – 30/04/2006

EC contribution €2 million

Introduction

The main focus of public concern regarding new variant CJD has, up to now, been its direct transmissibility from food chain sources. The goal of the European funded project TSE-SOIL-FATE however, was to research the highly important aspect of soil contamination. Soil may act as a reservoir for TSE proteins and could therefore pass the infective agents through to associated crops and grazing animals. Potential sources of soil contamination are many and varied including agricultural such as bone meal fertiliser, burial of infected carcasses and grazing of infected herds. From industry, there may be effluents from gelatine production and slaughter houses. Means of dispersion and migration of the protein are multiple. The soil, as the beginning of many food chains, is a potent source of the protein through decomposers like bacteria and fungi and plants as producers. Contamination of the water supply may also be a concern.

The project's objective was to ascertain if prions persist in the soil and if they do remain infectious, for how long would they last. Researchers from the TSE-Soil-Fate consortium studied the environmental aspects, including the degrading of contaminants by worms and dissemination by insects. Once these potential risks are understood, informed decisions could be made regarding the most suitable form of disposal for contaminated material. Knowledge of the persistence of prions in the soil environment allows accurate risk assessments to be made regarding the suitability of the burial route for disposal.

Safe Means of Study

Study of an infectious and extremely resilient agent presents inherent problems for the study teams. However, TSE-SOIL-FATE used a non-infectious modified protein molecule that mimics the actual prion in its physico-chemical and biological behaviour. The movement of the protein can be tracked through the soil and food chain using radioactive isotope labelling to trace the molecule. Fluorescence resonance energy transfer (FRET) is a means of molecule identification that relies on the detection of energy released on interaction with another molecule. As such, it was an ideal tool to detect the protein when it bonded to soil particles.

Mobility of prions in soil

Prion mobility will determine how readily the infective agent becomes incorporated into the food chain or enters the water cycle. The teams used lysimeters, which are devices for collecting water from the pore spaces of soils allowing the soluble constituents to be analysed. Two types of soil representative of those from Europe were used, these were a 'free draining' sandy soil, and a clay, because this has notoriously poor drainage qualities and proteins bind very easily onto the soil particle surface.

Mechanisms of adsorption

The means by which prions bind onto soil particles can be an important indicator as to how other environmental factors such as pH, ionic strength, microorganisms and other biotic agents, and sterilisation, may affect this process. Furthermore, when the protein separates or desorbs from the particles, its structure and properties may be changed. Ultimately, this may hold the key to how mobility of the agent can be restricted and then removed from the soil.

The adsorption capacity of clay for prion protein is very high but it decreases with increased acidity. This could be a function of the change in structure of the protein or a result of its change in orientation on the surface. It was found that the retention of the protein by the clay is reliant on one end of the molecule that contains the terminal nitrogen atom. This coincidentally is the part most sensitive to action by enzymes. The fate of prions in soil may well be affected by the action of protease enzymes from, for example, bacteria. With this in mind, a future objective of the team is to combine microbiological studies with those of soil scientists. Heat and the addition of salt (NaCl) were also found to affect the adsorption behaviour of prion protein. Ultimately, when this line of

research is complete, there will be a more complete picture as to how soil properties and structure are able to influence the mobility and structure of prion protein.

Binding to soil organic matter

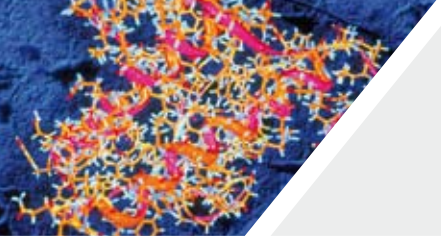
The manner in which prion protein interacts with organic matter and the study of the mechanics of the reactions, in the presence of different molecules that may compete for the adsorption site is vitally important. Catechol is a component of all plants and it forms long chains or polymers, as found in oil and plastic. The way protein is then trapped within the giant molecule is under study together with the effect of different conditions such as pH and concentration. Organic matter is an important component of fertile soil and its presence affects the dynamics of prion protein spread and dissemination. The effect of removal of organic matter and the study of the conformational and biological activity changes upon organic interaction is also an objective of the project. Appropriate research tools utilised include X-ray diffraction (XRD) which identifies the structure of molecules through crystallisation, and infra-red spectroscopy which identifies a molecule by the energy it emits on heating. Also relevant if prion behaviour within soil is to be understood, is cation exhange capacity which is a measure of the ability of different positively charged particles to swap positions on organic matter.

Can micro-organisms break down the prion protein?

In the quest to find an answer as to how to disable this rogue protein that is so potentially destructive, it is logical that researchers should look to natural sources to solve the problem. Natural decomposers in soil possess a range of enzymes that can break down many molecules. Micro-organisms possess growth inhibitors that ward off advancing competitors.
Antibiotics are probably the best example of this phenomenon. A typical brown soil in a pasture in France was studied and encouragingly, it was shown that both fungal and microbial communities can exhibit proteolytic activity in bulk soil.

Earthworms

The role of earthworms in recycling and increasing soil fertility is well-known. Tough organic matter is broken down into the basic nutrients required by plants in digestive systems no longer than a few centimetres. As well as their own array of enzymes, earthworms have a bacterial and fungal flora associated with their digestive system. 16S cDNA analysis allows the classification of thousands of genes in a single experiment and can simultaneously analyse multiple targets labelled with different dyes. The technique of Fluorescent In Situ Hybridisation (FISH) was used to characterise the microbial population of one of the earthworm species involving fluorescently labelled DNA probes to detect the presence



Full title Biotic and abiotic mechanisms of TSE infectivity retention and dissemination in soil

Duration 01/01/2003 – 30/04/2006

EC contribution €2 million

of relevant genes.
In one study alone, three species of earthworm have revealed more than 1 970 strains of bacterial and fungal cultures. An amount of 1 260 were tested for protease activity and of these, 200 were selected for prion protein degradation. These results appear to be promising and research on this line continues. The fate of prions as they pass through the digestive tracts of invertebrates like earthworms has yet to be fully investigated in the search for a biotic agent to degrade prion protein.

Important Implications

The implications of these studies are far-reaching and may enable guidelines to be drawn up to control the potential contamination of water supplies and the food chain from soil by TSEs.

Classification of risk and safety factors will be facilitated and the relative importance of each factor will be assessed while taking into consideration the vast variations in soil, climate, flora and fauna that exist throughout Europe. There will be a sound basis on which the consequences of soil prion contamination for industry and agriculture can be gauged.

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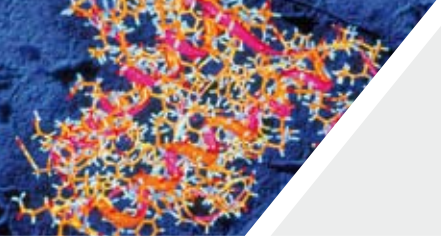

Full title Pre-clinical improvement of combined immunotherapy and chemotherapy for the new variant Creutzfeldt-Jakob disease

Duration 01/10/2002 – 30/09/2005

EC contribution €1.59 million

Introduction

Much has been learned about the proteinaceous infectious particles called prions that cause TSEs. However, little progress has been made in developing an effective treatment to cure the disease in humans. Spurred on by progress in treating cancer tumors with immunotherapy, the Netherlands Cancer Institute (NKI) headed a large-scale research project to investigate the potential of immunotherapy in treating vCJD. Immunotherapy focuses on encouraging the body's own immune system to fight existing infection while at the same time preventing new infection from occurring. The project also aimed to assess the power of combining immunotherapy with more traditional methods of treatment, namely chemotherapy.

