ABOUT THE BOOK

This book is an outcome of the efforts of Vigyan Prasar to reach out to common people and spread the message of science in a popular but correct language. This book is unique and first of its kind on a topical subject of current international interest and continuing and expanding debate. "The mad cow disease" has sparked off serious panic and extensive media blitz on a perceived future threat of an extent probably next only to the AIDS and Ebola virus scares during this century. Apart from many scientific articles published, the Press and Internet are full of information and comments on the mad cow disease. These range from authentic and scientific to bizzare and untrue. This book could be the first attempt to collate all essential and correct information in an organised overview of the mad cow disease and the prevailing scenario. The book describes the history and genesis of the mad-cow disease the science of this disease and what is known. It then describes similar diseases that occur in humans and other animals. In the fourth chapter the current concept of infective protein particles or "prions", supposed to be the causative agent is discussed. The book also covers the socio- economic aspects of the mad cow scare and tries to enlist answers to some common questions, that may come to everybody's mind. To enhance the usefulness of the book, it also includes a glossary and an extensive bibliography. In essence, the book tries to convey all that is known about this disease and its nature, in a correct and conceptually scientific framework. Nevertheless, this book is not meant to be the last work on the mad cow disease but is only a humble attempt to organise most of the information currently available and present the same in an easy-to-understand language.

MAD, MAD, MAD COW

MAD, MAD, MAD COW

An Overview of the Mad Cow Disease

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Contents

List of Abbreviations

APHIS	Animal and Plant Health Inspection Service
BSE	Bovine Spongiform Encephalopathy
CJD	Creutzfeldt-Jacob Disease
CNS	Central Nervous System
CFS	Cerebrospinal Fluid
CVL	Central Veterinery Laboratory
DNA	Deoxyribonucleic acid
EEC	Electroencephalogram
EU	European Union
FFI	Fatal Familial Insomnia
FSE	Feline Spongiform Encephalopathy
HPLC	High Pressure Liquid Chromatography
IU	Infective Unit
MAFF	Ministry of Agriculture, Fisheries and Food
MBM	Meat-and-Bone Meal
RNA	Ribonucleic acid
SAF	Scrapie Associated Fibrils
SBO	Specified Bovine Offal
SDS	Sodium dodecyl sulphate
SEAC	Spongiform Encephalopathy Advisory Committee
SEs	Spongiform Encephalopathies
TME	Transmissible Mink Encephalopathy
TSE	Transmissible Spongiform Encephalopathy
USAD	United States Agriculture Department
ZSE	Zoonotic Spongiform Encephalopathy

PREFACE

Vigyan Prasar, as part of its continuing efforts, towards promoting a rational outlook and stimulating a scientific temperament in society, brings out communication materials like publications, video films, audio-cassettes, slide-sets, posters/ charts etc., on various aspects of science and technology. Particular emphasis is laid on natural phenomena (like eclipses, comets, etc.)and subjects/issues of common interest which have a direct bearing on day-to-day life of human beings. Often such natural phenomena or issues get entangled with myths, legends or unfounded beliefs. Vigyan Prasar's efforts to deal with such matters and other activities are briefly described in the section "Introducing Vigyan Prasar"

When the bovine spongiform encephalopathy (BSE), a strange brain disorder, commonly known as the mad cow disease, sparked off serious concerns and extensive media coverage it was thought to be a fit case for being taken up by Vigyan Prasar. Hence this volume! The possibility that there might be a link between a new variant of the Cruetzfeldt-Jacob disease (CJD) — a brain disorder found in humans — and BSE created a real scare particularly in beef-eating countries. A causal link between CJD and BSE is yet to be established. However, the future threat posed by the mad cow disease was perceived next only to the AIDS and

Ebola virus scares during this century. The onset of the BSE also posed a real challenge to the scientists, i.e. that of identifying the agent which causes this disease. The bibliography given at the end gives some idea of the intensive research that has gone into this strange disease. Now we know that this disease, like other transmissible spongiform encephalopathies is transmitted by an infectious proteinous particle called prion.

Though the disease did not strike in India, people still became concerned mainly for two reasons. First that the disease might eventually spread throughout the world as it was transmissible i.e. it was possible to spread the disease to other animals by exposing them to tissue from an affected animal. Second, the decision of the British Government to eliminate the affected cattle — mostly, cows — by incineration. This is because cow occupies a special place in the Indian psyche and it is worshiped in many parts of the country.

Initially we started putting the available information on our Homepage on the Internet. Soon a large body of information emerged on the probable causes of the disease and its consequences on human health and economy.

The information on the disease ranged from authentic and scientific to bizarre and at times totally unfounded. Given this background it became imperative on the part of Vigyan Prasar to convey what is known and what is not known about this disease and its implications, in a conceptual and scientifically correct frame-work. Accordingly we requested Professors Kunal B. Roy and

Santosh K. Kar of the Centre of Biotechnology at the Jawaharlal Nehru University, in New Delhi, to take up this task. They have organised most of the information available on the disease and analysed it in the context of modern day scientific knowledge. The section 'Common questions' may appear to be a little repetitive but it has been done with a specific purpose i.e. to enable even a lay person to get an idea of the nature of the disease and its implications without having to delve through all the scientific details.

The book would have attracted a lot more attention had it been brought out in the midst of the raging debate on this topic. But we decided not to sacrifice thoroughness at the altar of opportunity. The mad cow disease is no longer an issue as hot as it once was for the media. But it is far from being dead and gone. We feel that the book is still timely and will serve its envisaged purpose. Many people still do not have a clear picture. The curiosity still remains, and to our knowledge this would be the first book on the mad cow disease written by professional scientists. The 1997 Nobel Prize awarded to Stanley Prusiner fo r characterisation of the BSE agent, prion, will also create renewed interest in the mad cow disease.

A CD-ROM has also been prepared based on the contents of the books with added sounds, visuals and animation.

Narender K. Sehgal
Director, Vigyan Prasar

Introducing
VIGYAN PRASAR

Vigyan Prasar (VP) was set up by the Department of Science and Technology, Government of India, as an autonomous registered Society in 1989 for taking up large-scale science popularisation tasks. Its broad objectives may be summarised as follows:

To undertake, aid, promote, guide and coordinate efforts in popularisation of science and inculcation of scientific temper among the people and to increase the knowledge, awareness and interest about science and technology among all segments of the society.

To provide and promote effective linkages on a continuing basis among various scientific institutions, agencies,educational and academic bodies, laboratories, museums, industry, trade and other organisations for effec-tive exchange and dissemination of S&T information.

To undertake development of materials—audio, visual, audio-visual and printed—methods and modes of communication, so as to enable the masses to better understand, appreciate and comprehend abstract scientific principles and practices.

To organise research work, courses, workshops, seminars, symposia, training programmes, fairs, exhibitions, film-shows, popular discussions, street plays, quizzes, song-dance-dramas etc., in furtherance of the objectives of the Society.

After its establishment Vigyan Prasar remained dormant for a few years. Only in 1994 some activities could be taken up in right earnest. One among the first few programmes initiated by Vigyan

Prasar was the 'Ready-to-Print' Science Page project. The idea was to prepare a well laid-out newspaper-size page with one or two features and several smaller items on scientific and technological (S&T) developments taking place in India, appropriately supported with photographs, illustrations, graphics etc., and to supply it to newspapers to carry as it is. Initially, such pages in Hindi and English were planned for release once a month. Subsequently, a children's page, science pages in other major Indian languages and a feature packet service were also added. Today, these pages are being carried once or twice a month by more than 30 editions of some 20 newspapers spread all over the country. In fact, today Vigyan Prasar's are the largest circulated science pages in the country. The combined print order of all these newspapers exceeds 2.5 million copies. These pages have led to fresh demands for enhanced science coverage in other newspapers.

Vigyan Prasar's publications programme is gradually taking shape. A number of important series has been launched; some more are planned. The first major English publication brought out by Vigyan Prasar, viz., *"Memoirs of Ruchi Ram Sahni: Pioneer of Science Popularisation in Punjab,"* under its series on Pioneer Science Popularisers in Pre-Independence India has generated positive awareness among science communicators and enthused researchers about the need to unearth other such personalities in other parts of the country. Already names of a number of individuals who did pioneering work in the field of science popularisation in pre-independence India have come to light.

Popular science classics written by Great Masters in the past, which have inspired generations of students of science, are no longer seen in the hands of our younger generation. This is not because these books have gone out of context, but because they are no longer available. Vigyan Prasar under its Popular Science Classics

series intends to reprint these books and bring them out in low-priced affordable editions so that more and more children can have them. Already two such classics (Michael Faraday's *Chemical History of a Candle* and C.V. Boys' *Soap Bubbles And the Forces Which Mould Them)* have been brought out and more are on the way.

Inspired by the focal theme for the National Science Day-1995, viz., 'Science for Health', Vigyan Prasar initiated a Health Series. Under it publications on all common diseases, along with possible management of their curative and preventive aspects would be brought out. The first three titles on *Sexually Transmitted Diseases, Asthma and Jaundice,* have already been released.

Under its series of Monographs on India's Scientific Heritage Vigyan Prasar intends to bring out publications on specific science and technology areas in which India's contributions have stood the test of time, as also have made an impact on modern-day science. The first monograph in the series *'The Rustless Wonder : A Study of the Iron Pillar at Delhi'* was released on 30 January 1997. The second volume *' Where Gods Come Alive : A Study of the South Indian Bronze Icons '* would be going to the press shortly.

The Total Solar Eclipse of October 24, 1995, provided Vigyan Prasar a rare opportunity to organise a country-wide awareness campaign, aimed at dispelling age-old myths and superstitious beliefs related to eclipses, and to develop among people an urge to learn about their known scientific aspects. Vigyan Prasar jointly with the National Council for Science and Technology Communication (NCSTC) organised a number of activities:

i. Telescope-making workshops for students and teachers.

ii. Development and production of books, a total solar eclipse chart and an activity kit for children.

iii. Production of several video films and their telecast.

Vigyan Prasar conceptualised and implemented a novel idea for ensuring that people did come out and watch the total solar eclipse. It circulated a total solar eclipse pledge. People in thousands from all corners of the country sent in signed pledges. Many individuals and voluntary agencies got these pledges translated into regional languages on their own and distributed the same in large numbers. All this led to a chain of activities throughout the country. The efforts made by VP, NCSTC and other agencies created a situation where millions of people came out and watched the spectacular event. This was a unique experience and made Vigyan Prasar's name a household word throughout the country.

Under its audio-visual programme, Vigyan Prasar developed a set of video films and several radio programmes on the occasion of the total solar eclipse of 24 October 1995. This event-based effort was enormously satisfying for the VP family and generated a very good response from the public at large.

Vigyan Prasar has recently begun building an Information System called VIPRIS — acronym for **VI**gyan **PR**asar Information System — to meet a long-standing demand from different quarters, particularly the science communicators, to establish a repository of background data and information on various aspects of S&T which would be accessible easily. The computerised system would be built on a modular basis, and aim to meet the information needs of science communicators of all kinds.

At this stage, under VIPRIS, we have a fortnightly clippings service, an electronic bulletin board service (BBS), two pages daily on Doordarshan's teletext service and weekly science news on the radio. Several other

products and services including training, development and production of CD-ROMs, generation of data bases on different subject areas etc., are being planned

The first phase of the database on " Environment & Safety Laws : Regulations & Guidence Documents " has been completed. VP launched its Home-Page on the Internet on 12 September, 1996. An online electronic popular science magazine 'ComCom', has been planned as part of the Home page. The other sections of the Homepage are, About Vigyan Prasar, Daily Weather Report, Sky map of the days/month links with other related homepage, S&T vacancies in India, News from S&T laboratories, S&T databases etc. It has provision for Hindi HTML and support for Web browsers / users to download the Hindi Plug-in and install it in their system. It has also a discussion forum with support to display and keep visitors' view.

Vigyan Prasar has also produced audio-cassette sets of the 108-part radio serial 'Manav Ka Vikas' (jointly produced by the NCSTC and All India Radio) in 18 Indian languages.

A number of video programme has also been produced. Recent ones among them have been on "Herbal petrol" and "Comets"(in connection with the coming of the comet "Hale-Bopp"). Several other programme are possibly under production.

This is not all. Vigyan Prasar does many other things. But for now this should suffice.

Narender K. Sehgal
Director
Vigyan Prasar

Mad Cows and Englishmen*

By C. Marsden

There's a whisper in the pastures,
Of our green and pleasant land.
There's a milker with the staggers,
There's a cow can hardly stand.
An illness brings them to their knees.
It's quickly dubbed 'Mad Cow's Disease'
Just a few, Sir. All's in hand.
It's our view, Sir, you'll understand
That there's nothing much the matter,
And this scare's just idle chatter.
The boffins they got down to work,
And found a direct line,
Between scrapie (a disease of sheep)

And the new disease in kine.
Well, soon the news begins to leak;
A hundred cases every week!
Don't worry, Sir, we've got it pat;
We'll stop it spreading just like that.
It really won't be hard to do
Now that we know to what it's due.

To get an extra pint a day,
The food producers had agreed,
The best and most efficient way,
Was ground-up meat waste in their feed.
Though cooked, unlike other diseases,
Scrapie had leapt across the species.

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* Reproduced with the permission of Prof. C. Marsden

The numbers, Sir, will soon go down.
You musn't fret, you needn't frown..
We've banned the feed; the danger's past,
We told you that it wouldn't last.

Meanwhile, in labs, in zoos, on farms,
Mice and monkeys, pigs and mink,
Across the land ring new alarms;
From cats to kudu; what's the link?
In all, the same sad signs are found;
BSE has got around!