Helping the immune system

Prion Proteins are found in healthy humans and animals. By mechanisms not yet completely understood, this cellular PrP (PrPc) is transformed into a pathogenic form, PrPSc which may be responsible for the degradation of the nervous system and death. Unfortunately, because PrPsc is derived from the host's own proteins it is not recognised as a foreign body and the immune system's defences fail to deal with it.
The immunotherapy treatment proposed by the NKI and its partners is based on PrP antibodies. More specifically, anti-PrPc Antigen Binding Fragments (Fabs) are injected into the patient in order to encourage an immune system response. The Fabs are designed to attach to PrPSc and provoke a response from the host's own defences.

Success in the laboratory

With funding of over one and a half million euros from the Quality of Life Programme, the TSE-IMMUNOTHERAPY project consortium began testing anti-PrP Fabs in vivo in mice. First the mice were infected with various forms of TSE, from BSE to scrapie to nvCJD, after which they were treated with the Humanized Mouse PrP antibody D18 Fab (HuM-D18 Fab). Samples of brain tissue were analysed using Western blotting and Electron Microscopy (EM) techniques at first to assess the level of PrPSc infection then later on to determine the extent of HuM-D18 Fab infiltration.

The NKI and its partners hailing from The Netherlands, Sweden, Italy and Israel were heartened by the outcome of their experiments, which showed that HuM-D18 Fab notably slowed the spread of the disease. Statistical analysis of the data revealed that the mice treated with HuM-D18 Fab also lived longer than the control group of TSE-challenged mice. Furthermore, no detrimental side effects of the treatment were observed.
However, the HuM-D18 Fab did not penetrate all regions of the brain, and the areas it did not reach were those most affected by the brain cell degeneration associated with PrPSc. This highlighted the need to devote considerable effort to determining the best method of drug delivery, which entails successfully traversing the blood-brain barrier.

Experimentation with the timing of the Fab injection indicated that a threshold is passed at approximately 70-80 days following prion innoculation, after which the treatment has no impact. Hence, as with most diseases, the earlier treatment is initiated, the better.

Following up on the initial success with HuM-D18 Fab, similar trials were performed with four additional Fabs: D13, R1, R2 and R72. While R72 Fab did not exhibit any therapeutic capacity, R1 Fab and R2 Fab were able to eliminate PrPSc, though not as effectively as D18 Fab. D13 Fab had the least desirable results - killing all the mice in less than ten days, drawing attention to the need for such preliminary research to gauge the neurotoxicity of these agents.

Benefits of Cholesterol

The next phase of the research carried out by the NKI and its international team narrowed the focus to the molecular level to learn how to remove existing PrPSc from cells. Yet again, experience gained in fighting cancer proved insightful as MEK

(Mitogen-Activated Protein Kinase) inhibitors demonstrated their ability to expel PrPSc from prion-infected cells.

Furthermore, the group showed that natural cholesterol can modify PrPc cellular processes so as to inhibit its transformation into lethal PrPSc. In addition, a natural steroid hormone produced from cholesterol, dehydroepiandrosterone (DHEA), was able to significantly delay the onset of scrapie in mice and, consequently, the time to death.

Finally, the study showed that supplementing the body's existing reserves of interferons, proteins produced by the body's immune system to fight viruses, tumors and other foreign bodies, is another potential weapon in the fight against prion disease. Specifically, interferon-gamma (IFN-γ) was able to successfully clear PrPSc from TSE-compromised cells. Similar results were also obtained for quinacrine and brain-derived neurotropic factor (BDNF).

Progressing towards a cure

The length of time from the original infection to the disease appearing may be extensive: it can take years for the symptoms of vCJD to manifest themselves. Since this incubation time varies greatly among individuals, it is not yet known with precision how many cases of vCJD are lying dormant. In addition, it has come to light that the disease can also be transmitted by other means, including blood transfusions, corneal grafts, etc.
This made work of the NKI and its partners all the more pressing. While a definitive cure was not found, significant progress was made towards identifying promising treatment regimes.
In the context of the TSE-IMMUNOTHERAPY project several new lines of research were opened up that will be pursued actively following the project's conclusion. These include therapies based on Fabs, MEK inhibitors, cholesterol, DHEA, interferons, quinacrine and BDNF. Care must also be taken to optimise drug delivery mechanisms in order for the immunotherapeutic and/or chemotherapeutic agent(s) to reach, and thus protect, all parts of the brain against PrPSc.

More information can be found at: http://tse-immunotherapy.vitamib.com.

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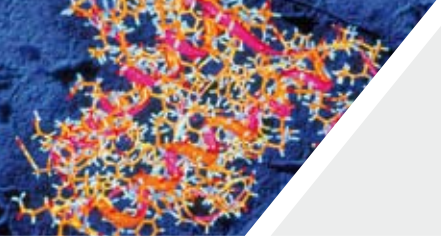

Full title Passage from intestine to brain : assessing the role of dendritic cells in capturing, expanding and disseminating prions

Duration 01/11/2002 – 30/04/2006

EC contribution €1.52 million

Introduction

The ImmunoTSE project involved seven research laboratories from four European countries: Belgium, France, The Netherlands and the UK. The research focused on the possible role of dendritic cells (DCs) in the early development of transmissible spongiform encephalopathies (TSEs). DCs are part of the body's immune system that present fragments of 'foreign' material (antigen) to other cells in the immune system so that the body can mount a defence against the infectious agent. In their precursor (antigen capturing) form, these cells can be found in the periphery of the body, in the skin and on other 'external' surfaces, including the gut.

The investigators hypothesised that if human BSE is acquired from the consumption of infected meat, prion infection may take place orally or elsewhere in the gut. Further, that DCs may play a pivotal role in prion infection – in transmitting, rather than processing – infectious material. Prions may also come into contact with other immune cells known as follicular dendritic cells (FDCs), where they accumulate. FDCs also serve an antigen presenting function. The investigators also looked at other parts of the immune system to see how these components might affect infection with prions.

Microscopic Evidence of the First Step in the Invasion

Using sophisticated immuno-electron microscopy techniques, one of the partners explored the role of DCs in the transport of the rogue protein (PrPSc) across the cells lining the intestinal epithelium (the surface layer of cells lining the gut). Using mouse models prions were observed in gut epithelial cells as early as one day after infection. After crossing the epithelium, they were then seen to congregate in vacuoles, (sac-like structures), within follicular dendritic cells (FDCs). Subsequently, they were found in increasing numbers in the so-called germinal centres in the lymph nodes, where the prion protein replicates. Germinal centres of lymph nodes are structures in which part of the adaptive immune response to infection takes place.

The mechanism by which prions reach the brain from lymphoid organs has not, however, been elucidated precisely but it has been suggested that the capacity of DCs to deliver TSE agents to the nervous system depends on the strain of TSE involved.

Impaired Immune Systems

Indirect evidence as to the importance of DCs in PrP transportation was tested by comparing the progression of scrapie in mice following DC depletion as well as in wild-type mice. The hypothesis was that if DCs are involved in the transportation of the pathogens at least to the gut-associated lymphoid tissues (GALT) then their absence would result in the blocking of the transport mechanism. Early studies did show that DC depletion resulted in a dramatic reduction of PrPSc in GALT, highlighting the role of DC. Furthermore, studies showed prion-infected DCs that are alive can accelerate the onset of disease compared to dead prion-infected DCs.

Along the same lines, mice deficient in complement proteins succumb more slowly to scrapie infection because the propagation of PrPSc to the brain is delayed. Complement is a set of proteins in blood, the normal role of which is to bind to foreign particles (antigens), then act as a link to bind these antigens to cells of the immune system, including phagocytes which ingest and destroy them. The complement protein C1q binds to densely-aggregated prion protein, and activates other complement proteins so that they also bind. It is suggested that complement proteins bind PrPSc, and help it to bind to DCs and FDCs, where PrPSc encounters native (normal) prion protein and causes it to change to form more PrPSc.

The potential role of DC migration in scrapie pathogenesis was further investigated by comparing infection of wild-type and 'plt' mice (the in vivo model used). The 'plt' mouse is a

natural mutant that is deficient in the subset of chemokines responsible for DC migration from the skin or mucosa to the lymphoid system. Chemokines are signalling molecules of the immune system. The research strongly implied that the spread of infection to secondary sites was independent of DC migration. In other words, the absence of DC-migratory mechanisms in 'plt' mice seemed not to affect the spread of prions post-inoculation. The end result was similar to that observed with wild-type mice. Evidently, the role of DCs in pathogenesis requires further investigation to resolve these apparently conflicting findings.

Cell tracking

Two sophisticated methods were used to track DCs and associated prion protein in observations in vivo and in vitro. Flow cytometry is a widely used method for analyzing expression of cell surface and intracellular molecules, characterizing and defining different cell types in heterogeneous cell populations, assessing the purity of isolated subpopulations, and analyzing cell size and volume. It allows simultaneous multiparameter analysis of single cells. Confocal microscopy is an imaging technique used in this project to increase contrast in electron micrographs. It can also be used to reconstruct 3-D images by eliminating out-of-focus light.

In vivo, the position of DCs at the site of prion entry, and in the lymph organs during replication, was recorded. The network of lymph nodes and interfaces between DCs and nerve fibres was observed in scrapie-infected animals together with prion expression in DCs. In vitro, a culture of mouse DCs and neurons together with prion protein was studied, to observe the transmission of protein from dendritic cells to nerve fibres. Results supported the argument in favour of the implication of dendritic cells in prion diseases.

Therapeutic Approach

Although some lines of research in this project shed doubt on the relative importance of dendritic cells in the transport of prion protein, there is considerable evidence that both DCs and FDCs are involved to a large extent in the early development of TSEs. This has exciting implications for efficient therapeutic approaches which could be based on the targeting of both dendritic and follicular dendritic cells, possibly through the complement immune system, aiming to hinder the initial invasion by prion protein.

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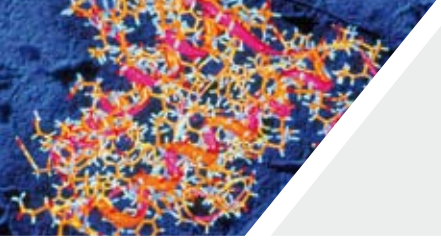

Full title Immunological and structural studies of prion diversity
Duration 01/06/2006 – 31/05/2009
EC contribution €1.96 million

ImmunoPrion is a project at the cutting edge of current scientific knowledge on Transmissible Spongiform Encephalopathies (TSEs). The project is built around three key issues: the strain diversity of TSE agents; the crossing of the species barrier (in other words, the ability of a strain from one species to infect another); and the evaluation of the host innate and acquired immune responses. Understanding strain is fundamental to our understanding of this unique set of diseases. ImmunoPrion is therefore investigating the fundamental features of TSEs, to enable the development of detection and control strategies for prion strains.

The diseases caused by TSEs differ in terms of incubation period, distribution of infectivity and other factors depending on the host species. It may be that vCJD, CJD, BSE, and Scrapie are all 'strains' of disease caused by pathogenic prions. Sheep in particular show multiple variants, or strains, of scrapie. Strain may itself be defined by the interaction of host and infecting prions, the 3D structures of which are determined by their genetic code.

This in turn leads to the concept of the species barrier. This idea suggests that infection may depend on the 'fit' between host and infecting prion. The strain causing BSE has also been shown to infect domestic cats and some species of antelope such as Kudu, which were amongst some of the earliest cases of BSE. Pigs, however, do not appear to develop the disease.

Sudying the diversity of strains

The project's first goal is to provide a better definition of prion strains based on the structural properties of scrapie. Project teams have begun by producing in vitro synthetic variants of prions, because they constitute a reliable and homogenous source, mimicking natural strain diversity, which is important for characterising naturally infectious strains. They will then switch to naturally infectious strains in mice, hamsters and sheep.

The project team members are also developing a new imaging technique in order to achieve high-resolution 3-D characterisation of prion strains. So far it has not been possible to characterise the conformational changes of PrP (prion protein) at high resolution by conventional experimental methods of x-ray crystallography or NMR spectroscopy. All these methods have failed because of the insolubility of the transformed protein. However, by utilising electron crystallography, limited structural information has been obtained for two infectious variants of the prion protein.

The same technique is to be developed for the identification of the 3-D structures of macromolecular prion assemblies in cell organelles of gut follicular dendritic cells (FDCs), following oral contamination with different prion strains from rats and sheep. This will allow researchers to determine the exact cartography of the major strains of scrapie, and will result in a well-standardised map of prion diversity, which will be used to relate the morphological specificities to the pathogenic profile.

Stopping the prion contagion

The mechanisms involved in the species barrier phenomenon have not yet been fully understood The ImmunoPrion project is attempting to acquire a better understanding of the species barrier by carrying out research using rodents, to probe the transmission of TSE agents of animal origin to humans. This model will allow direct testing on prions of potential concern, including BSE, CWD (Chronic Wasting Disease, in deer) and other emerging scrapie strains.

ImmunoPrion also aims to understand how innate immunity and complement (a non-adaptive component of the immune system) mediate interactions between specific strains of prions and the cells of the host immune system. Researchers will investigate markers of acquired immunity in infected hosts in order to establish protocols for the early diagnosis of prion strains.

These scientists believe that a rational food safety policy cannot be attained without a detailed

scientific knowledge of TSE pathogenesis. This is the reason for the importance of mouse models in many of the research activities undertaken within ImmnoPrion. The project also recognises the strong immunological connotations involved in the work. This is in a large part due to the growing appreciation of the key role played by the immune system during the early stages of the disease. It appears that the immune system is an accomplice of the TSE agent during lympho-invasion. However, it seems that it may also act as a highly sensitive alarm and first line of defence (see also ImmunoTSE)

ImmunoPrion includes a partner responsible for organising contact between the project participants and the political, industrial and academic fields, in order to promote the project's goals and participate in the establishment of markers and methods for the detection of prion strains. All European countries aim at the highest possible standards of consumer protection, which include the safety of food products. To this end, the project's objectives are organised around the idea that a rational food safety strategy must prevent, predict and protect. Therefore, the results of this research will have a major impact on the development of improved food safety measures.