Two hundred cases every week;
That's normal, Sir, we've reached the peak.
We really have no doubts at all,
That numbers now will start to fall.
It's now well known that cooking heat,
Does not destroy this cruel infection.

So every burger that you eat,
May bring this plague in your direction.
And still the weekly numbers grow;
(Compensation helps, you know).

Now really, Sir, we must insist,
You stop this aggravating list.
Scrapie always let us be,
So why this fear of BSE?
scrapie first can be transferred
feeding cows infected tissue
It really isn't too absurd
To see transferral as an issue.

From sheep to cow, from cow to cat,
Why shouldn't man be part of that?
What you say, Sir, is quite absurd..

Rather than hear another word,
We'll shut our ears; we'll hear no more.
Your briefcase, Sir; your coat; the door!
BSE starts slow but steady.
It takes a while to incubate.
Some cows that have the bug already,
are sure to end up on our plate.

Eighty percent of those infected,
May reach our tables undetected.
A minute risk, Sir, we confess,
But there's a way to stop this mess
The abbattoirs will throw away
The bits worth pennies anyway.
Mad cow disease can go no further.
No use working up a lather.

We found the source and broke the chain.
The numbers will go down again.
That's fine; it sounds very pretty,
BSE, goodbye, so long.
But people, here's the nitty gritty.
The Min of Ag. may have it wrong.
500 cases weekly show

Their estimates were somewhat low.

You doubt us, Sir! But can you prove
That BSE's still on the move?

Please eat your beefsteak with impunity
We're quite convinced of man's immunity.

All well and good, but please explain
Why cows have gone on falling ill.
Since feed's no longer in the chain,
6000 head they've had to kill.

If fodder didn't cause their doom
They must have caught it in the womb.
Not so, Sir, not at all, no way!
And what is more we're glad to say,
We've tested all the bits you eat;
There's no infection in your meat now;

Its soon apparent that the test
For meat infection in the cow
Decidedly was not the best
And dangerous levels of infection
Escaped the scientist's detection.

How many times, Sir, must we say,
That BSE will go away?
We've briefed the vets, we've paid the farmers,
What you don't know now - can't harm us!
Man also hosts a malady,
at much resembles BSE.

It also has no remedy;
It's known for short as CJD.
In 4 years it's increased twofold.
Don't you think we should be told?
But Sir, that's simply happenstance,

A curious effect of chance,
farmers dead? Two kids infected?
That's all? No cause to be dejected.

It we did what you say we should,
The farming lobby'd have our blood.
So cool the figures, calm the press,
The country can't afford the mess.
What! Slaughter whole herds and restock?
The industry would die of shock.

And paying all that compensation,
Would rapidly bankrupt the nation.
So is it just coincidence,
Or are these deaths the first of many?
Will BSE, slow death, advance,
In humans and their progeny?
One thing is sure; our precious State,
Won't tell us till it's much too late!

***

History of the Mad Cow Disease

In the summer of 1985, a strange neurological disorder was observed for the first time among adult cattle of both the sexes in a small farm in Surrey, a county in south-east England. The affected cattle were becoming uncoordinated and there were changes in their temperament making them either more nervous or aggressive. Despite normal appetite, the cattle lost body weight and the females produced less milk. All of them died within two weeks to six months of showing the clinical symptoms. Since the diseased cattle behaved abnormally, the new affliction was reported to the world in November 1986 as "mad cow disease." But the loss of muscular co-ordination or inability to move was not due to any madness and therefore the name "Mad Cow Disease" given to this disease was not very appropriate.

When the cattle showing this disorder were sacrificed and tissue from their different organs were examined under the microscope for any abnormalities, the brain tissues appeared unusually spongy. The neurons in the brain contained a large number of vacuoles. No other tissue showed any detectable abnormalities. This sparked off intense research into the cause of this disease. Gerald A.H. Wells and John W. Wilesmith of the Central Veterinary Laboratory (CVL) in Weybridge, England, identified this disorder to be bovine spongiform

encephalopathy (BSE). It belongs to a family of diseases earlier known as the transmissible spongiform encephalopathies (TSEs), which include scrapie in sheep and goats and Creutzfeldt–Jakob disease (CJD) and Kuru in humans. But such a condition in cattle had not been reported earlier. Diagnosis of this disease is purely symptomatic. Post-mortem confirmation of the clinical signs is provided by the characteristic brain lesions and presence of vacuoles in the neurons, or detection of the fibrillary proteins which vary, depending upon the particular spongiform encephalopathy. The causative agent of BSE has yet to be fully characterised. What has been clearly established is that this agent does not cause any detectable immune or inflammatory response in the host. It has not been observed under a microscope.

In 1990 the British government established the Spongiform Encephalopathy Advisory Committee (SEAC), consisting of experts in epidemiology, microbiology and neurology, to examine the implications of the mad cow disease for animal and human health.

The public panic about the "mad cow disease"was set off by an official announcement in the British press that 10 cases of fatal human neurodegenerative disorders known as CJD, may be linked to the BSE epidemic that had killed thousands of cattle in the UK. This panic soon spread throughout the world. The SEAC examined these 10 cases of CJD, which predominantly seemed to have affected the young. It

opined that the most likely explanation for these unusual cases of CJD was exposure to the mad cow disease, and this was a matter of great concern. According to James Ironside, an neuropathologist of the CJD Surveillance Unit in Edinburgh, these 10 cases of CJD, out of a total of 40 that were reported, indicated the emergence of a new variant of CJD. These cases were initially diagnosed as psychiatric disorders such as anxiety and depression. This was followed by signs of a movement disorder. However, older patients suffering from CJD first experience dementia. Moreover, CJD generates a characteristic pattern of electrical activity in the brain, but this was absent in the 10 cases examined by the SEAC. Ironside also observed that the patients' brains showed a very widespread distribution of protein plaques with densities far higher than normally found in usual CJD afflicted brains. Some of these 10 affected young individuals (of whom 8 have reportedly died) were known to be keen consumers of beef burgers and one regularly visited a relative's dairy farm. The BSE agent had already been transmitted experimentally to cats, pigs and other exotic ruminants via infected beef. So, despite the absence of any direct evidence, one could not say with certainty that there was no risk to humans from infected meat. The SEAC, therefore, concluded that there was every need to be cautious.

Epidemiological studies of the mad cow disease led to the conclusion that it probably originated from the scrapie agent which has been present in sheep in the UK for at least 200 years. It was suggested that the

scrapie agent jumped the species barrier and moved into cattle when they were fed with protein supplements made from leftover parts of butchered sheep—a practice followed in Britain to provide better nourishment to cattle.

These protein supplements, called specified bovine offals (SBOs), were prepared by following a particular sterilisation procedure. In the early 1980s, the process by which the leftover parts of butchered animals were converted into animal feed was changed. Earlier a solvent extraction step was used to extract fats from livestock carcasses before they were used for preparing protein supplements for animal feed. But as the prices of petroleum-based solvents used in this extraction process went up, this practice of solvent extraction was discontinued. It is believed that the scrapie agent present in the carcasses of infected sheep was getting destroyed during the process of solvent extraction. But with the omission of this step the infectious agent remained in the feed and got transmitted into some of the cattle. And in 1986 the mad cow epidemic struck. Once it became evident that the animal carcasses-derived protein supplement could be the cause of this new disease, the British government banned its use in animal feed in 1988. The rate of BSE cases has, since then, declined. While it has been established that scrapie is as infectious via the oral route as via intracerebral inoculation, it is not known whether the BSE agent behaves in the same way. Nevertheless, these facts point to a species jump from sheep to cows. The real scare is whether a further

species jump from cow to man can occur through the meat diet.

The mad cow epidemic in the UK peaked in 1993 when more than 1,000 cases were reported per week. By then more than 160,000 infected cows had been identified, constituting over 50 per cent of the total dairy herds in the UK. Even though the use of protein supplements containing sheep and cattle offals had been banned in 1988, it could not be strictly enforced till 1991-92. The mad cow disease is a slow incubating encephalopathy which manifests itself within two to eight years; there is no way of knowing that the animals are infected before the onset of clinical symptoms. Therefore, it took a long time to control the disease.

By May 1996, the mad cow disease had been reported from 10 other countries (Table1.1). In one group of countries—namely, France, Portugal, Republic of Ireland and Switzerland—the disease was detected in native cattle. It is believed that they got infected via cattle feed imported from the UK, containing the contaminated protein supplements. Since the disease manifests after a long incubation period, the native cattle must have been given the feed before the ban was imposed. In another group of countries—consisting of Falkland Islands (Las Malvinas), Oman, Germany, Canada, Italy and Denmark—the mad cow disease afflicted only cattle imported from the UK. The United States government took several steps to prevent the disease from entering their country. Consequently,

Table - 1.1

Cases of BSE Reported in Various Countries

Country	*Numbers of cases*
UK	1,61,663
Switzerland	205
Irish Republic	123
Portugal	31
France	13
Germany	4
Italy	2
Oman	2
Canada	1
Denmark	1
Falkland Islands	1

Source : Stekel et. al, *Nature*, 9 May, 1996 p. 119.

to date not a single case of the mad cow disease has been reported in the USA.

About 15 per cent of CJD cases are inherited. In such families mutations have been found in a specific gene which codes for a prion protein (PrP). Scrapie also has been shown to involve a cellular prion protein. The normal PrP is a glyco-protein found on the surface of neurons present throughout the central nervous system. It is not infective but its aberrant or mutated form, which aggregates easily, is capable of causing infection. This prion protein is present in skeletal muscles (steak) and also on the surface of lymphocytes which are present in milk. Pasteurisation temperature will not destroy this infective agent, although there is no firm evidence at present that milk ingestion can cause the disease.

Scientific data so far have been too meagre either to confirm or deny this apprehension. There are alternative viewpoints on whether prion-protein alone is responsible for the neurodegeneration associated with these diseases. There could be other hitherto undetected molecules which actually help destroying the brain tissue. This particular aspect is a black box. Fortunately, animal models have been developed for many of the prion diseases. However, extensive research is necessary before it can be conclusively established that the species barrier jump accounted for the fatal damage. Such evidence may be long way in coming. In the meantime we feel that it is a worthwhile exercise to collect and collate the available knowledge about these puzzling diseases . While not claiming to be an exhaustive

or complete account, it is hoped that this report will create awareness about the mad cow disease.

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2

Bovine Spongiform Encephalopathy

Bovine Spongiform Encephalopathy, popularly known as the mad cow disease, is a fatal neurological disorder among cattle. The disease afflicts only adult cattle of both the sexes. It has a long incubation period ranging from two to eight years. Its diagnosis is symptomatic. Post-mortem confirmation of it was reported for the first time from Britain in 1986—typical brain lesions and the presence of vacuoles in the neurons, or detection of specific fibrillary proteins in the brain. Bovine spongiform encephalopathy affects the central nervous system (CNS); when examined under a microscope, the brain tissue of the affected animal appear to be "spongy." All cattle after showing clinical symptoms of BSE die within a period of two weeks to six months. The affected cattle appear to be alert but are in fact agitated and lose weight. Fine muscular contractions involving various small muscle groups, especially the neck, can be observed; these are occasionally punctuated by large muscular jerks. The affected animal shows problems of motor coordination; and often demonstrates frenzied movements such as aimless head butting. BSE became a serious problem in Great Britain by the year 1993, when more than 50 per cent of the milking herds were reported to be infected. While BSE showed the highest incidence in southern England, where more than 60 cases were reported in a single herd, it had generally spread all

over the British Isles. The disease was also reported from Switzerland, France, Germany, Denmark and even from Canada and Portugal. Epidemiological studies in the UK found the causative agent of BSE to be present in the protein supplements, prepared from leftover parts of butchered animals, which are fed to cattle. Thus, the cases reported from other countries are believed to be caused by the cattle feed imported from the UK into these countries.

BSE is caused by an unconventional infectious agent described as a "slow virus" or a "self-replicating protein", and more recently as a "prion protein" an aberrant conformation of the normal cellular glycoprotein. Research is increasingly supporting the hypothesis that a prion molecule after coming in contact with its normal protein counterpart in the brain can induce it to refold into the abnormal conformation. The process keeps on repeating itself, thereby creating a large number of replicas of the prion molecule from the normal protein molecule. Such a type of neurodegenerative disorder has been found in other mammalian species as well. These include the Creutzfeldt–Jakob disease, kuru and Gerstmann-Straussler-Scheinker (GSS) disease in humans; transmissible mink encephalopathy in mink; chronic wasting disease in mule, deer and elk; feline spongiform encephalopathy (FSE) in cats; and scrapie in sheep and goats. The incubation period of all these diseases, following transmission either by inoculation or by feeding, has been found to vary with the dose of exposure, the route of infection, the species of the host and recipient, as well as the nature of the pathogen.

The neurological disorder caused by BSE differs widely from one case to another, manifesting itself through a broad spectrum of symptoms. This has made the study of the disease much more difficult. There are different scientific hypotheses concerning the origin of BSE. Besides the theory that in England it may have been caused by feeding cattle protein supplements made from the carcass of scrapie infected sheep. An alternative view holds that BSE was prevalent in undetected level among cattle in Britain even prior to 1985; but because of the low incidence, it went undetected. For the last several decades, it has been a common practice in this country to use products such as meat-and-bone meal (MBM) as a source of protein. Scrapie which has a long incubation period upto—five years in sheep—has been endemic in the UK for centuries. But changes in the 1980's method of preparation of protein supplements — stoppage of the solvent extraction process, including steam heat treatment —may have played an important role in the appearance of BSE in the cattle population.