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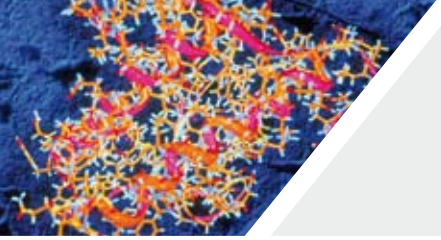

Full title Understanding prion strains and species barriers and devising novel diagnostic approaches

Duration 01/11/2006 – 31/10/2009

EC contribution €2.72 million

The existence of stable strains of prions that differ in their basic structural and clinical properties has been the subject of ongoing research. Furthermore, it is feared that there may be new emerging strains requiring alternative methods of control. Research underway in the project StrainBarrier attempts to tackle these issues. A set of objectives to evaluate and develop methods to identify different existing strains has been drawn up, the overall goal being to be able to classify new strains and predict their behaviour within populations.

Genetics of the species barrier

The species barrier has so far been quite effective in limiting the propagation of the TSE agent between animals. Sheep scrapie has exhibited almost no cross-species transmission. However, the spread of BSE into human and other populations has demonstrated how easily some strains of the agent cross the species barrier. It is thought that this unprecedented spread is controlled by the PrP gene. The StrainBarrier project proposes to elucidate this mechanism, and its effects, by the study of a broad variety of molecular, biochemical, structural and functional characteristics of several prion strains before, and after infection in several types of animal. This will also be done in vitro using the PrP gene from different species. The main question that the project hopes to answer is whether specific TSE strains are more or less dangerous for humans after infection of another species.

Strain Structure

Protein functions are determined by their three dimensional structure. An example of this is an enzyme, which can exert its action when changing its shape. This shape, or structure can studied by treating proteins with substances that alter the interactions between protein subunits: for example, using proteolytic enzymes, different acidic and alkaline environments or heat. This principle is applied in this project and the resulting protein fragments and configurations will be used to develop strain signatures. Also, new microscopy techniques will be used within cells and exosomes (small cell fragments) to study the native structure of pathogenic prions.

Prion pathogenesis may be the result of the infectious PrPSc form possessing a different structure from the constitutively expressed PrP, either preventing PrP from functioning normally or resulting in PrPSc interfering with other cellular processes. However, the exact mechanism is not known at present. Furthermore, it may be that different strains are encoded by different alleles of the prion gene. A better understanding of prion structure will give a better understanding of strains.

Interaction with the Host Cell

The propagation of rogue prion proteins can be observed in cultured host cells and there are many important parameters that influence this process. These include prion receptors, or sites on the host cell where the protein can attach. The discovery and manipulation of these sites may provide an answer to prion infection by disabling the protein and disallowing its entry to cells during its pathway of infection. Movement of the protein will also be a focus of study together with dependence on cholesterol rafts, lipid structures in the cell membrane implicated in cell signalling mechanisms. Once inside the cell, prion protein may be prone to destruction by a battery of enzymes that are housed in special structures, lysosomes. Lysosomes are one of the cell's "housekeepers"; they get rid of old components and even destroy worn-out cells when necessary. The teams also intend to characterise strain-dependent cytopathic effects using cultured cells of sheep, cattle and goats; specifically, cells destined to give rise to neuron cells known as neuronal stem cells.

Designer Cells

Novel tools in terms of cells and molecules that are specially designed are crucial to this project. They include the development of versions of the prion protein with small significant differences (isoforms). Furthermore, it is not

always necessary to study the whole protein. Significant fragments of the molecule produced after enzyme action can be observed. So-called immortalised neurons i.e. modified nerve cells that can bypass senescence and therefore continue to reproduce and grow without restriction, will also be used. Naturally occurring nerve cells (astrocytes) are thought to play a key role in pathogenesis and produce cytokines that are involved in the immune response in the later stages of infection. These will be included in the research as well as special neuronal lines that give rise to various cell types determined by slight variations in the prion protein gene.

Next steps

Improved knowledge of strain properties relating to species barriers will enhance understanding of the effects of the diseases on a population basis and their potential to produce silent or subclinical infections. Promiscuous strains present a public health threat if they propagate through the food chain while remaining either undetected or misidentified. StrainBarrier, through the powerful consortium of partners with proven experimental and industrial achievements, aims to tackle these problems and help to eradicate the pathogen from our food chains and environment.

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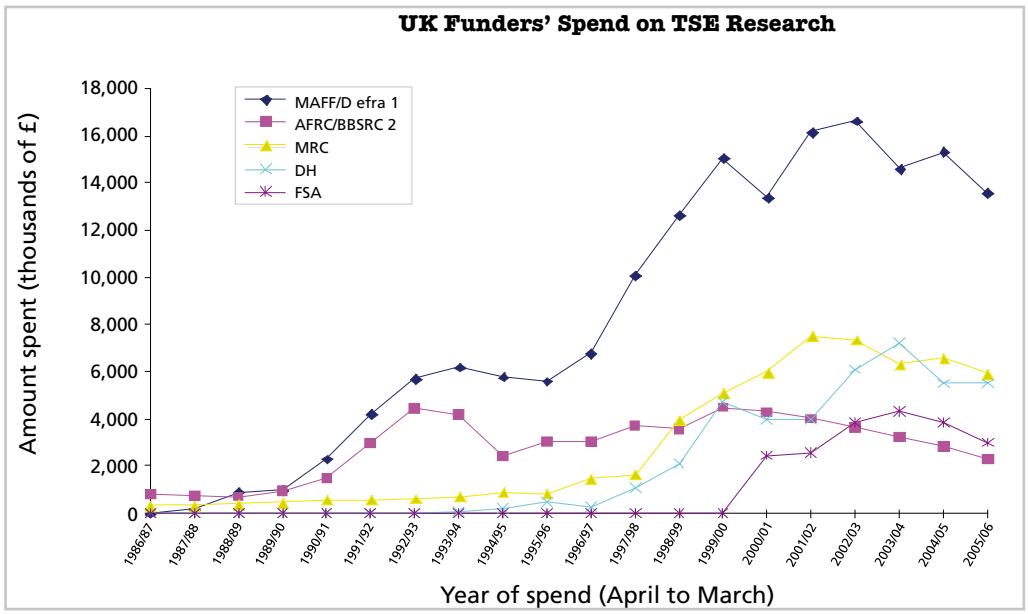
A summary of the public research effort in United Kingdom

The development of Transmissible Spongiform Encephalopathy (TSE) research in the UK

Bovine Spongiform Encephalopathy (BSE) was first recognised as a new disease in UK cattle in 1986. The BSE epidemic reached its peak in 1992 with over 36 000 confirmed cases in that year alone. A new form of Creutzfeldt-Jakob disease (CJD) in humans, called variant CJD (vCJD) was reported in 1996 in the UK, and was quickly shown to be linked to BSE.

Transmissible Spongiform Encephalopathies (TSEs) have been known about for hundreds of years, for example, scrapie in sheep was documented in 1732. Scrapie research has been receiving funding for some time, but in the UK, TSE research was developed in the mid-1980s, and rapidly expanded after the identification of vCJD in the 1990s. This research has been crucial in ensuring that the UK government measures to protect animal and public health from TSEs are based on the latest scientific knowledge. Examples of how research has informed measures that have been effective in controlling the BSE epidemic include the following.