The outbreak of BSE in the UK was not without precedent. In 1965 a similar disease affecting mink, called transmissible mink encephalopathy (TME), was identified. This extremely rare disease was found to be caused by the exposure of ranch—reared animals to infected unprocessed offal. Since sheep and goats are the only known natural reservoirs of such infectious agents, which cause scrapie in them, a causal relationship between TME and scrapie was accepted. But BSE appears to have started in a different way. The cattle

were not fed unprocessed offals. The protein supplements given to them had undergone some chemical processing. Moreover, these had been fed to them not sporadically, but continuously from 1981-82 until July 1988 when the British government banned the feeding of meat-and-bone meal to all ruminants.

There is no evidence that BSE spreads among unrelated cattle or from cattle to other species of animals by physical contact. Epidemiological evidence suggests that mother-to-foetus transmission is possible. According to preliminary results of research carried out by the Ministry of Agriculture, Fisheries and Food (MAFF) in the UK, maternal transmission of bovine spongiform encephalopathy does occur at low levels. A study conducted by researchers from the epidemiology department at the Central Veterinary Laboratory in Weybridge, found that the risk of transmission of BSE from cow to calf was 10 per cent. It was observed that 1 per cent of calves born to cows which die of the same disease contracted from their mothers. This study, which was started in 1989 continued till the end of 1996. According to SEAC, no further action was needed to protect public health as Britain's eradication plan for BSE already recognised that maternal transmission was a possibility.

In this study researchers observed two groups of animals . One group consisted of over 300 cattle which were offspring of mothers with confirmed cases of BSE. The other group comprised same number of animals born in the same herd and in the same calving season but whose mothers were at least five years old

and did not show any clinical signs of BSE. The animals in the two groups were observed until the age of 7 years or until BSE or any other disease intervened. Of the 273 animals, whose mothers were infected, 42 were histologically confirmed to have contracted BSE themselves. In the other group, out of the same number of animals born to mothers who did not show any clinical signs of the infection, 13 were confirmed to be suffering from BSE. The disease showed up in both groups because the cattle studied were born around the time in 1988 when the ban on ruminant feed had just been imposed; and therefore even though the control group of animals showed no clinical signs of BSE at the start of the study, some might have been exposed to infected feed prior to its commencement .

On the basis of this study, the Spongiform Encephalopathy Advisory Committee observed that there was some evidence of the enhanced risk of maternal transmission in the last six months of the BSE incubation period.

However, this study did not throw any light on the route of maternal transmission, which can be in utero, at birth, or soon after birth. Evidence of maternal transmission in the case of scrapie in sheep has come from two sources. First, infection has been detected in the uterus of a few affected sheep. Second, embryo transfer experiments have shown evidence of in utero transmission. When the embryo from a sheep infected with scrapie was transplanted into the uterus of a healthy ewe, the lamb that the latter gave birth to went on to

develop scrapie. Similar experiments are underway in cattle, but the results are not yet available. However, so far, no infection has been detected in bovine placenta, milk or blood.

There is a school of thought which is still sceptical about maternal transmission as route among any species. Researchers belonging to this school argue that the data for scrapie were derived from only a small number of animals. While agreeing that the new data on BSE show a striking correlation, they point out that this can be explained by genetic susceptibility in the cattle population. Further, on examining an entire herd which has been fed on the same feed, from the same infected sack, it turns out that only one or two cows per birth cohort come down with BSE. This raises the question: Why did not all of them get BSE? There is some genetic variation in the PrP gene. While analysis suggests that this is not associated with BSE, much more research is needed to establish this. These researchers also point out that the comparison group consisted of the offspring of elderly cows. It is possible that the elderly cows had some resistance to BSE as a result of ongoing genetic selection .

The Spongiform Encephalopathy Advisory Committee categorically states that there is no evidence from any of the studies of TSE to indicate that infection can be transmitted through milk. However, more research is being undertaken in this direction. The BSE causing agent, prion, has been found only in brain tissue and the spinal cord of infected cattle. In an

experimental study involving calves that have been fed large doses of material derived from BSE-infected cattle, traces of these causative agent were detected in the small intestine and retina of the animals. Detection of the presence of BSE agents in tissue or body fluids is complicated due to the lack of a definitive laboratory test. The only conclusive way to establish that an animal is suffering from BSE is by examining the brain tissues after slaughtering the animal. Therefore, there is problem of detection of transmission right till the appearance of the clinical signs.

Whether bovine spongiform encephalopathy has a greater potential for cross-species transmission than scrapie and other non-human transmissible spongiform encephalopathies is an important question. It is relevant irrespective of validity of the proposition that bovine spongiform encephalopathy is derived from scrapie, since passage through cattle may confer new transmission properties. Observations in experimental animals suggest that the transmission of a spongiform encephalopathy to one foreign host can result in an infection which can then transmit to a second initially unsusceptible, foreign host. Actually, a similar experiment might have been done serendipitously by exposing cats. Feline spongiform encephalopathy appeared in the 1980s, apparently as a new disease. The agent causing this infection was examined by strain typing and found to have the same strain phenotype as bovine spongiform encephalopathy. Assuming that cats have been equally exposed to sheep scrapie and bovine spongiform encephalopathy, the observations suggest that bovine

spongiform encephalopathy has a different, and possibly broader host range, than circulating scrapie strains. We do not yet know whether this apparently novel host range of bovine spongiform encephalopathy extends to humans.

The molecular factors that influence cross-species transmission remain incompletely defined. But the seminal experiments by Scott et al. established that the genotype of the prion protein gene is the major factor. Indeed, the species barrier to transmission between mouse and hamster was overcome by transferring the hamster PrP gene into a mouse by genetic engineering. Although recent reports have suggested the involvement of additional factors, homophilic interactions between PrP in the inoculum and PrP in the host seem necessary for transmission to occur, with amino acids 96-167 on the PrP possibly being most important. What inferences can be drawn by comparing the PrP sequences of humans and cattle? It would be comforting if the degree of homology between them was lower, or at least no greater, than that between humans and sheep. Unfortunately, this is not the case. Overall, the bovine PrP sequence is closer to the human sequence than the sheep sequence, and the 96-167 region of PrP has only five differences rather than six. However, a second important region of the PrP molecule , i.e., the C-terminal end, is equally diverged.

Collinge *et al.* have recently provided evidence that mice transgenic for the human PrP gene can be used

as a model for human PrP related diseases. Similarly mice, on a mouse PrP background but lacking their own PrP gene, are available and should be tested for susceptibility to bovine spongiform encephalopathy by various routes. A positive outcome, particularly if the mice were relatively resistant to sheep scrapie, would be evidence that bovine spongiform encephalopathy infected by can transmit to humans. Bruce *et al.* have described "strain phenotypes" based on the lesion profiles and transmission characteristics of various isolates of scrapie and bovine spongiform encephalopathy. The bovine spongiform encephalopathy phenotype is distinct and, moreover, stable even on passage through another species such as cat or antelope. If the present cases of Creutzfeldt–Jakob disease have been caused by bovine spongiform encephalopathy, it is likely that this will show up in strain typing tests. As a matter of urgency, therefore, necropsy material from all the present cases should be strain typed. Any presence of the bovine spongiform encephalopathy phenotype should be regarded as strong evidence that the bovine spongiform encephalopathy has been transmitted to humans.

CJD and BSE

The Creutzfeldt-Jakob Disease Surveillance Unit was set up in Britain in 1990. One of its aims was to alert the public to the fact that humans are affected by exposure to the agent responsible for bovine spongiform encephalopathy. A previously unrecognised but consistent disease pattern had emerged among young adults. The most likely explanation that has been proffered is exposure to the bovine spongiform encephalopathy agent,

before the specific bovine offal was banned in 1988.

Creutzfeldt-Jakob disease has now been identified in four farmers and two adolescents in Britain, where BSE has been an epidemic for the past several years. Is there a link between CJD and BSE? This is the question being debated by different groups according to pre-existing biases and professional goals. The media has sounded alarm bells mainly because its goal is to produce an exciting story. The government has taken a low key, cautious approach so as to prevent unwarranted panic in people. Medical scientists, on their part, have been somewhat unpredictably divided over this question.

Creutzfeldt-Jakob disease typically begins in the sixth or seventh decade of a human's life, with loss of memory, or less commonly behavioural changes or higher cortex functional deficits such as dysphasia or dyslexia. Over several weeks, the mental deterioration progresses to frank dementia. This is accompanied by abnormalities of vision or coordination, rigidity and involuntary movements like myoclonic jerks, which often occur in synchrony with periodic spike waves observed in an electroencephalography. Death usually occurs within six months. At necropsy, the brain shows a spongiosis, with neuronal loss and gliosis in the cortex, deep nuclei and cerebellum. Amyloid plaques are found only in about 5 per cent of CJD cases. Creutzfeldt–Jakob disease contracted from use of infected growth hormone also begins in a similar way but with ataxia or other abnormalities of coordination.

The Table-2.1 shows the incidence of Creutzfeldt-Jakob disease in France, Germany, Italy, the Netherlands, and the UK during 1993 and 1994. The data are based on registries in these countries, which have used similar methods of surveillance and criteria for diagnosis since the start of a European collaborative study in 1992. The incidence in the UK, with a much higher risk of exposure to bovine spongiform encephalopathy, was similar to that in other European countries.

The epidemiological evidence concerning occupational exposure derives from two sources . First a meta-analysis of three case-control studies, conducted in Japan, the UK and the United States. Second an on-going European case-control study based on the registries of Creutzfeldt-Jakob disease started in 1992. The meta-analysis included data on 178 cases of Creutzfeldt-Jakob disease and 333 controls. In 95 cases, information on occupational exposure to cattle was available (26 subjects had been exposed). The corresponding relative risk of exposure (adjusted for age and sex) was 1.7 (95 per cent incidence interval 0.9 to 3.1).

In contrast, the newly reported variant syndrome of CJD is clinically characterised by psychiatric symptoms and progressive neurological deficits, with an unusual neuropathological profile. This syndrome parallels Kuru, another epidemic spongiform encephalopathy among humans in the eastern highlands of Papua New Guinea.This disease is thought to have

Table-2.1

Incidence of Creutzfeldt-Jakob Disease in European countries in 1993 and 1994*(per million person)

	1993		1994	
	No. of Cases	Incidence	No. of Cases	Incidence
France	29	0.50	47	0.81
Germany	19	0.47+	58	0.73
Italy	31	0.54	40	0.53
Netherlands	10	0.68	16	1.04
UK	32	0.56	54	0.95

* *Includes probable and definite cases according to criteria adapted from Masters et al.*

\+ *Extrapolated from figures for June to December 1993.*

been orally transmitted through the practice of eating the brain tissues of infected individuals. All types of human and animal spongiform encephalopathy studied so far, including kuru, scrapie, and bovine spongiform encephalopathy are experimentally transmissible among animals.

It cannot be said with any degree of certainty whether the cases of Creutzfeldt-Jakob disease among adolescent farmers in Britain are the result of infection with bovine spongiform encephalopathy . Supporting this idea is the fact that the so-called sporadic Creutzfeldt-Jakob disease is typically a disease of late middle age, with only a handful of cases among people aged under 25. The disease, however, should have shown a more uniform age distribution if the source of infection was ingestion of cattle products, which explains the concern about "back-to-back" cases in young people. On the other hand, each of the four farmers, had at least one infected cow in his herd, raising the possibility of contact infection from the animals or from the contaminated meat-and-bone meal feed given to them.

Undermining the link between CJD and BSE is the fact that adolescent Creutzfeldt-Jakob disease has occurred in countries where there are no reported cases of bovine spongiform encephalopathy. Moreover, the type of illness experienced by all the patients was typical of the sporadic disease, rather than the predominantly cerebral illness, which is seen in patients infected peripherally from an external source such as use of contaminated pituitary hormones.

Statistical analyses will not be helpful in the evaluation of these cases, even if the observed occurrence can be shown to significantly exceed the expected occurrence in adolescents and farmers. This is because the number of cases are too few. In due time, however, statistics will provide the most important evidence for or against a clear association between Creutzfeldt-Jakob disease and bovine spongiform encephalopathy. Therefore, it is crucial that the ongoing surveillance programme of Creutzfeldt-Jakob disease be sustained for at least several more years if an association between Creutzfeldt-Jakob disease and bovine spongiform encephalopathy is ever to be properly assessed.

In November 1995, the *British Medical Journal* published a debate on the possibility of a link between bovine spongiform encephalopathy and Creutzfeldt-Jakob disease. In view of all of the earlier negative epidemiological and laboratory evidence concerning the risk of human infection from scrapie —a disease among sheep prevalent in the UK for two centuries—and the failure to detect infection in the muscle of cattle with bovine spongiform encephalopathy, the conclusion that human infection might be occurring from the ingestion of beef was rather unusual. More so because no unusual dietary history characterises these CJD cases and that a "normal" British diet has been sufficiently contaminated to cause any new infections. However, it must also be emphasised even today the link with cattle products is itself only a presumption. The contemplated move to slaughter 11 million British cattle to eliminate

the risk of zoonotic Creutzfeldt-Jakob disease, could turn out to be a premature one. Further research might establish that the true villains were pigs or chickens who were also fed contaminated nutritional supplements, but were brought to market at such a young age that the disease did not have sufficient time to manifest itself.

A good deal of work remains to be done in order to establish the link between bovine spongiform encephalopathy and Creutzfeldt-Jakob disease. Several studies have already been initiated. Of course, none of these will be of any help to those who have been already exposed to the infectious agent in the early 1980s, before precautionary measures were put in place to minimise the risk to humans.

Epidemiology of BSE

There are different scientific hypotheses concerning the origin of BSE. One theory is that BSE existed in undetectable levels in the British cattle population even prior to 1985. Another view which stems from epidemiological data, suggests that BSE in Britain was caused by feeding cattle protein supplements produced from the carcass of scrapie-infected sheep.

When BSE was first observed in 1985, about 200-300 infections had probably occurred already. Based on British epidemiological data, the age at infection is 1.3 to 1.8 years, the mean incubation period is five years and the rate of maternal transmission to calves is approximately 10 per cent. But the actual incidence in

dairyherds is much higher, probably because the cows are fed more protein and remain on feed for a longer period of time. There is no evidence to date of the genetic susceptibility (breed differences) of cattle to BSE.

BSE is considered a "common source" epidemic, meaning thereby that animals contract the disease from a common element in their environment. Semen, chemicals, autosomal inheritance, and pharmaceuticals have been ruled out as the common source. In trial circumstances, maternal transmission can occur. And in created extreme situations, such as where infected material is injected into the brain of another animal, it appears that the species barrier between animals can be crossed. Development of infection appears to be related to the size of the inoculum. The infectiveness of BSE is similar to that of scrapie. It is estimated that the dose of BSE infected brain needed to transmit the disease to mice orally would be 125,000 times greater than the dose required to transmit it to cattle by intracerebral injection.

Though infection is dose dependent, the required dose for BSE transmission among cattle is small. An oral dose of 500 mg to 1g of infected brain appears sufficient to cause infection.

The BSE agent has been found only in the brain tissue, spinal cord and retina of cattle naturally afflicted with BSE. It has not been found in meat or milk. Similarly, it is not transmissible through these products. Traces

of the BSE against are detectable in the small intestines of calves which have been fed large doses of feed made from BSE-infected animals.

Research work published in *Nature*, an international science journal (vol. 378, pp.779-783, 1995), suggests that transgenic mice by expressing human PrP in addition to their own can generate 'human' prions. When inocula from a CJD case were injected into these mice by the intracerebral route, the mice produced human PrP^{sc}. But mice expressing only human PrP when injected with pooled BSE brainstem homogenate did not develop a neurological disorder like BSE for 264 days following inoculation. The time period for which the mice were observed may be too short, but such a study certainly provides an opportunity to observe whether bovine prions can actually induce the formation of aberrant human PrP.

To understand some aspects of the epidemiology of BSE, the explanation regarding infection from contaminated feed is inadequate. Vertical transmission (VT), the transfer of infection from the dam to the calf, may well explain some of these anomalies (the rate of vertical transmission can perhaps be calculated using current data). An attempt is made here to explain the BSE epidemiological pattern seen in the UK. The analysis is based on the official data from the BSE epidemiology unit at the Central Veterinary Laboratory, official documents, and statistics published in peer-reviewed scientific journals.

Attempts have been made since 1988 to produce models that better fit the reported epidemiology of BSE. The initial model assumed that BSE was derived from scrapie in sheep and transmitted from the slaughtered animals, via the infected tissue in meat-and-bone meals used to feed cattle. The rapid increase in the incidence of BSE in the UK during the 1980s fits well with the epidemiological pattern of an infectious disease. From this model, it is possible to estimate the number of cattle that will be infected, as also the transmission rate (R) of the disease and how this would changes as the epidemic progresses.

Initially, the pattern that seemed to characterise the transmission of BSE was unofficially described as the "nugget" theory. It assumed that infectivity was not evenly spread in the feed but was concentrated in "nuggets", which therefore affect one but not necessarily another eating from the same bag of meal. This might explain why the age distribution of BSE cases remained relatively steady throughout the epidemic. It appears that the cattle contracted infection at a specific age from nuggets of similar infective sizes. The theory also suggests that nuggets were relatively uncommon throughout the epidemic and that the number of nuggets required by a cow to cause infection was one or close to one.

Other transmission routes, although not originally thought to be likely, could also have been responsible for the outbreak of BSE epidemic. For instance, it may not have been the cow that ate the infected material but

its offspring which showed symptoms of the disease. Such a scenario (discussed below as the vertical transmission model) would be expected to lead to a similar increase in the incidence of the disease and would be difficult to separate from the direct transfer 'nugget' model. Also, horizontal transmission, from one bovine to another, through soil or other material carrying the BSE agent could be involved. It now seems likely that there is more than one mode of transmission of BSE. As the ban on the feeding of MBM to cattle becomes effective , these models may become more important. Therefore this and various other epidemiological models should be discussed.

Specific problems that have arisen with the nugget model are :

1. The number of nuggets per bag of feed would be required to be very small indeed. Infection is expected to be maximum when the brain, spinal cord and other soft tissue offal are used. It would be expected to spread through the product made as a batch. How does one find a specific nugget in the feed which has been eaten by one cow but not by another; as it would be difficult in the experimental processes to separate infectious fractions from the non-infectious ones.

2. As the epidemic progresses the number of "nuggets" involved should increase. Moreover, the number of cattle in any particular herd which consumed the single nugget should have risen in tandem with the national rate of increase in the

incidents of BSE. However, that was not seen to happen.

Vertical transmission (VT) model

Investigations have shown that the older, milking cattle were fed the maximum amount of meat-and-bone meal. This is the feed, with its high calcium content, is believed to increase the milk output of the animals. In USA MBM is also given to calves as a milk substitute. It is also fed to female cattle between the periods either to shorten the time required for heifers to become pregnant, or to increase of their growth. The question then is : Which cattle would be expected to become infected as age was not related to the infective dose? It would suggest that the dams would be expected to become infected rather than the calves. The possibility then arises that the calves actually became infected from the dams either inutero or when very young by physical transfer or through the colostrum.

This VT epidemiological model then suggests that the cattle seen dying at peak of 5 years are not really those that became infected from the feed but rather from their mothers.

The epidemiology of BSE cannot be explained by a single factor or a simplistic model. The age at which an animal becomes exposed to the disease, the time taken to get infected, the dose that is consumed, and the distribution of factors in cattle must surely be expected to alter the pattern of the disease seen at the national level. It is important to separate the epidemiological

factors and the effects that they have on the disease. BSE will be transmitted at a progressively lower rate through animal feed as more time lapses after the 'feed ban'. A greater proportion of the subsequent cases of infection will be due to transmission through some other route. In order to envisage the trajectory which BSE epidemic in the UK will take in the future, it is essential that the facts be separated statistically.

Research into BSE

Genetic susceptibility of cattle to BSE

Considerable work has been done on this aspect. Currently, there appears to be no association between genetic make-up and the clinical disease.

Longitudinal studies of the brain pathology of BSE

In such studies scientists follow the changes in pathology as the disease progresses. Many cattle which had been slaughtered because they were suffering from BSE did not show the expected changes in brain histopathology.

Clinical and epidemiological correlates of the vacuolar profile in BSE

Attempts have been made to compare the post-mortem changes in the brain with those found in the animal while alive.

Transmission of BSE and natural scrapie into sheep and goats by intracerebral and oral routes.

Such transmission has been carried out successfully.

Sheep and goats have died following infection via both these routes.

Strain typing of BSE pathogens in mice and comparisons with natural sheep scrapie.

BSE was found to be of the same strain type throughout the epidemic and even after inoculation into mice.

Survey of CJD cases in the UK

This study includes complex investigations to find out whether specific foods are associated with the occurrences of the disease. The main problem is that bovine tissue are eaten by such a high percentage of the population in Britain that it would be many years before the control and test groups could be followed up.

Selective studies of neurological disorders in cattle to aid clinical differential diagnosis of BSE

Since the incidence of BSE is declining, a method has to be devised by which veterinary officers can clinically distinguish BSE cases from other neurological disorders.

Electrophoretic analysis of body fluids to identify diagnostic markers in BSE and scrapie

This follows the work which showed that scrapie urine contained a small precipitated molecule.

Identification of putative nucleic acid components of the etiologic agent of the TSEs

This is being carried out at the University of Edinburgh Centre for Genome Research.

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Transmissible Spongiform Encephalopathies

Popularly known as 'Mad Cow disease' is now scientifically termed as bovine spongiform encephalopathy because the post-mortem appearance of the cattle brain resembles a sponge with large vacuoles in the cortex and cerebellum. Several diseases in other animals and humans, caused by similar uncharacterised agents which produce spongiform changes in the brain, have now all along with BSE been grouped as transmissible spongiform encephalopathies or TSEs. Specific types of TSE's include scrapie (which affects sheep), transmissible mink encephalopathy, feline spongiform encephalopathy, and three rare diseases in humans — kuru, Creutzfeldt-Jakob disease (CJD), and Gerstmann-Straussler-Scheinker (GSS) disease. These are discussed in some detail below.

Creutzfeldt-Jakob disease (CJD) :

The onset of CJD usually occurred between 40 to 80 years of age and death usually occurs within 3 to 12 months. Major clinical symptoms are dementia and myoclonus, sometimes accompanied by motor disorders and uncoordinated cerebellar functions. Cerebrospinal fluids (CSF) are usually normal, with a slight increase in protein content. Mild cortical atrophy and ventricular dilation may be present, but such changes are not diagnostic. Only small percentage of cases are familial,

most being sporadic, ruling out any strong genetic basis. In a few cases transmission is known to have occurred by corneal transplant or the administration of contaminated growth hormone.

CJD was first described in 1920-21 when it was known as "spastic pseudosclerosis" or subacute spongiform encephalopathy. The disease is rare—annual incidence is approximately one case per million—with some temporal and spacial clusters in Slovakia, Hungary, UK, USA and Chile. High incidence among Libyan Jews (26 per million) has been reported. These figures are certainly conservative since not all human deaths suspected to have been caused by CJD are followed up with necroscopies, the only histopathological analysis performed. The onset of the disease is accompanied by changes in sleeping and eating patterns. Over a few weeks, it progresses to a clear neurological syndrome, with symptoms like vibrating muscular spasms, dementia, loss of higher brain function, and behavioural abnormalities. Cerebral and cerebellar functions are severely compromised, sensory and visual functions get impaired, and 90 per cent of the patients die within a year.

CJD is diagnosed through a clinical assessment of patients with pre-senile dementia and ECG patterns showing triphasic one cycle per second activity, or slow wave bursts with intermittent suppression (also found in animals with TSE). Post-mortem diagnosis, currently carried out by the histological examination of

brain tissue under light microscope, is not always reliable. More advanced methods to demonstrate CJD (or other TSE) involve electron microscopic examination of brain tissue for prion protein antigens, or intracerebral injection of tissue into animals, which then go on to die of the disease.

Transmission of CJD has been claimed to occur from other CJD infected patients, but neither the infectious agent nor the route of infection is well-established. For example, two young epileptic patients contracted CJD from inadequately sterilised (only with alcohol and formalin vapour) cerebral electrodes which had previously been used on a CJD patient. Transmission of CJD by corneal transplant or injection of contaminated growth hormone has also been reported. Some cases of transmission reported in the literature seem too improbable. For example, contraction of a CJD by neurosurgeon, a mortuary attendant; two men living 200 meters apart and sharing a general practitioner; three patients who had been operated in the same neurosurgical unit within a period of eight months; an individual who married into an infected family (although spouse did not suffer from CJD). Four farmers who had BSE-infected cows on their farms have died from CJD in the UK since 1993. By 1996 a total of 10 CJD cases were reported in this country.

In April 1996, a group of international experts met in Geneva and reviewed the public health issues relating to BSE. At this meeting the emergence of a new variant of CJD was reported in Britain. Reviewing the

clinical and pathological data from the 10 British cases , the group observed that the disease had occurred at younger ages than is usual for classical CJD and had distinct clinical and pathological differences. It concluded, that while there is no definite link between BSE and CJD, circumstantial evidence suggests exposure to BSE may be the most likely explanation. Although transmission from animal sources is a distinct possibility, human exposure to scrapie affected sheep is poorly associated with CJD. The geographic distribution of V-CJD, hitherto reported only in the UK, needs to be investigated.

A familial form of CJD, accounting for about 10 per cent of cases, is also known. Genetic analysis in these cases establishes mutation in the gene coding for a normal protein, such that the mutant protein folds abnormally and tends to aggregate. When such an aggregation occurs in the brain, the consequence is lethal. This mutation when inherited causes familial CJD.

Kuru

Kuru is found among the Fore tribe of the Okape district in the eastern highland of Papua New Guinea. The practice of ritual cannibalism existed among this tribe until around 1956. The disease has mainly affected adult women, and children of both sexes. Kuru is contracted by eating infected tissue. The brain of dead tribal members was eaten by women and children, while men mostly ate the muscular tissue. The cohort of children born since 1956 has not suffered greatly

from kuru as the practice was stopped subsequently.

Clinically, the disease is characterised by progressive cerebellar ataxia, resulting in uncoordinated movements, neurological weakness, palsies, and decay in cortical functions. Most patients dying of kuru are not demented—a major clinical difference between this disease and CJD. They are thought of as two distinct spongiform encephalopathies. Kuru studies were instructive since they showed for the first time that a slowly progressing neurological disease among humans can be infectious and is transmitted from person to person. For this discovery, D. Carleton Gaiduschek alongwith B. S. Blumberg was awarded the Nobel Prize in 1976.