- Findings from epidemiological research led to the introduction of a ban to prevent the feeding of mammalian meat and bone meal to any farmed animal. Since then, the number of BSE cases has continued to decline every year. There were a total of 104 confirmed cases in 2006 in Great Britain, compared to over 36 000 in 1992.



- Studies in which cattle and sheep have been challenged with BSE and in which tissue samples have been collected at various points in the course of disease development, have determined which tissues contain infectivity and should be kept out of the food chain to protect human health.

UK funding of TSE research

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). Some UK TSE research receives funding from other sources such as the Scottish Executive and charities (e.g. the Wellcome Trust).

Up to, and including, the financial year 2005/06, these bodies have spent over EUR 340 million on research into TSEs, as is shown in the figure below.

The UK Government departments and agencies are also able to fund non-UK-based researchers, and this has aided collaborations and links between the UK and the rest of the world. UK researchers are also well represented as participants and coordinators in EC Research Framework Programme projects, and at present, nine UK research institutions are members of the EU-funded network of excellence NeuroPrion.

Funding has also been allocated for the provision of valuable TSE tissues and reagents for research. For instance, the TSE Tissue Archive (http://defraweb/corporate/vla/science/science-tse-arc-intro.htm) has been established to receive, collate and store a supply of animal tissue samples and fluids in support of international TSE research groups.

Coordination of research at the UK level

The TSE funders are members of the TSE research and development (R&D) Funders Coordination Group, which provides a forum to ensure that TSE research is addressing priority issues and is following a coherent strategy. The Funders' activities include holding a biennial workshop attended by researchers from all the UK research groups. The aims of this workshop are to disseminate research findings to the funders, and to encourage collaboration between the different research groups.

UK strategy for TSE research

The UK funds research to improve the understanding of TSEs at several levels, ranging from the biochemical, genetic and cellular aspects of these diseases to epidemiology and public health issues. The current UK research policy aims are outlined in detail in the strategy for research and development on human and animal health aspects of TSEs (available online at http://www.mrc.ac.uk/Utilities/Documentrecord/index.htm?d=MRC002112). In addition, the UK undertakes surveillance of human and animal TSE disease. The UK's research aims are continually reviewed in light of new evidence and emerging findings.

Further information

More information on the research into TSEs supported by the major UK public bodies is available from: http://www.mrc.ac.uk/OurResearch/ResearchPortfolios/TSE/UKTSEPortfolio/index.htm

Further information is available on the four main centres of TSE research in the UK:

- the MRC Prion Unit: www.prion.mrc.ac.uk
- the National CJD Surveillance Unit: http://www.cjd.ed.ac.uk/
- the Roslin Institute: http://www.roslin.ac.uk/research/TSE_index.php
- the Veterinary Laboratories Agency: http://defraweb/corporate/vla/science/science-tse.htm

A summary of the public research effort in France:

Groupement d'intérêt scientifique "Infections à prions" (GIS Prion).

The study of TSEs intensified in France in 2000 when the Ministries of Research, Agriculture and Fisheries, and the State Secretariat for Health founded, in partnership with AFSSA, AFSSAPS, CEA, CNRS, INRA, INSERM, InVS and the Pasteur Institute, a committee of scientific, medical and technical experts to investigate, promote, coordinate and finance research programmes on prion diseases.

How much funding is involved?

With €17.42 million in financial support, the first action of the Group d'Intérêt Scientifique Infection à Prions (GIS PRION) was to finance, or co-finance, a series of French-based infrastructures, including a laboratory designed for the study of infectious biological material requiring containment in Saclay, also used by the NeuroPrion project.

In the last seven years, GIS PRION has seen the finalisation of a number of calls for proposals. To date, €15.35 million have been allocated to 140 projects in various fields. Partners have met extensively to coordinate activities in various thematic networks, including those concerning Materials & Methods, Decontamination, Meat & Bone Meal, and Epidemiology.

GIS PRION will continue to run until December 2008. In addition to coordinating and standardising the actions of research partners, and aiming at the prevention and treatment of infections with prions, GIS PRION determines the prioritisation of funding for prion research in France.

A breakdown of actions

The general framework and structure implemented by GIS PRION can be characterised by four principle priorities:

- **Priority 1:** the mobilisation of scientific teams to conduct new detection tests
- **Priority 2:** the understanding of the nature of infectious agents, and the physiopathology of prion diseases
- **Priority 3:** the development of epidemiologic and therapeutic research on prion diseases
- **Priority 4:** research development on alternative methods of disposal and decontamination

In Priority 1, whilst some diagnostic tests were available, these had certain limitations. More than €5.3 million were allocated for the improvement of BSE research, as well as to find quick and significant diagnostic methods.

More than €10.8 million were allocated for Priority 2. The funding was used to understand: pathology; the development of the disease; and the concept of species barrier. It is crucial that the specificity of the prion disease is understood, that the factors able to prevent the passage of the disease from one species to another are elucidated and that the barrier is reinforced.

With respect to Priority 3, the research involves: investigating the origin of the disease; searching for active molecules that

can inhibit the reproduction of pathological prions; assessing intervention methods on the immune system; and treating the disease itself. Financial support totalled €4.58 million.

Priority 4 relates to the development of novel research, where new research teams are created. Their research can move in one of two directions: develop one or more methods that make the materials biologically and chemically inert to ensure safe storage; or develop methods that allow animal proteins to be chemically transformed or absorbed by microorganisms.

With support from key partners, GIS PRION is instrumental in ensuring knowledge exchange and collaboration with research programmes from the EU and abroad.

Further information can be obtained from:
http://infodoc.inserm.fr/serveur/Prions.nsf

A summary of the public research effort in Germany: TSE Forschung

The origins of TSE research in Germany

Transmissible spongiform encephalopathies (TSEs) are transmissible diseases of the brain that can be found in both humans and animals. They include Creutzfeldt-Jakob disease (CJD), which affects humans and was first discovered and described by the German medical doctors Hans Gerhard Creutzfeldt and Alfons Jakob between 1920 and 1921. Systematic TSE research began in Germany with the setting up of a working group based at the Robert Koch Institute in Berlin. Following the Bovine Spongiform Encephalopathy (BSE) outbreak in cattle in Great Britain, a nationwide research infrastructure was set up at the beginning of the 1990s. In 1994, the Ministry of Education and Research established a German network for TSE, which ran for six years with the last projects finishing in the summer of 2000. During this period, eleven scientific working groups from nine institutions received funding from the Ministry of Education and Research and the Ministries of Health and Agriculture respectively. The money received amounted to 10 million DM (€5.1 million). The ministries provided further funds for TSE research, as did the German Research Council (DFG). This national support enabled a variety of German Research groups to participate in EU-funded consortia under the umbrella of various EU framework programmes.

How is TSE research currently being funded in Germany?

National research funding for TSE has risen substantially following the first appearance of genuine cases of BSE in Germany, with the Federal government currently investing €13.8 million. Three Federal Ministries (Education and Research, Health, and Agriculture, Food and Consumer Protection) have provided funds for the establishment of the German TSE Research Platform and for funding the research groups therein, as well as additional funds for the public research sector including the Friedrich-Loeffler-Institute, Robert Koch Institute and the Paul Ehrlich Institute. Federal and state ministries allowed substantial project funding, as did the European Union, the German Research Council (DFG) and other private funding bodies. At present 7 German research institutions (in total 13 research groups) are members of the EU-funded network of excellence (NoE) 'NeuroPrion'.

The German TSE Research Platform was set up by the Federal Ministry of Research and Education as a science-driven, independent research institution in response to the BSE crisis in Germany. It is a communication platform for all German TSE research groups (currently around 60-70 working groups involving more than 200 scientists) and enables systematic coordinated research in the field as well as facilitating the exchange of research results. Three sample archives for human and animal tissue material were established within the Platform. All promising projects, including those of private industry, are provided free of charge with the required prion-containing samples of human and animal origin. Thus, for the first time, researchers can turn to a central facility to obtain samples not only when located in Germany, but also when based abroad. Consequently, the archives

were, in part, also implemented into the NoE 'NeuroPrion'. Furthermore, the TSE forum plays an important role as the interface between science and society. In informing the public on the current state of scientific knowledge regarding the TSEs, the Platform promotes a rational discourse on prion-induced diseases.

What kind of TSE research is being carried out in Germany?

The German TSE Research Platform is currently supporting studies into seven areas of research. The field of prevention and consumer protection is concerned with minimizing risk and carrying out risk assessment of food and animal feed. This includes the development of highly sensitive tests for detecting the TSE agent in meat, sausages, milk and animal feed products. Other work involves the safety of blood and blood products as well as drugs, and everyday items such as soap and other cosmetics. Studies into the destruction and inactivation of the TSE agent within hospitals and the protection of workers in laboratories, slaughterhouses and animal waste processing units are being undertaken. In the field of diagnostics, accurate and fast practical detection methods are being developed to test for the infectious agent in the blood or urine of living organisms.

Research is being carried out into the possibility of effective therapies for slowing or halting the progress of the disease in people, who may have been infected but who do not yet show any clinical signs. Epidemiological studies are being carried out for human and animal diseases, in order to both monitor and attempt to predict the course of the disease. This includes a clinical and epidemiological investigation into CJD at the universities of Göttingen and Munich. The German pathogenesis study of experimentally infected BSE cattle, based at the Friedrich-Loeffler-Institute on the island of Riems, aims to research the course of infection from ingestion of the infectious agent to the outbreak of the disease. Samples will be gathered from infected, but not yet symptomatic animals, a prerequisite for the development and evaluation of BSE tests, especially live animal tests ('ante-mortem tests').

Basic research is being undertaken to investigate the nature of the causative agent, the prion, the identification and characterisation of its structure and function, as well as the origins and effects of the disease. Molecular determinants of infectivity are identified and new cell culture models will support the study of biogenesis of prions. This important work will allow scientists to gain a greater insight into the TSE agent and how it crosses the species barrier. In addition, susceptibility to the disease as a result of genetic factors is also being investigated.

Last but not least, projects dealing with the protection of the environment have been carried out to study the behavior of the TSE agent in soil, biological waste and mud, particularly with regard to whether the disease can enter the human food chain via contaminated soil.

The German TSE Research Platform was established as an interdisciplinary project, which did not exist in this fashion in Germany, nor, to the best of our knowledge, anywhere else. Six years after its implementation, the project German TSE Research Platform has shown that its special set-up contributed significantly to the unification and effectiveness of German TSE research, e.g. the establishment of stable networks, more and new co-operations in the field, increase of interdisciplinary research and an increase in international cooperation. As a matter of fact, Canada has taken over many elements from the German TSE platform in its present attempts to address the BSE problem there within their Networks of Centres of Excellence 'PrioNet Canada'. The results produced within the framework of the German TSE Research Platform are not only important for the understanding of prion diseases and the progress of prion research worldwide, but have shown to be instrumental regarding basic and applied research on dementia and neurodegenerative diseases in general.

Further information can be obtained from:
http://www.tse-forum.de/tse_forum

List of all projects funded by the Directorate General for Research of the European Commssion in the area of Transmissible Spongiform Encephalopathies, 1996-2008

Project Code	Project Acronym	Project Title	Project Coordinator	Start Date	Duration (years)	Budget (EUR)
Animal Genetics						
FAIR5-CT97-03305	Ovine / Caprine Genetics in Scrapie	Improving prospects for scrapie control in sheep and goats by study of host genotypes, TSE isolates and their in-vivo and in-vitro interaction	Jean-Michel Elsen	1/1/1998	4.5	1,385,000
FAIR5-CT97-03311	MASSES	Analysis of molecular factors affecting variability in BSE and scrapie susceptibility	John Williams	1/1/1998	4.5	1,000,000
FAIR5-CT98-07017		Genome scan for loci controlling scrapie incubation time in mouse and sheep	Jean-Michel Elsen	3/1/1999	4	327,800
Cellular Pathogenesis, Transmission and Nature of the Agent						
BMH4-96-0601	PrP in glial cell activation	Basic mechanism of neurodegeneration in transmissible spongiform encephalopathies: role of amyloidogenic prion protein PrP in glial activation	Jan Langeveld	6/1/1996	3	350,000
BMH4-97-2679	Molecular and experimental neuropathological analysis of prion neurodegradation, strain variation and transmission risks	CJD and BSE: an integrated molecular and experimental neuropathological analysis of prion neurodegradation, strain variation and transmission risks	John Collinge	10/1/1997	3	900,000

Project Code	Project Acronym	Project Title	Project Coordinator	Start Date	Duration (years)	Budget (EUR)
BMH4-98-3265	Cellular pathogenesis	Cellular pathogenesis of prion diseases	Hans Kretzschmar	6/1/1998	4	605,000
BMH4-98-6006	Signal transduction role of normal PrP	Investigation of putative signal transduction processes of normal prion protein and their role in spongiform encephalopathy pathogenesis	Brian Anderton	6/1/1998	3	600,000
FAIR5-CT98-06022		Role of PrP in prion spread and establishment of central nervous system infection	Dominique Dormont	8/1/1998	3.5	1,400,000
BIO4-98-6045	Yeast Prions	Maintenance and transmission of yeast prions: a model system	Michael Tuite	6/1/1998	3	805,000
BMH4-98-6050		The bovine prion protein: from structure analysis to the molecular mechanism of conformational transitions	M Sorgato	6/1/1998	3	900,000
BMH4-98-6051		Structure, function and interactions of prion proteins and prion protein domains	Hans Kretzschmar	7/1/1998	3	1,000,000
BIO4-98-6055	Trafficking Pathways	Trafficking pathways of normal and pathologic isoforms of the prion protein	Sylvain Lehmann	7/1/1998	3	1,200,000
BIO4-98-6060	Cerebellar Network Alterations in disease	Cerebellar networks alterations in prion diseases	Herbert Axelrad	7/1/1998	3	945,000
BMH4-98-6027		The role of plasma membrane structure in prion propagation, transport and pathogenesis	Krister Kristensson	1/1/1999		500,000
Diagnosis						
BMH4-98-6016		Analysis and function of 1433 isoforms. early diagnosisof Creutzfeld-Jakob disease	Alistair Aitken	7/1/1998	4	564,000
BIO4-98-6046		Diagnosis of transmissible spongiform encephalopathies using PrPSc/PrPc specific antibodies	Hans Kretzschmar	7/1/1998	4	1,545,000
BMH4-98-6048		Quantitative analysis of MR scans in Creutzfeldt-Jakob disease	Alan Colchester	11/1/1998	4	890,000