Gerstmann-Straussler-Scheinker (GSS) disease

GSS disease is an autosomally dominant condition rarely present in families. The disease is similar to CJD except that it has a more extended onset and duration. A tendency towards cerebellar ataxia is the initial predominant neurological sign. A large number of amyloid plaques are present among the spongiform encephalophatic changes observed in the brain. It has been transmitted to monkeys and rodents by intracerebral inoculation and to hamsters merely by the insertion of the human abnormal PrP gene from chromosome 20 into the hamster genome.

Alpers disease

Alpers disease represents a group of very rare progressive degenerative disorders of the central nervous system afflicting infants and children. Histopathologically, the disease is similar to CJD. And like the latter, it can be transmitted easily to hamsters but not to guinea pig by intracerebellar inoculation. Unlike CJD there is fatty degeneration of the liver in Alpers disease.

Fatal familial insomnia

Fatal familial insomnia (FFI) presents with an untreatable insomnia and dysantonomia. Details of its pathogenesis are largely unknown. Onset is much earlier than in the case of CJD or GSS disease. Visible end results at post-mortem are non-inflammatory lesions, vacuoles, amyloid protein deposits and astrogliosis. As the name implies, it runs in families.

Feline spongiform encephalopathy

FSE was first reported in May 1990 in a five year old male Siamese cat. Since then many cases have been reported in the UK. The epidemiology of FSE is unclear at present. Attempts to find previous cases among demented or neurologically degenerate cats have been unsuccessful. Its histopathology is similar to that of other TSEs. It is now believed that FSE has been caused by the presence of the BSE agent in feline food. However, the owner of the cat first reported with FSE

denies having fed his pet tinned cat food, insisting that always fresh meat was given.

Zoonotic spongiform encephalopathy (ZSE)

Zoonotic TSE has been reported since 1986 in an eland, a nyala, an Arabian oryx , a kudu, a gemsbok, a cheetah, a puma, and an occlot in a British zoo. Various offspring of the mother kudu have died of the disease, and the possibility of vertical transmission cannot be ruled out. They became unwell after the appearance of bovine spongiform encephalopathy on British farms. They might have been infected from the same source as the cattle or from BSE contaminated food stuff. No TSE in these species of animals had been reported before the outbreak of mad cow disease.

Scrapie

Scrapie is probably the oldest known TSE. Affecting sheep in many parts of the world, it has been prevalent for over 200 years. Scrapie probably originated in Spain and spread to the whole of Western Europe. The export of strictly bred sheep from Britain in the 19th-century is thought to cause the rapid spread of the disease in other countries. Sheep imported into New Zealand or Australia are kept in quarantine for several years before being allowed to mingle with local animals. The procedure seems to have been successful in keeping the disease at bay. It makes the entry of an infected animal into these countries difficult. Yet, some sporadic outbreaks in low numbers in unconnected flocks have

taken place. The practice of slaughtering infected flocks in an attempt to eradicate scrapie is followed in certain countries such as the US, but such measures have been largely unsuccessful in the UK. Failure to recognise the symptoms and report it well in advance to the authorities appears to be the main factor in the persistence of this disease. Among the well-known symptoms of scrapie are irritability, restlessness or excitability of the sheep at the onset, with scratching, biting or rubbing of the skin (hence the name scrapie); patchy loss of wool, tremors, loss of weight, weakness and in some animals impaired vision. The disease is always fatal. Only a small number of animals in a herd show the clinical symptoms. Lambs of scrapie–infected sheep are likely to develop the disease later in life. The infective agent has been found to be present in the membrane of the embryo but not in the colostrum or milk of the mother. Many cases of scrapie appeared following the accidental contamination of a louping-ill vaccine. However, the mode of transmission in most cases of scrapie seen on farms is unknown.

In 1936 it was shown that scrapie could be transmitted to a healthy sheep by intraocular inoculation of a tissue homogenate of scrapie infected sheep brain. Intracerebral injection requires an incubation period of as low as two months but on farms only sheep older than four years shows signs of the disease. There are two likely methods of transmission in sheep,

1. Pasture contaminated with placental tissue carrying the infectious agent, when ingested can cause the infection.

2. Though not confirmed unequivocally, transmission from an infected mother during birth is a distinct possibility.

With considerable foresight, Herbert B. Parry suggested that it might be a genetic disorder and could be eradicated by selective breeding.

Transmission of spongiform encephalopathies

These diseases get transmitted when nerve tissue from an infected animal is transmitted intracerebrally into a healthy animal. TSE infection has been found in most tissue tested so far. However, the kind of tissue which become infective, and their capacity to cause infection, varies with the species of the animals. The detection of infection in the buffy coat of blood cells has led to the apprehension that CJD may be transmitted through blood transfusion, although no such case has been reported so far. The scrapie agent has been found in the central as well as peripheral nervous tissue and lymphoid tissue. After injection the animal remains asymptomatic for a long period before clinical symptoms develop. During this so-called incubation period, many tissue of the body become infectious. But even at this low titre, they can cause infection if inoculated intracerebrally.

Dose experiments

Scrapie infected brain tissue is exposed to various agents like irradiation or chemicals and 10-fold multiple dilutions are made. Identical volumes of these diluted infective portions are then injected into different un-

infected animals of the same species. The infective dose of greatest dilution, which causes the animal to die even after years of incubation, is defined as an infective unit (IU). The brain of an animal dying of TSE commonly contains between one million and ten thousand million IUs per gram. The oral infective dose of scrapie for a mouse is about 4x10,000 IU, which is about one hundred thousandth of a gram (10^{-5}g) of infected brain tissue. The incubation period of the disease is proportional to the dose of infection. With a very small dose, the animal may die of old age before clinical symptoms of TSE appear. Since the infection shows up in peripheral nerve tissue as well as macrophages or lymphocytes, the exact mode of spread of TSE within the body is unclear. Experiments show that whatever may be the infective agent, it can easily travel from the site of injection or absorption to the brain.

The properties of TSE infective agents can be altered by the host passage in the following ways:

1. Infectious agents from one species can infect animals of another species, but usually with a higher dose.

2. When an animal is infected with TSE agents from another species, it can then go on to infect a greater range of animals than the original species, but with a different dose.

3. Within a species, an infected animal can transmit infection to its healthy counterpart, with a much

lower dose

4. The histopathologic changes observed in an inter-species jump are not the same as those occurring in an intra-species infection. Moreover, the incubation period of inter-species infection is much longer.

Histopathologic changes

Characteristic lesions under the light microscope consists of spongy changes in the neurophil nerve cells and astrocytes, with degeneration and astrocytosis. These changes occur in the grey matter of the cerebrum and cerebellum. The distribution of histopathology, as shown by inbred strain of mice, varies with the strain of the disease and the site of inoculation. For instance, if the infection reaches the brain through the optic nerve, the spongy degeneration is clustered around the occipital lobe. Amyloid plaques may be seen between cells in some TSEs (e.g. kuru, hamster scrapie) and can be stained with Congo red. Electron microscope shows twig like structures, 12-16 nanometers in width and 100-500 nanometers long, which are found only in TSEs and are called scrapie-associated fibrils (SAF).

Nature and resistivity of infective agents to TSEs

Chemical disinfectants, DNase, RNase, UV light, ionising radiation, heat (cooking temperature), and chemicals that react with nucleic acids, all have little effect on the infectiveness of the TSE agent. It decreases dramatically following autoclaving at 135°C for 18 minutes or treatment with 1 M Sodium hydroxide (NaOH), but

neither treatment fully destroys the agent. Internment of the infective tissue in the soil for three years does not destroy its capacity to induce infection. Some proteases and phenols can dramatically decrease the infectiveness of the TSE agent but cannot completely destroy it.

The TSE agent is now thought to be a cellular protein, PrP, which has acquired an abnormal property of association due to abnormal folding and/or mutation. PrP is a sialoglycoprotein produced from a gene normally found in the genome of animals. This gene, which is on chromosome 2 in mice but on chromosome 20 in humans, may be conserved between strains but altered between species. PrP in a normal brain has glycosidic modifications and is held onto the membrane of the cell by a covalent attachment to phospholipid. In scrapie, however, the PrP is present to a greater extent within the membrane and has a different structural form (PrP^{sc}). This modification, whatever may be its exact nature, renders the protein resistant to heat and to most proteases. Only when treated with proteinase K, PrP^{sc} splits into particles of 27-30 KDa. It has also been shown to induce conformational changes in normal PrP, leading to abnormal folding and aggregation. The PrP of patients with GSS disease is mutated such that proline is replaced by leucine at position 102. When this mutated gene is inserted into the genome of a hamster, about one in twenty animals spontaneously develops TSE. CJD cases in Libyan Jews and Slovaks, which are familial, also carry mutation in PrP gene unlike non-familial CJD cases. PrP is thus greatly involved in the infection, and the pathogenesis of the disease, but it is not clear how

PrP becomes the infective agent. Knock out mice have been developed that do not carry the PrP gene and they cannot be infected with scrapie.

Chemical and physical inactivation of TSE infectious agents.

Knowledge yielded by extensive research on inactivation procedures to date can be summarised as follows:

1. The BSE agent survives exposure to formalin saline.

2. The BSE agent is inactivated within 30 minutes by a concentration of sodium hypochlorite known to be effective with the scrapie agent. A concentration of 20,000 ppm (parts per million) of available chlorine for 1 hour is recommended.

3. A solution of sodium dichloroisocyanurate containing the same level of available chlorine as in an effective solution of sodium hypochlorite is not effective with BSE agent.

4. Exposure to 2 M sodium hydroxide for 2 hours does not inactivate the scrapie agent but is effective against the BSE agent.

5. BSE and scrapie agents have survived porous-load autoclaving at 134-138° C for more than 18 minutes.

6. BSE agents have been inactivated by a 30-minute exposure to gravity displacement autoclaving at 132° C.

7. Microwave irradiation does not inactivate the scrapie agent.

8. Mouse brain infected with the 22Å strain of scrapie agent which has been immersed in ethanol for 48 hours remains infectious after porous-load autoclaving at 136°C for 18 minutes. When infected brain is immersed in saline for 48 hours, it was inactivated by the same autoclaving process.

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4

Prion Disease

Human and animal neurodegenerative disorders grouped under the common title of "TSEs", are now also known as "Prion diseases". Their etiology and pathogenesis involves modification of the "prion protein." These diseases can be transmitted among mammals by an infectious proteinous particle called 'prion'. Despite intensive researches over past three decades, no nucleic acid has been found in 'prions'. Instead, a modified isoform of the host-encoded prion protein, designated as PrP^{sc}, is known to be essential to demonstrate infection. Prion diseases occupy a unique place in medical history in that they can manifest themselves as infectious communicable as well as genetic or inherited diseases and sometimes even appear as completely spontaneous or sporadic.

History and background

Scrapie, a disorder affecting sheep, has been prevalent for over 200 years. However, until recently it was not clearly known how the disease was caused or transmitted; except that it caused degeneration of the brain tissues, which themselves or their extracts were infectious. In 1972, Tikav Alper and her colleagues made an astonishing observation that scrapie-infected brain tissue retained their infectivness despite exposure to

ultra-violet or ionising radiation which are known to destroy nucleic acids. This led them to suggest that the scrapie infectious agent might lack DNA or RNA. Spurred by their observation, Stanley B. Prusiner of University. of California, at San Francisco, resolved to purify the elusive infectious agent from scrapie-infected brain so that its chemical nature and composition could be established. In 1982, after years of painstaking research, Prusiner and his colleagues obtained extracts of hamster brain containing almost pure infectious material. A series of tests thereafter quickly established that the infectivity of scrapie did not depend upon the presence of DNA or RNA — procedures known to degrade nucleic acids did not reduce the infectivity. On the other hand, procedures that denature or degrade protein reduced the infectivity. It was Stanley Prusiner who coined the term "prion" for the proteineous infective agent to distinguish it from other pathogens like bacteria, fungi or viruses, all of which bear a nucleic acid genome for replication and survival. Very quickly his Laboratory established that scrapie prions consist of a single protein, which was named "prion protein" (PrP). For his work on prion protein Stanley Prusiner has been awarded Nobel Prize in Physiology and Medicine for the year 1997.

Nature of prions

A spate of investigations or almost an explosion of research followed to understand the nature of this unique protein and the mechanism by which it causes infection and to identify similar proteins for other known transmissible spongiform encephalopathies. This veritable

explosion of research in identifying the gene which codes for PrP and established that the gene resides in the chromosomes of hamsters, mice, humans and all other mammals that were examined. Most puzzling was the finding that PrP genes are also expressed in normal healthy animals. Although the function of PrP is unknown, it certainly does not make the animals sick. The clue to this puzzle came from the observation that PrP found in infected brain was resistant to breakdown by the usual cellular proteases, while the normal PrP was fairly sensitive to these enzymes. Prusiner and his colleagues thus established that scrapie causing PrP is a variant form of normal PrP. They designated the former as Prp^{sc} (scrapie) and the latter as PrP^{c} (cellular).

Transmissible or inherited?

A scrapie-like illness in humans takes a unique form. Sometimes it runs in families and sometimes it appears to have been transmitted. If PrP^{sc} is the infectious agent for such illness, how it can be responsible for both the hereditary and transmissible variants of the illness? In 1988 Kwren Hslow and Prusiner acquired clones of a PrP gene obtained from a man who had died of Gerstmans-Straussler-Scheinker disease. When they compared this gene sequence with the sequence of a normal PrP gene from a healthy individual, they discovered a point mutation. In other words one nucleotide base pair at codon 102, out of more than 750 base pairs, had changed causing the amino acid proline in normal PrP to be substituted by leucine. Discovery of the same mutation in a large number of GSS patients established

the genetic linkage between mutation of the PrP gene and the disease. It was thought that PrP^{sc} is a mutated variant of PrP, which explained the inheritance of the disease. Subsequently many investigators discovered 18 mutations in families with prion diseases. The mutation, of course, can also explain the sporadic appearance of the disease. The normal wear and tear of life constantly exposes our genome to chemical mutagens and irradiation. In the process, it might happen, although rarely, the PrP gene get mutated, albeit a rare possibility, leading to the expression of the PrP^{sc} variant. And when enough PrP^{sc} accumulates to cause extensive damage in brain tissue, the clinical symptoms appear.