Project Code	Project Acronym	Project Title	Project Coordinator	Start Date	Duration (years)	Budget (EUR)
BMH4-98-7024		Development and control of PrPsc based test in humans and animals using cerebrospinal fluid and brain tissue	Hans Kretzschmar	10/1/1999	3	567,000
BIO4-98-6064		Development of cell culture models of infectious forms of TSE	Herbert Laude	7/1/1998	3.5	694,000
BMH4-98-6003		Transgenic mice expressing human prion protein use for characterisation of human encephalopathies, and sensitivity for detection of infectivity	Jean-Jacques Hauw	1/1/1999	3.5	800,000
FAIR5-CT97-03315		Development of a novel diagnostic to assist quality assurance procedures in European meat production	Heinz Schroeder	1/1/1998	4.5	730,000
FAIR5-CT97-03304		Generation of ovine and bovine PrP transgenic mice for the development of improved bioassays for BSE and scrapie agent detection	Martin Groschup	2/1/1998	5	682,500
BMH4-98-3727		Laboratory supported diagnosis of Creutzfeldt Jakob Disease	Sigrid Poser	3/1/1998	3.5	225,000
FAIR5-CT97-03314		Relationship between conformation of PrP, infectivity and pathogenicity of bovine spongiform encephalopathy (BSE) as a basis for diagnosis	Detlev Riesner	1/1/1998	3.5	1,414,000

Project Code	Project Acronym	Project Title	Project Coordinator	Start Date	Duration (years)	Budget (EUR)
FAIR5-CT97-03306		New approaches to the diagnosis and control of transmissible spongiform encephalopathies	Mark Rogers	4/1/1998	5	1,650,000
FAIR5-CT98-07008	Best test	Establishing the resources for and examination of transcription changes occurring during BSE infection : a route to early diagnosis of infection and a detailed study of early molecular pathology	John Williams	4/1/1999	5	883,000
FAIR5-CT98-07006		Development of a pre-clinical test to differentiate between scrapie and BSE infection in sheep	Lucien Van Keulen	4/1/1999	5	838,730
QLK2-CT-2001-01523	TSE Lab	Human TSEs: The European Diagnostic Laboratory	Herbert Budka	9/1/2002	3	1,734,350
QLK4-CT-2001-01763	Prionmrdiagnostics	In vivo magnetic resonance diagnostic surrogate markers in prion diseases	Bruno Barbiroli	1/1/2003	3	239,820
QLG3-CT-2001-01606	CJD Markers	Early clinical diagnosis of human spongiform encephalopathies by analysis of biological fluids	Inga Zerr	10/1/2002	3	235,200
QLRI-CT-2001-01457	Fiatest	A diagnostic test for abnormal prion protein in live animals based on fluorescent immunoassay using capillary electrophoresis and HPLC	Michele Lees	9/1/2002	3	1,850,720
QLRT-CT-2001-01333	EurovolTSE	Improved bioassays for TSE agents based on the bank vole, a wild rodent species higly susceptible to scrapie and its possible role in scrapie epidemiology	Umberrto Agrimi	10/1/2002	3	800,000
QLK3-CT-2001-02345	FCS Prion typing	Development of a rapid high-throughput assay for sensitive and specific detection and strain typing of Creutzfeldt-Jakob disease based on fluorescence correlation spectroscopy	Armin Giese	9/1/2001	3	699,980

Project Code	Project Acronym	Project Title	Project Coordinator	Start Date	Duration (years)	Budget (EUR)
Epidemiology and Surveillance						
BMH4-CT98-3698	NEUROCJD	Co-ordination of national surveillance programmes for CJD in the European Union	Robert Will	1/04/1998	3.5	535,000
BMH4-97-2034	Prionet	Human transmissible spongiform encephalopathies (prion diseases): neuropathology and phenotypic variation	Herbert Budka	6/1/1997	3	360,000
BMH4-98-6015	Prionet	European centralised facility for human transmissible spongiform encephalopathies (prion diseases).	Professor Herbert Budka	8/1/1998	3	1,120,000
BMH4-98-7022		A study of genetic factors in CJD	Cornelia Van Duijn	1/1/1999	3	229,200
BMH4-96-0856		Clinicopathological features and pathogenesis of fatal familial insomnia	Elio Lugaresi	4/1/1996	3	75,000
FAIR5-CT98-07021		The establishment of a European network for the surveillance of ruminant TSE and the standardisation and harmonisation of the process and criteria for the identification of suspect cases	Mark Rogers	5/1/1999	3.5	2,454,000
QLK2-CT-2000-01709	EUROCJD	CJD: Epidemiology, Risk Factors and Diagnostic Tests	Robert Will	10/1/2000	3	386,000
QLK2-CT-2000-00837	Prionet	'Human Transmissible Spongiform Encephalopathies: The Neuropathology network'	Herbert Budka	10/1/2000	3	400,000
QLK2-CT-2001-02248	NEUROCJD	CJD: Epidemiology, Risk Factors , Diagnostic Tests and Genetics	Robert Will	10/1/2001	4	1,000,000

Project Code	Project Acronym	Project Title	Project Coordinator	Start Date	Duration (years)	Budget (EUR)
QLK2-CT-2001-01441	SEEC-CJD	Surveillance for vCJD in Central and Eastern Europe and China; Risk Assessment, Tranms-mission and Surveil-lance	Robert Will	8/1/2001	3+	800,000
Inactivation						
BIO4-98-6030	Gelatin Process	TSE spiking experi-ments for process validations: evaluation of different sources of infectivity and spiking approaches	Herwig Reichl	7/1/1998	3	405,000
BIO4-98-6065		Inactivation of the causative agents of transmissible spongi-form encephalopathies by thermophilic and hyperthermophilic proteases	Neil Raven	7/1/1998	3	1,000,000
FAIR5-CT98-07015		Low levels of TSE infectivity in blood: Determination of titre and Evaluation of removal	Herwig Reichl	2/1/1999	3	187,000
QLK1-CT2000-00009		Evaluation of inactiva-tion / removal effect of the gelatin manufac-turing process on TSE infectivity	Ad Grobben	2/1/2001	2	277,590
FAIR5-CT98-07019		TSE agent inactivation, product quality evalu-ation and sterilisation process simulation in rendering processes for the production of feed grade animal proteins	Radulf Oberthur	4/1/1999	3	769,660
Pathogenesis Transmission and Nature of the Agent						
QLK5-CT-2001-02332	SC Gut	Studies on the alimen-tary pathogenesis of BSE agent and natural scrapie in sheep and mice	Charles Press	12/1/2001	5	1,370,000
FAIR5-CT97-06013		PrPSc distribution and kinetics in lymphoid tissues of sheep with natural scrapie	Lucien Van Keulen	1/1/1999	5	719,550
QLG3-CT-2001-02353	PrP and Neurode-generation	Molecular basis of neurodegeneration in Transmissible Spongi-form Encephalopathies	Michel Reourna	12/1/2001	3	997,830
QLK4-CT-2001-02493	TSE Soil Fate	Biotic and abiotic mechanisms of TSE in-fectivity retention and dissemination in soil	Herve Quiquampoix	1/1/2003	3	199,260

Project Code	Project Acronym	Project Title	Project Coordinator	Start Date	Duration (years)	Budget (EUR)
QLK4-CT-2001-02723	Fate Pride	Factors affecting the evolution of prion diseases in the environment	K Vala Ragnarsdottir	1/1/2003	3	1,690,000
QLG3-CT-2001-01871	Oxprion	Investigating the role of oxidative stress or diet on prion disease susceptibility	Jean-Yves Cesbron	9/1/2002	3	910,000
QLG3-CT-2001-01030	PrPScNeuropathways	Pathways and mechanisms in the spread of PrPSc to the central nervous system	Walter Schulz-Schaeffer	9/1/2002	3	1,769,438
Risk assessment including TSE in other species and societal aspects						
FAIR5-CT98-07023		Role of environmental and host factors on the horizontal and vertical transmission of scrapie in naturally infected sheep flocks	Lucas Gruner	2/1/1999	4	800,000
BMH4-97-2216		Creutzfeldt-Jakob disease in the European Union- incidence and risk factors	Robert Will	5/1/1997	3	440,000
FAIR5-CT97-03301	REMCORD	Measures to reduce contamination of meat and environment with CNS tissue during slaughter and processing of cattle and sheep	David Harbour	3/1/1998	3	938,500
BMH4-98-6029		Risk assessment in primates of TSE transmission to humans through food and blood products	Gerhard Hunsmann	10/1/1999	2	2,000,000

Project Code	Project Acronym	Project Title	Project Coordinator	Start Date	Duration (years)	Budget (EUR)
FAIR5-CT98-07004	EUCNSRISK	Contamination of meat, and exposure of abattoir workers, by CNS material during standard butchering prevalent in the member states of the European Union	David Harbour	1/1/1999	2.5	888,986
FAIR5-CT97-03308		Separation, identification and characterisation of the normal and abnormal isoforms of prion protein from normal and experimentally infected fish	Liana Bolis	3/1/1998	4	1,000,000
BMH4-98-7026		Infectivity of blood components in experimental nvCJD: towards a risk assessment for human blood	Gerald Eder	5/1/1999	3	1,155,000
BMH4-98-6057		Building a common database on scientific research and public decision on TSE in Europe	Pierre Benoit Joly	7/1/1998	3	547,000
BMH4-98-7028		Public perceptions of BSE and CJD risk in Europe, their interplay with media, policy initiatives and surveillance issues. Drawing the lessons for information policy.	Carlos Dora	7/1/1999	3	879,800
QLK2-2002-30531	Safestun	Review of current stunning methods for cattle and sheep that avoid the risk of dissemination of brain particles into the blood and carcass, and maintain good welfare and operator safety	Andy Knight	12/1/2002	0.5	140,000
QLK1-CT-2002-01096	BSE Primates	BSE Transmission Through Food and Blood Products: A Study in Primates to Assess the Risk for Humans	G Hunsmann	12/1/2002	5	3,930,000
QLK5-CT-2001-00866	TSE in fish	Evaluation of the posisble transmission of prions (scrapie and BSE) to different fish speciesl	Theodoros Sklaviadis	12/1/2002	4	1,300,000
QLG3-CT-2001-01223	EurosurgyCJD	Surgery and the risk of CJD	Jesus de Pedro Cuesta	9/1/2002	3	856,590

Project Code	Project Acronym	Project Title	Project Coordinator	Start Date	Duration (years)	Budget (EUR)
Sample Banks						
FAIR5-CT97-06056		Concerted action for the settling up of multicentric epidemiological databases and biological samples banks for small ruminant scrapie	Frederic Lantier	7/1/1998	4	600,000
Small Ruminants						
QLK5-CT-2001-01309	BSE in sheep	European project to study BSE strain in sheep	O Andreoletti	1/1/2003	5	2,660,000
QLK5-CT-2001-00959	SRTSE-Network	European network for surveillance and control of TSE in Small Ruminants (with emphasis on epidemiology, pathology and diagnostic tests)	Kumar Sivam	10/1/2002	4	1,520,000
QLK5-CT-2001-01733	Scrapies-freesheep	Monitoring the effect of scrapie control policies that use genetics in different countries	Francis Barillet	12/31/2002	4	1,110,000
Therapeutics						
BMH4-98-6040		Prion diseases: mechanisms of transmission and identification of targets for potential therapeutics	John Collinge	8/1/1998	3	1,500,000
BMH4-98-6011		In vitro investigation of PrP induced neurodegeneration: Development of a system for testing potential therapeutic agents	Alun Williams	7/1/1998	3	1,100,000
FAIR5-CT98-07020		PrP dimerization and oligomerization as a model for the evaluation of TSE transmission modalities and as a target for a therapeutic intervention against TSEs	Stefan Weiss	3/1/1999	3.5	1,285,814

Project Code	Project Acronym	Project Title	Project Coordinator	Start Date	Duration (years)	Budget (EUR)
BMH4-98-6054		Development of TSE therapies based on prion protein binding oligosaccharides	Ruth Gabizon	8/1/1998	3	1,000,000
QLK2-2003-30584	TSE Therapeutics	New perspectives for Prion therapeutics (Paris conference 1-3 December 2002)	Sylvain Lehmann	9/1/2002	1	85,000
QLK2-CT-2001-02085	HMs as antiprion drugs	Heparin mimetics as anti-prion drugs	Ruth Gabizon	10/1/2001	3	1,500,000
QLK2-CT-2001-01924	EU Prions	Strategies for the prevention and treatment of prion disease	John Collinge	11/1/2001	4	2,340,000
QLK2-CT-2001-01628	TSE Immunotherapy	Clinical improvement of combined immunotherapy and chemotherapy for vCJD	Peter Peters	10/1/2002	3	1,590,053
QLK2-CT-2001-01399	Priovax	Vaccination against prion disease	Ulrich Kalinke	9/1/2002	3	1,662,690
QLK5-CT-2001-01044	ImmunoTSE	Passage from intestine to brain: assessing the role of dendritic cells in capturing, expanding and disseminating prions	Pierre Aucouturier	11/1/2002	3	1,522,760
Other						
QLG1-CT-2001-30045	International TSE conference	International Conference on TSEs	Jan Fraser	1/1/2002	1	69,780
Framework Programme 6						
Food CT 04 506579	NeuroPrion	Prevention, control and management of prion diseases	Jean-Philippe Deslys	9/1/2003	5	14,400,000
Food CT 06 023183	Strainbarrier	Understanding prion strains and species barriers and devising novel diagnostic approaches	Albert Taraboulos	11/1/2006	3	917,360
Food CT05 36353	GoatBSE	Proposal for improvement of goat TSE discriminative diagnosis and susceptibility based assessment of BSE infectivity in goat milk and meat.	Alex Bossers	12/1/2006	4	3,850,000
Food CT07 023144	Immunoprion	Immunological and structural studies of prion diversity	Patrice Marche	6/1/2006	3	1,960,000
TOTAL EC Contribution						102,164,961

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TRANSMISSIBLE SPONGIFORM ENCEPHALOPATHIES - the European Union's Research Response to a Major Public and Animal Health Challenge

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This booklet highlights the research response of the European Commission to the spectrum of diseases known as the Transmissible Spongiform Encephalopathies (TSEs), which include BSE (primarily a disease of cattle), scrapie in sheep and goats (small ruminants) and variant and classical or sporadic CJD in man. It also provides information on three of the larger research programmes carried out in Member States of the European Union. The booklet focuses on key projects funded by the Directorate-General for Research from the mid 1990s to the present day, and in doing so, aims to give an overview of the important work carried out in the past, as well as of current research priorities.

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