How can PrP^{sc} transmit the disease from one animal to another? To establish this, one has to demonstrate that PrP^{sc} can replicate without the participation of nucleic acid, and that it is not necessary for a DNA or RNA to be associated with prions to establish the infection in a new host. Stanley Prusiner and his colleagues conducted a crucial experiment. They created genetically engineered mice carrying many copies of a mutated PrP gene. These animals produced high levels of mutant PrP and eventually all of them displayed symptoms of brain damage and died. The researchers then injected brain tissue from these dead mice into a set of genetically altered mice who were making low levels of the same mutant PrP protein. While uninoculated mice did not become ill, indicating that low levels of aberrant PrP were safe, many of the inoculated mice developed the disease. Moreover, brain tissue from the diseased recipient when transferred to their healthy counterparts

once again caused illness. If the aberrant protein was unable to transmit infection, none of the inoculated mice would have fallen ill.

Mice lacking the PrP gene develop normally, without any apparent physiological or behavioural problem. Thus, loss of the PrP function is unlikely to be the cause of the disease . Nor do knockout mice develop infection when inoculated with prions. Further, animals expressing a reduced level of PrP are resistant to infection, while those over expressing PrP are prone to prion diseases.

Although this experiment does establish transmissibility, it is not clear how infection can come about unless the abnormal protein can replicate itself without the involvement of nucleic acids. It has been suggested that the domino effect can explain propagation of PrP^{sc} in the neurons of the brain. The process begins when a molecule of PrP^{sc} come into contact with a normal PrP and induces it to refold into a scrapie conformation. Of course, it is being assumed that the only difference between PrP and PrP^{sc} is conformational, since it has been established that the infectious variant has the same amino acid sequence as the normal form. This change initiates a cascade of events in which newly converted molecules continue to alter the shape of other normal PrP molecules until PrP^{sc} accumulates to a dangerous level. (It is, of course, possible that molecules that start off being identical can later be modified in ways that alter their conformation and activity, but extensive investigation has yielded no such evidence). Such replication of shape

but not of the molecules explains the development and transmission of the infection without the inclusion of nucleic acid in the infective agent. The fact that PrP is sensitive but PrP^{sc} is resistant to many proteases supports such a view. However, several researchers have isolated various strains of 'prions' and reported differential effects of various isolates on mice. Only the pathogens containing nucleic acid have been found to occur in multiple strains. This has led them to believe that a virus or virino is at the root of scrapie and similar diseases. All attempts to detect nucleic acid in infective 'prion' preparations have failed. Prions if they can take various shapes or multiple conformations instead of just two, and if aggregation and infectivity iscontrolled by conformation of the molecule then, multiple strains and the differential effect, be explained. A recent finding that a protein in yeast, UreZp, might change its conformation by affecting its activity (in one shape the protein is inactive but in the other it is active) lends strong support to such a view.

Protein of various shapes

PrP^{sc} is a protease resistant form of PrP and both are extensively post-translationally modified. No chemical differences between the two forms of this protein have been detected, yet the infectivity ratio between PrP and PrP^{sc} is 1:100,000. How can the PrP^{sc}protein be so highly infectious when PrP is not?

The conversion of the normal form of prion protein (PrP) to its protease resistant isoform (PrP^{sc})

is a key process in the development of scrapie and related prion diseases. Infectious particles are specially rich in PrP^{sc}, and it is this protein, either by itself or in association with other hitherto undetected molecules, which is responsible for the disease.

Recently in an elegant experiment Dr. B. Caughey and colleagues at the Rocky Mountain Laboratory, Montana, have been able to convert bovine PrP to a protease-resistant isoform (PrP^{sc}) in vitro using bovine PrP^{sc} as a catalyst. The parts of the protein molecule needed for this conversion and for the replication or inter-species transmission of the disease are being investigated. This is being done by using recombinant DNA technology to produce native and mutated PrP protein in cells and in transgenic mice.

PrPs from the various spongiform encephalopathies have been sequenced and found to differ from each other in varying degrees. For example, scrapie PrP protein differs from BSE-PrP protein at only seven amino acid locations, whereas the BSE-PrP differs from human CJD-PrP at more than 30 loci. These differences might explain the concept of strains and the species barrier. The latter was discovered as early as 1960 by Pattison, who found it hard to transmit scrapie between sheep and rodents, as though something was preventing prions made by one species from inducing disease in animals of another species. Understanding this barrier is particularly important today in view of the "mad cow disease" epidemic in Britain. It is still not clear if this barrier is strong enough to prevent the spread of prion disease from

cows to humans.

To understand the nature of the barrier, researchers at Pruisner's Laboratory generated transgenic mice expressing the PrP gene of Syrian hamster in them. The mouse gene differed from the hamster gene at 16 positions out of 254. Normal mice inoculated with the hamster prion rarely acquire the disease, but the transgenic mice became ill in two months. Apparently, the barrier resides in the amino acid sequence of the PrP proteins of the two species; more homologous the amino acid sequences of the PrP of donor and host, more easily the host will acquire the disease.

Structure of PrP

The major obstacle to our understanding the mechanism by which PrP—PrP^{sc} conversion occurs is that the secondary and tertiary structures of both PrP and PrP^{sc} are unknown. X-ray crystallography has been hindered by the restricted availability of the protein and its aggregated insoluble nature. It is hoped that recombinant PrP protein will help to bypass these problems. If both PrP and PrP^{sc} are the same molecule but differ only in shape, then the question is : How do these shapes differ?

Recent studies have provided some clues to this aspect. Circular dichroism, FT-IR, and fluorescence spectroscopic measurements on native PrP and PrP^{sc} give estimates of an enhanced alpha-helical secondary structure in PrP, and increased beta-sheet conformation

in PrP^{sc} or its active peptide fragment, PrP 27-30. Thermal or irreversible chemical denaturation study on PrP 27-30 indicates a complex melt of this fibrillar form of protein. Such data and computer simulations of the secondary and tertiary structure of PrP have led F. Cohen and his colleagues to propose a four helix bundle model of PrP. It includes a mechanism involving the switch of one or more of these helices to the beta sheet structure when the normal isoform changes to protease resistant forms .

Further Reading

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Socio-economic Issues

The mad cow disease panic was triggered by a statement in December 1993 from the eminent neuropathologist Sir Bernard Tomlinson, who announced to the world that he had personally decided to forgo the humble hamburger for the fear that he might succumb to the ravages of CJD. Further, there were two reports that farmers, whose farms had BSE afflicted cows, had died of a CJD - like disease. The British press also reported the death of two teenagers who had been eating beef from farms with, reported cases of BSE. A dramatic debate raged in the media. While the government tried to assure the public that beef could be safely consumed, scientific experts and consumer groups disputed this claim. Local education authorities and schools removed beef from their menus. Butchers and supermarket chains reported a drastic slump in beef sales in the pre-Christmas season. Finally, a report by an independent expert advisory committee expressed concern of a possible link between BSE and CJD. By this time panic had spread all over the world, and British beef was banned in Europe and other countries. In March 1996, the British government announced new beef safety measures.

Is beef really a risk?

The world wide incidence of human spongiform disease, CJD, is about 1 per million. It has not been found to vary between meat-eating and vegetarian populations. The incidence in Britain is no higher than that in other countries. Yet a possible link between BSE and CJD is being suspected because of the unusual characteristics of the recent cluster of CJD cases in the UK. A consistent and previously unrecognised pattern of symptoms has been identified in 10 cases. Six of these cases were diagnosed in 1994 and four in 1995. The disease manifested by this cluster is referred to as variant CJD (V-CJD). These cases occurred among younger people in comparison to conventional CJD patients. The average age for classical CJD cases is 63, while among V-CJD cases, it was 27 years. Moreover, the two are histologically distinct. The key histological feature in V-CJD is the presence of multicentric amyloid plaques during the post-mortem examination of sections of the brain. V-CJD cases exhibit typical EEG recording; also, the duration of illness in such cases is only 13 months as against the usual 6 months in classical CJD cases.

The British Spongiform Encephalopathy Advisory Committee concluded that "the most likely explanation at present is that the cases are linked to exposure to BSE before the introduction of the specified bovine offal ban in 1988." While the British government stressed that there was still no proof of a definite link, it accepted that the new evidence gave cause for concern. Scientific opinion is not unanimous about

the current safety of beef. If there is a real risk, it should have been greater for British consumers before the 1988 ban on SBOs was introduced. However, because of the lengthy incubation period for CJD (5-15 years), it will be some time before even tentative estimates can be made of the number of people that are likely to have contracted CJD from beef before 1988, assuming that transmission and a species jump had indeed occurred!

Prevention of BSE in the UK

Since 1989 Britain has taken a number of measures to eradicate BSE, including: (a) making BSE a notifiable disease; (b) prohibiting the inclusion of ruminant-derived proteins in ruminant feed; (c) destroying all animals showing signs of BSE; (d) prohibiting the consumption of milk from affected or suspect cows by either animals or humans (except for milk from a dam to its calf); and (e) stopping human and animal consumption of certain bovine organs like brain, spinal cord, spleen, thymus, tonsils and intestine. Concern about instances of failure to achieve complete removal of the spinal cord from the diet led to measure for the exclusion of the spinal column (excluding the tail) from the mechanical recovery of meat in the UK.

The British Minister of Agriculture, Fisheries and Food (MAFF), stated in November 1995 that the measures taken were recognised by the World Health Organisation, the International Veterinary Organisation, and the EC Scientific Veterinary Committee as being sufficient. The independent Spongiform Encephalopathy

Advisory Committee, which advises the UK government on all aspects of BSE and CJD, also endorsed these measures. It stated that in its view, there was no firm evidence of a link between BSE and CJD and believed that control of animal feed was the most effective way to eradicate BSE. In January 1996, the following measures were announced: (a) carcasses of animals aged more than 30 months must be deboned in licensed plants under the supervision of the (British) Hygiene Service and the trimmings classified as SBOs; (b) the use of ruminant meat-and-bone meal in farm animal feed was prohibited. No changes in current food safety advice were recommended. The SEAC stressed the importance of proper enforcement of current measures and recommended that the process for the complete removal of the spinal cord must be subject to constant supervision.

In May 1996, the MAFF proposed steps towards the complete exclusion of meat-and-bone meal from all farm animal feeds. The term used to refer to the banned items was changed from 'SBO' to "SBM" (Specified Bovine Material). The new removal requirement specified the whole head of bovines as SBM. In addition, meat from animals over 30 months of age was not to be sold for human consumption, such animals were to be rendered and incinerated. The cattle in this category were those at highest risk, and it was expected that these measures would reduce BSE cases by 10-15 per cent, in addition to the 40 per cent yearly reduction reported following the ban on SBO. The MAFF estimated that 1 million high risk cattle would be slaughtered during the first year.

European Union's response

Several countries suspended imports of British beef following the announcement about the possibility of a link between BSE and CJD. On 25 March, 1996, all the members of the European Union (EU), except Denmark and Republic of Ireland, banned British beef. Germany banned imports of beef from Switzerland as well, which had reported cases of BSE.

Prevention of BSE in the US

To prevent BSE from entering the United States, the APHIS has restricted the import of live ruminants and ruminant products from countries where BSE is known to exist. Other products derived from ruminants, such as fetal bovine serum, meat-and-bone meal, offal, fats and glands, also cannot be imported into the United States from these countries, except under a special permit for scientific research purposes.

The APHIS leads the US interagency effort to co-ordinate the surveillance of BSE. Officials of the USDA's Food Safety and Inspection Service notify APHIS of cattle showing signs of neurological disorders at slaughter. State diagnostic laboratories and public health officials also submit the brains of rabies-negative cattle to the National Veterinary Service Laboratories. (NVSL) for testing.

In addition to international import restrictions, the APHIS has intensified surveillance efforts to detect the accidental introduction of BSE into the United

States. More than 250 APHIS and State veterinarians specially trained to diagnose foreign animal diseases, regularly conduct field investigations into suspicious disease conditions.

The APHIS veterinary pathologists and field investigators have also received training from their British counterparts in diagnosing BSE. These pathologists examine brain tissues of cattle over 2 years of age which show signs of neurological disease.

Detection hurdles

At present, the only way of making a firm diagnosis of CJD (or BSE) is to examine the brain tissue after death. A brain biopsy while the patient (or cow) is alive is hazardous and may fail to show the presence of the disease. A pre-mortem diagnostic test which can identify humans and animals afflicted with transmissible spongiform encephalopathy needs to be urgently developed. In September 1996, *The Times* (London) reported that American scientists had developed such a test for CJD. The test, developed by scientists at the California Institute of Technology, Pasadena, and the National Institute of Neurological Disorders, Bethesda, Maryland, detected CJD in 68 out of 71 suspected patients and in 26 out of 30 animals with suspected TSE. A total of 186 humans and 94 animals were tested, according to the *New England Journal of Medicine*. The test requires a spinal tap, a generally safe but unpleasant procedure. Samples of the spinal fluid are then checked for the presence of telltale protein (a simpler version

which can be carried out in the farm or in a doctor's office is being developed). The test correctly identified 96 per cent of patients with CJD and also ruled it out in a similar percentage of those suffering from other types of dementia. This new test does not identify any infective agent. Instead, it works by detecting a substance in the spinal fluid, the 14-3-3 protein, which is thought to leak from the brain cells damaged by CJD or mad cow disease. The 14-3-3, a normal brain protein, appears in unusually high quantities in the spinal fluid of people with CJD, cows with BSE or other animals with similar illness. This probably results from the destruction of nerve cells in the brain and explains why it also shows up soon after a stroke. Dr. Michael Harrington of the California Institute of Technology, USA, developer of the test, acknowledges its drawbacks. The test can reveal the disease only about the time symptoms start to appear and not during its long incubation period. Thus, the test cannot be used for screening and it can even produce false positive results in people who have recently suffered a stroke or encephalitis caused by herpes simplex virus.

The test, details of which have been published in the *New England Journal of Medicine* (vol. 335, pp.924-30, 1996) is basically an immunoassay for the 14-3-3 brain protein. In patients with dementia a positive assay in cerebrospinal fluid strongly supports diagnosis of Creutzfeldt-Jakob disease . This finding does not support the use of the test in patients without clinically evident dementia.

It is not yet clear why the detection of 14-3-3 protein in cerebrospinal fluid is a useful and specific biochemical marker for transmissible spongiform encephalopathy. The role of this protein in the pathophysiology of CJD is yet to be determined. This highly conserved protein is found in a broad range of species, including yeasts, plants, insects and mammals. It is a normal neuronal protein having several isoforms and possibly plays a role in the conformational stabilisation of other proteins. Since misfolded prion proteins are the central feature of spongiform encephalopathy, an intriguing possibility is that the 14-3-3 protein may be centrally involved in the molecular pathological features of CJD, BSE and scrapie. Prion protein purified from the brain of a patient with CJD does not cross react with the antibody against the protein 14-3-3, confirming that the latter is not the prion protein. The 14-3-3 protein is abundant in an extract of normal human brain but is not found in normal serum or serum from CJD patients.

In June 1996, scientists from nine European countries met in London to discuss the latest developments in research into BSE and its possible links with CJD in humans. In his address to the gathering, Prof. Philippe Lazar, Chairman of the European Science Foundation Standing Committee of European Medical Research Council observed:

"There is now widespread acceptance that transmission of BSE to humans may have occurred. If this is so, I would find it unlikely that we have seen all the cases

we are going to see, if the incubation period is of the order of five to ten years. I would not be surprised if there were tens of cases next year. If it becomes larger than that, then one would begin to be worried".

Some scientists apprehend that tens of thousands of people might have been infected with BSE and that there may be a CJD epidemic in the coming years. Peter Smith, Head of Epidemiology at London School of Hygiene and Tropical Medicine said: "If this is indeed a BSE related epidemic I would expect more cases to occur over the next years. It would not be unknown, with diseases with incubation periods of five years, to persist for five years or more. If it was much longer, one would begin to be worried". It is against this scenario of fears of hidden and misdiagnosed cases, that scientists are making efforts to increase their monitoring of CJD cases. Experts believe that cases of the variant CJD, which is linked to BSE in cows, might have been mistaken for Alzheimer's disease or other kinds of dementia. They advocate similar checks to be made of very young children who have died of neurological disorders. In fact, the World Health Organisation is considering a systematic detection of V-CJD in children and elderly through a large-scale surveillance of CJD around the world. In view of the newly reported pre-mortem test developed by American scientists, effective surveillance becomes a possibility. However, research on prevention and extensive surveillance require substantial funding.

Before BSE crippled the British beef industry it enjoyed extensive exports. Table-5.1 shows the volume

Table - 5.1
Beef exports from the UK

Country Receiving	1990	1995
France	67,000	80,000
Italy	4,000	42,000
Netherlands	9,000	17,000
Spain	1,000	7,000
Other EU countries	16,000	45,000
South Africa	3,000	27,000
Other Non-EU countries	14,000	28,000

(Derived from the Meat and Livestock Commission)

of beef exported from the UK. Approximately 400,000 cattle used to be exported per year, a large proportion of which were calves for veal production. Many of the cattle exported to the Netherlands were in fact just fattened and the meat resold to France, Belgium and Italy. After the scare about BSE infected beef causing CJD, the European Union banned the import of cattle and meat products from the UK. Both export and indigenous consumption have crashed and the livestock/beef industry is in doldrums. The economic damage is enormous. Even more serious is the threat to public health. It is estimated that meat from about 1.8 million infected cattle on British farms will have been consumed by the year 2001. Most of the damage has perhaps already been done. There was under reporting of BSE cases in 1992 and 1993 to the extents 60 per cent. Number of BSE cases by age, predicted by Sterel et. al.(*Nature*, 9th May 1996, p.110), shown in Table 5.2, however, does not take any under reporting into account. To prevent this calamity, Britain is preparing to slaughter and then incinerate 15,000 cows per week for next six years. On 16 April, 1996, Douglow Hogg, the Agriculture Minister, announced in Parliament that: "A previously announced programme to kill and dispose of all cows over 30 months of age at a rate of 15,000 per week will begin on April 29."

The British press is full of stories about the economics of the slaughter but does not dwell at all on the humanitarian, ethical or environmental issues. After all, incineration or mass burial of 15,000 cows per week can pose serious environmental risks that has not

Table - 5.2

Predicted number of BSE cases by age

Age	1995	1996	1997	1998	1999
3	330-490				
4	1520-1860	1000-2000			
5	3360-3870	1510-2120	1090-2280		
6	3120-3650	1790-2410	800-1320	590-1420	
7	3260-3620	1260-1630	720-1080	320-590	230-640
8	1260-1420	1290-1520	500-730	290-480	130-260
9	370-440	570-760	590-860	230-390	130-260
10	150-160	190-240	290-420	300-480	110-210
11+	90-120	100-150	130-230	230-390	210-450
Unkn	420-490	250-340	130-220	60-120	30-60

Actual number of cases reported in 1995. These would represent at least 90% of the total (and probably over 98%).

Age	Number reported
3	435
4	1160
5	3221
6	2942
7	2957
8	1020
9	311
10	117
11+	67
Unknown	523

been studied so far. The slaughter is more a public stunt than a public health necessity. The British farmers have agreed to slaughter because they will be compensated. The EU countries plan to subsidise the slaugther to the tune of 400 million US dollars per year for six years.

How far can we go down the road of slaughter? Mass killing would be insane and never a right answer. The British government has known about BSE for the last 15 years but did not do anything until the epidemic struck. Britain should concentrate on the surveillance and detection of BSE and fund more research for its cure and prevention. After all, it is responsible for the outbreak of this disease among cows by feeding them protein supplements made from scrapie infected sheep and even BSE - infected cows.

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Common Questions and Answers about BSE and TSEs

In this chapter an attempt has been made to provide answers to a set of commonly asked questions regarding BSE and TSEs. The answers are based on a review of the scientific literature and direct communication with scientists studying this problem.

What is BSE?

Bovine spongiform encephalopathy, or BSE, is the appropriate name of a fatal brain disease known to exist in beef and dairy cattle in the United Kingdom (the UK includes England, Scotland, Northern Ireland, and Wales). In this country, the disease was inappropriately named "mad cow disease", a label which has been perpetuated by the media worldwide. The cows infected with BSE are not "mad." Some researchers have suggested that a more appropriate name might be "Sad Cow Disease" because the loss of muscular co-ordination and locomotive function caused by BSE is indeed, pathetic. Efforts are underway to fully understand why the disease became an epidemic in the UK. This information will help ensure that other countries do not acquire BSE.

How widespread is BSE and what has the British government done to control its spread?

Between 1986 and September 1995, an estimated 1,56,000 heads of cattle in more than 32,000 herds were diagnosed with BSE in the UK. The number of cases, which peaked in December 1992, which has declined to less than one-third of that figure and is still declining. A series of precautionary steps taken in the UK have resulted in the decline of newly reported cases. For example, in July 1988, the UK banned the feeding of ruminant-derived protein to ruminants.

What is the incidence of CJD?

CJD occurs at a consistent rate of approximately one case per million people per year around the world, including countries with no cases of BSE. In fact, BSE is found only in limited areas. The incidence of CJD does not vary among vegetarians and meat-eaters, which seems to indicate that its cause is not meat consumption. Scientific evidence suggests several possible causes of CJD, including genetic predisposition and certain surgical procedures.

Is BSE a viral or a bacterial infection?

Scientific evidence suggests that BSE is neither a viral nor a bacterial infection. It seems to point to a protein material or "prion" as the cause of the disease.

How do cattle get BSE?

Scientists conclude that cattle may get the disease from eating protein in feed that was probably contaminated with a spongiform disease agent. Scientific evidence indicates that BSE does not spread from cattle to cattle or from cattle to other species by physical contact.

What are the symptoms of BSE?

Cattle with BSE have impaired co-ordination and are very nervous. In the advanced stages, infected cattle stand away from the rest of the herd and exhibit severe muscular twitching and weight loss.

What are the clinical signs of BSE?

Cattle affected by BSE develop a progressive degeneration of the nervous system. Affected animals may display changes in temperament, such as nervousness or aggression, abnormal posture; loss of muscular co-ordination, and difficulty in lifting their bodies, decreased milk production, loss of body weight despite continued appetite. At present there is no treatment, and the affected cattle die. The incubation period ranges from two to eight years.

Following the onset of clinical signs, the animal's condition deteriorates until it dies. This usually takes from 2 weeks to 6 months. Most cases in the UK have occurred in dairy cows between three and five years of age.

What causes BSE?

The causative agent of BSE as well as other transmissible spongiform encephalopathies is yet to be fully characterised. Three main theories on the nature of the agent have been proposed:

1. An unconventional virus.
2. A "prion" or abnormal proteinase K-resistant protein, devoid of nucleic acid and capable of causing a cell to produce more abnormal protein.
3. A virino or "incomplete" virus composed of naked nucleic acid protected by host proteins.

The agent has the following characteristics:

- It is smaller than most viral particles and is highly resistant to heat, ultraviolet light, ionising radiation and common disinfectants that normally inactivate viruses or bacteria.
- It causes no detectable immune or inflammatory response in the host.
- It has not been observed microscopically.

Can BSE be confirmed in a live animal?

Currently, there is no test to detect the disease in a live animal.

How is BSE diagnosed?

Microscopic examination of brain tissues is the primary laboratory method used to confirm a diagnosis of BSE.

Are there other similar diseases in humans and other animals?

Yes. TSEs are caused by similar uncharacterised agents that produce spongiform changes in the brain. Specific examples include: scrapie which affects sheep and goats; transmissible mink encephalopathy; feline spongiform encephalopathy; chronic wasting disease of mink, mule deer and elk and kuru, CJD, GSS diseases and fatal familial insomnia in humans.

What are the common characteristics of the TSEs?

They are as follows:

- An incubation period ranging from a few months to several years.
- The presence of scrapie-associated fibrils in the brain.
- The ability to transmit the disease to mice by an intracerebral inoculation of brain tissue from the host.

Have TSEs been identified in other animals?

Yes. They are: scrapie in sheep and goats; transmissible mink encephalopathy; and chronic wasting disease of mink, mule deer and elk.

Have other TSEs been identified in other countries?

Yes. Scrapie is reported in most countries of the world. Transmissible mink encephalopathy has occurred on mink ranches in Finland, Russia, the United States, Canada and Germany. Chronic wasting disease has been diagnosed in elk, mule and deer in the United States and in elk exported from the United States to Canada. Other cases of spongiform encephalopathy have been reported in kudu, eland, nyala, gemsbok, domestic cats, and a few exotic cats.

What caused BSE in Britain?

The epidemiological data suggest that BSE in the UK was caused by feed containing contaminated meat-and-bone meal as a protein source. The causative agent is suspected to be derived from either scrapie-affected sheep or cattle with a previously unidentified TSE.

Has BSE been confirmed in countries other than the UK ?

Yes. In addition to the UK, BSE has been confirmed in native cattle in Ireland, France, Portugal and Switzerland. Over 99 per cent of all cases of BSE have occurred in the United Kingdom. BSE has also been identified in cattle exported from the UK to Oman, the Falkland Islands, Germany, Denmark, Canada and Italy. BSE has not been diagnosed in the United States.

Which tissues from BSE affected cattle are infective?

Brain, spinal cord, and retina from naturally-infected animals have been found to be infective. The lower ileum (intestine) from experimentally–infected cattle has been found to cause infection.

What do "SBOs" include?

Specified bovine offals (SBOs) refer to a group of tissues specified by regulations of the British government to be banned from animal and human consumption. SBOs include brain, spinal cord, spleen, intestines (duodenum to rectum), thymus and tonsil.

Which tissues from scrapie affected sheep are infective?

Highest levels of infectivity are found in the brain, spinal cord and lymph tissues. Moderate to minimal capacity to infect was found in a variety of tissues including those of the lung, liver, pancreas, adrenal and pituitary glands.

Does vertical or horizontal transmission of BSE occur?

There is no evidence yet to indicate that BSE spreads by contact from cattle to cattle or from cattle to other species. Epidemiological evidence suggests that maternal transmission, if it occurs at all, is rare.

What is CJD ?

CJD is a slow degenerative disease among humans affecting the central nervous system with obvious dysfunction, progressive dementia, and vacuolar degeneration of the brain. CJD occurs throughout the world at a rate of 1 to 2 cases per 1 million people per year. More rare are the related human TSE conditions of Gerstmann–Straussler Scheinker disease, Kuru and fatal familial insomnia.

Why did the Spongiform Encephalopathy Advisory Committee make the announcement of a possible link between BSE and CJD ?

When contemplating measures in response to an emerging disease, one runs the risk of either acting prematurely and causing an unnecessary panic or not acting soon enough resulting in increased risk to a certain population. Considering the seriousness of the situation, the Committee felt it scientifically, ethically and morally necessary to state what was known and not known about the disease so that consumers, regulatory officials and scientists could then take whatever action they felt was appropriate.

How do we know that the CJD cases were not caused by the scrapie agent?

Scrapie has existed in the sheep population in the UK for 200 years, and has never been shown to be a human health risk. There is no reason to suppose that this has changed.

What epidemiologic and *risk analyses have been conducted in response to the BSE outbreak?*

The USDA continues to analyse and report epidemiological findings and potential risks in the US. In 1991, the USDA issued two reports analysing risk factors associated with BSE in the UK .These were based on the British hypothesis that the disease was caused by feeding cattle scrapie-contaminated meat-and-bone meal. Because of some similarities in the animal industries across the two countries, the possibility of BSE occurring in the US could not be ruled out. However, the probability of occurrence was believed to be very low, as the amount of sheep offal was determined to be 0.6 per cent of all estimated products. Since 1991, the USDA has closely followed scientific findings and has updated its BSE risk factor analyses, first in 1993 and then as recently as February 1996.

Can you get BSE from eating beef?

Scientific evidence indicates that BSE cannot be transmitted from infected cattle to humans through physical contact or consumption of beef or dairy products. The World Health Organisation does not consider BSE to be a human health hazard based on current scientific evidence.

While the BSE infective agent can be detected in the brain, spinal cord and retina of BSE-infected cows, extensive tests have failed to detect it in muscle meat or the milk of infected animals. Consuming muscle meat or

milk, even from an infected animal, is not considered a risk.

Some scientists have suggested that a few people in Britain may have contracted CJD from eating BSE-infected beef. Is that true? In England, of a new form of CJD, called Variant CJD (V-CJD) resembles the New Guinea Kuru disease more closely than the typical CJD.

Recent research in England has used a new test which distinguishes the chemical fingerprint of the infectious prions and found similarities between BSE and V-CJD.

As mentioned earlier, CJD occurs at a consistent rate of approximately one case per million people per year around the world, including those countries where BSE has never been reported. It has a similar incidence among vegetarian groups and meat-eating groups, indicating that its cause is not meat consumption.

CJD occurs in humans in four forms: a familial or genetically inherited form; an acquired, or iatrogenic form due to inadvertent exposure to CJD-contaminated material (as in neurological surgery or corneal transplants); a sporadic form, which though the most common is of unknown origin; and the variant form which has been found in England and has been attributed to BSE-infected bovine products.

There is no unusual incidence of CJD in the US. In October 1996, the Federal Centre for Disease Control and Prevention (CDC) released a report on the

incidence of CJD in the US from 1979 to 1994. The CDC found no cases of V-CJD in the US. And the overall incidence of CJD was slightly less than one case per million population annually — consistent with the incidence of this disease worldwide.

Can animals get BSE from physical contact with each other?

No. Scientific evidence indicates that BSE does not spread from cattle to cattle or from cattle to other species by physical contact.

How widespread is BSE and what has the British government done to control its spread?

Between its reported beginning in 1986 and October 1996, an estimated 163,000 heads of cattle have been diagnosed with BSE in the UK. The number of cases peaked in December 1992, declining dramatically thereafter.

A series of precautionary steps taken in Britain have resulted in the decline of newly reported cases. For example, in July 1988, the government banned the feeding of ruminant-derived protein to ruminants. In addition, BSE has been made a notifiable disease and animals showing signs of the disease are destroyed. Currently, fewer than 30 cases are occurring per week.

What steps has USDA taken to prevent BSE from entering the US?

To prevent BSE from entering the United States, USDA's Animal and Plant Health Inspection Service has taken the following steps:

* Beginning in 1989, the APHIS banned the importation of live ruminant and ruminant products from countries where BSE is known to exist.

* Since 1991, there has been a voluntary ban in place on the use of rendered products from adult sheep in animal feed.

* In 1986, the APHIS established a programme for BSE surveillance in the US and provided specialised training to 250 APHIS veterinarians who conduct field investigations involving animals with any suspicious symptoms.

* The APHIS veterinary pathologists and field investigators have received training from their British counterparts in diagnosing BSE.

* More than 60 veterinary diagnostic laboratories throughout the United States are participating in the BSE Surveillance Programme (initiated in May 1990), alongwith the National Veterinary Service Laboratories in Ames.

* The APHIS veterinarians have, since 1989, kept

track of a number of cattle imported from Britain between 1981 and 1989 (before the ban on imports went into effect) to check their health status. A total of 499 animals were imported from the UK prior to 1989 and, as of November 1996, only about 40 of these animals remain alive. The surviving animals that are alive are monitored regularly and no signs of BSE have been found. A number of the imported animals were euthanised and tested and no BSE was found.

* Between 1986 and September 1996, more than 5,000 brain specimens from cattle exhibiting possible neurological symptoms have been studied by the APHIS. All samples tested have been negative for BSE.

* The US Food and Drug Administration's Centre for Veterinary Medicine is planning to introduce a regulation that will prohibit the use of potentially infectious animal tissues in the production of animal-derived protein supplements such as meat-and-bone meal. This preventive measure is being contemplated to ensure that BSE does not break out in the USA through infected feed as it apparently did in the UK

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Glossary

Alzheimer's disease : A form of degenerative brain disease resulting in progressive mental deterioration with disorientation, memory disturbance and confusion. The atrophy and eventual loss of neurons and the condition is probably genetic.

Amyloid : An abnormal protein deposited in tissues, formed from infiltration of unknown substances, probably a carbohydrate.

Amyloidogenesis : The production of amyloid.

Amyloid plaques : A glyco protein that builds up and deposits in the brain tissues in an amorphous way.

Amyloidosis : An incurable, multisytemic disease process of uncertain etiology characterised by the interstitial accumulation of amyloid fibrils. The affected tissue have a firm waxy texture.

Astrocyte : A cell comprising one of the major class of neuroglia, characterised by relatively large size, ample cytoplasm, microfilaments and extensive radiating processes found in both gray and white matter of CNS.

Astrocytosis : The spread of astrocytes into damaged tissue.

Ataxia : Unteadiness, lack of coordination or

disorganisation of movements in the absence of paralysis due to any of the serveral disorders of central nervous system.

Bioassay : The qualitative estimate of the amount or potency of a substance, such as, drug, or pathogens etc. based on its production of a specific type of biological activity or response either in vivo or in vitro.

Chronic wasting disease (of deer) : A disease found at Fort Worth Zoo only in deer and elk.

Clinicopathological correlations : Pertaining to correlations between clinical signs and symptoms and pathological findings.

Congo red : An azo-dye, sodium diphenyldiazo-bis-α napthylamine sulphonate, used as a biological stain and as an acid-base indicator. It is used to stain amyloid. The dye is found to inhibit the build up of PrP in infected tissue in culture.

Creutzfeldt-Jakob Disease (CJD) : A TSE that gives rise to a pre-senile dementia in human, supposed to be a human prion disease.

DNA (Deoxyribonucleic acid): The biopolymer that carries the genetic information. It is present in chromosomes and chromosomal material of cell organellels such as mitochondria and chloroplasts.

Dalton : A measure of the relative weight of a molecule.

Downer cows : Cattle that show neurological problems (an American term).

Electrophoresis : A technique of separating colloidal particle or macro molecules on the basis of their charge to mass ratio. It involves movement of a mixture of macromolecules like proteins through a gel matrix under an electric current. This is extensively used for separation of proteins .

Epidemiology : The study of the frequency, distribution and causation of disease, both infectious and non-infectious, in a population, based upon the investigation of factors in the physical and social environment.

Famillial Fatal Insomnia : A clinical disease found in humans possibly due to a specific change found in the prion protein, characterized by a fatal sleep disorder.

Genome : The total make-up of the genes of an organism (a life form or virus)

Glycoprotein : A protein that have attached to it some chains of carbohydrate. PrP carries two such carbohydrate chains . It has been suggested that it is different carbohydrate chains that actually separate the strains of TSEs.

Iatrogenic cases : Pertaining to or describing a complication, injury, unfavourable result, or other problem which can be directly attributed to medical care.

HPLC : High performance liquid chromatography by which molecules can be separated from each other.

Immunoblotting : It is a method by which antigens

separated by gel electrophoresis are transferred to suitable membranes are visualised by using antibodies.

Immunohistochemistry : Visualisation of antigens on sections of tissue by using antibodies and a specific chemical reaction.

Immunolabelling : Labelling of cells or sections of tissues by suitable antibodies.

Immunostaining : Staining of cells or sections of tissues by using antibodies.

Infective unit : This is the minimum amount of an infectious agent needed to establish the disease in a particular species.

Kuru : A disease found in the Fore tribe in New Guinea traced back to a custom of cannibalism. The disease occur due to eating of infected brain tissues. In general the women and children ate brain tissues rather than the men and so it was the women and children that died relatively rapidly of the disease.

Lateral (or horizontal) transmission : Transmission of a disease from one animal to another but not from the parent to the offspring.

Maternal transmission : The transmission of disease from the mother to the offspring (not necessarily before birth).

Murine models : A pathological condition or

physiological event occuring in a mouse but analogous to or illuminative of an event occuring in humans.

Neuroanatomy : The branch of neurology concerned with anantomy of nervous system, with the development of new reserach techniques, the field now includes gross descriptive anatomy, study of tracts and pathways, the microscopic and ultra micrscopic study of neural tissue and examination of living neural tissue in vivo or in vitro.

Neurogenesis : Neurogenetics is the field of knowledge concerned with genetic mechanisms underlying early embryonic development as well as cell differentiation, patterns of neural organisation and genetic disorders of nervous system.

Murine Model : A pathological condition or physiological event occurring in a mouse but analogous to or illuminative of an event occurring in humans.

PAGE : The technique for separation of macromolecules on the basis of their charge and/or mass at a specified pH on a poly acrylamide Gel matrix under the influence of a uniform electrical field.

Passage : The inoculation into a series of cultures or experimental animals or other growth media, using material from the initially infected samples.

Pathogenesis : The mechanisms involved in the development of a disease.

PCR : Amplificiation of a piece of DNA in a test tube using an enzyme called polymerase which keeps on catalyzing the addition of nucleotides to a polynucleotide

chain using a primer (a small DNA sequence) complementary to the DNA which is being amplified.

Phenotype :

1.Any identifiable structural or functional characteristic of an organism. It is determined by the combined influences of genotype and the environment.

2.The toal presentation of an organism, due to collective influences of its genome and the environment.

Plaques : A flat lesion. In microbiology—an area of clearing in a confluent cell culture, caused by viral lysis.

(a) Dental plaque : A specific but highly variable material resulting from colonisation and growth of micro organisms on the surfaces of teeth and consisting of numerous microbial species embedded in an extracellular matrix.

(b) Bacteriophage plaque : A clear area created on the surface of culture plate by a phage particle in a lawn of growing sensitive bacteria.

Polymerisation : Polymer is a substance which is formed by the combination or addition of several molecules of a simples substance, often forming chain. The method or reaction of formation of such polymers is polymerisation.

Prion protein : This is the protein that makes up the infectious agent claimed to be the infectious particle that transmits the disease from one cell to another and

from one animal to another. It can exist in various forms. For example PrP is the normal type of the protein that is found in a cell and PrP^{sc} (or PrP-scrapie) is found in the infected cells. The latter may also be called PrP-res, indicating that it is difficult to break down with proteinases.

Prion rods : The microscopic rods that appear when prions, that have been broken up with proteinase K but then allowed to come back together into crystalline forms.

Protease K : An enzyme that catalysis the hydrolysis of proteins.

RNA : A long, unbranched molecule usually consisting of four types of nucleotides linked together by 3'-5' phosphodiester bonds, synthesised in the nucleus by transcription of one strand of DNA.

Scrapie associated fibrils (SAF): Scrapie is an insidious, invariably, fatal spongiform encephalopathy of sheep and rarely goats characterised by intense pruritis and severe tremors. It is thougth to be an infection caused by a prion and is characterised by long period of incubation (2-7 years). The electron microscopy study of brain of these infected animals show presence of fine fibril like structures called Scrapie Associates fibrils or SAF.

SDS (Sodium dodecyl sulphate) : A compound of detergent category. In solution it can attach to proteins and hence allow them to be electrophoretically separated according to their molecular weights.

Species barrier : It appears that a much greater quantity of infectivity is needed to pass a TSE from one species to another than in case of interspecies transmission .

Sporadic cases : Infrequently occurring cases (Disease).

Strains : A clone of organisms that differ in one or more inheritable characteristic from other oganisms from the same species.

Transgenic mice : These are mice created by introducing foreign genetic material into the genome of mice.

Transmissible mink encephalopathy or TME : This is the TSE of mink and has been known for around 30 years, particularly in the mink farms of the USA.

Transmissible spongiform encephalopathy : A disease that can be transmitted from one animal to another and will produce changes in the brain that appear similar to a sponge (i.e. some of the cells are clear when seen down the microscope). Probably the trammitting substance in a prion.

Vertical transmission : The transmission of an illness from the parent(s) to the offspring.

Virino : A structurally complete mature virus.

Viroids : An infectious particle consisting solely of nucleic acid without any capsid structure.

Virus : An obligate intracellular parasite that is smaller than bacterial. Viruses can pass through filters that retain bacteria.

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