NATIONAL DAIRY DEVELOPMENT BOARD ANAND GUJARAT

ANIMAL HEALTH UPDATES

Animal Health Group

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Disease - Bovine Spongiform Encephalopathy (BSE)

Bovine Spongiform Encephalopahty (BSE) or "Mad Cow Disease" falls under a group of diseases called "Transmissible Spongiform Encephalopathies" (TSE). TSE is not known to be caused by any bacteria, virus, fungi or parasite but by prions. Therefore, TSE are also known as "prion" diseases. Scrapie is the original example of TSE. Prion diseases affect both animals and humans as shown in the tables below.

Animal TSE	Species affected
Scrapie	Sheep
BSE	Cattle
Feline Spongiform Encephalopathy (FSE)	Cats
Chronic Wasting Disease (CWD)	Deer

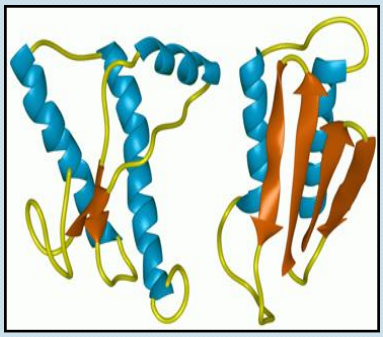
Human TSE
Familial Fatal Insomnia (FFI)
Kuru
Gerstmann - Straüssler -Scheinker syndrome (GSS)
Creutzfelt - Jakob Disease (CJD)
Variant Creutzfelt - Jakob Disease (vCJD)

BSE is an afebrile fatal neurological disorder affecting adult cattle characterized by a progressive degeneration of the central nervous system. BSE affects only adult animals and the incidence within a herd is low. It is believed to be the bovine equivalent of scrapie disease in sheep. Contact with scrapie-infected sheep is not associated with the development of BSE. BSE is of considerable importance as it is strongly linked to development of vCJD in humans and also on account of restriction on international trade of animals and many animal products.

Etiology

All TSE affect the brain and destroy neuron cells in large numbers creating vacuoles or microlacunae in the brain tissue which are visible with an ordinary microscope. The vacuoles are filled with amyloid-like deposits.

Normal animal cells make prion protein which is known as cellular prion protein (PrPc) (**P**rion **P**rotein **c**ellular). The word "prion" actually stands for "proteinaceous infectious particle" and so should only be applied to the pathogenic variants.



A schematic representation of normal prion (PrPc) (on the left) and misfolded Prion (PrPSc) (on the right)

Source :www.bio.davidson.edu

It is believed that this protein which is capable of changing shape is involved in the formation of memories.

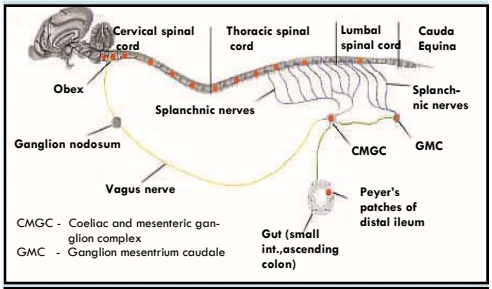
The prion protein has about 250 amino acids. Proteins are not linear molecules as the properties of different amino acids make the protein to fold into a particular shape or conformation. A protein's conformation is a factor in determining it's biological properties and functions. The theory of '*Protein only replication*' is that the abnormal prion PrPSc (Sc stands for Scrapie, the prion disease of sheep) can convert normal cellular prions (PrPc) to abnormal prions (PrPSc) by "flipping" their shape or conformation. These flipped rogue prions can go on to infect other cells or animals. A chain reaction occurs where the newly converted PrPSc converts other PrPc with which it comes into contact. This conformational conversion has been experimentally observed at the cell membrane surface of neuron cells in the laboratory.

Pathogenesis

There is limited information on the pathogenesis and development of BSE, but it is assumed that it is similar to that of Scrapie. The first PrPSc deposition has been observed in the brain stem 24 months post-infection. The onset of clinical signs and pathological change in the brain occur approximately at the same time.

Following ingestion or inoculation, PrPSc may accumulate in the lymphoid tissue (Peyer's patches) of intestine. From here they probably spread through two routes involving the autonomic nervous system (ANS) namely the (a) sympathetic and (b) parasympathetic innervation of the gastrointestinal tract (GIT) to the spinal cord. (*Christine et al., 2007*).

The PrPc in the neurons continues to be flipped and the resulting PrPSc accumulates in small vesicles in the cell. PrPSc is resistant to degradation by the enzymes contained in the lysosomes.



A schematic representation of pathogenesis: Regions of distinct PrPSc deposition are shown in red. Blue-sympathetic nerves; yellow-parasympathetic (vagus) nerve and green-mixed autonomous fibres are the most likely routes of BSE prions from gut into the brain via ANS.

Source : vir.sgmjournals.org

Occurrence

BSE was first described in 1986 in the UK. Since then till July'08, around 1,90,368 cases have been reported to Office International des Epizooties (OIE). The majority of cases (1,84,561) have been from the UK itself.

S.no	Country	Cases	First report
1	Austria	6	2001
2	Belgium	133	1997
3	Canada	14	1993
4	Czech Republic	28	2001
5	Denmark	15	1992
6	Finland	1	2001
7	France	984	1991
8	Germany	415	1992
9	Greece	1	2001
10	Ireland	1626	1989
11	Israel	1	2002
12	Italy	141	1994
13	Japan	34	2001
14	Liechtenstein	2	1998
15	Luxembourg	3	1997
16	Netherlands	84	1997
17	Poland	61	2002
18	Portugal	1043	1990
19	Slovakia	23	2001
20	Slovenia	8	2001
21	Spain	717	2000
22	Sweden	1	2006
23	Switzerland	464	1990
24	USA	2	2005
25	UK	184561	1987

Cases are now on the decline after strict control measures have been adopted. In 2007,the annual incidence rate (number of indigenous cases per million bovines aged over 24 months) was still the highest in the UK at 13.52 , but it has come down drastically from an annual incidence rate of 6636 in 1993. BSE has a long incubation period which is usually between 4 to 5 years. However, the youngest age at onset has been recorded at 22 months and oldest at 15 years. Though the clinical course is variable, case fatality is invariably 100%.

Risk assessment of BSE

The European Commission (EC) had initially adopted an approach for BSE risk assessment called the Geographical BSE risk Assessment (GBR). Under this, countries were classified from GBR I (highly unlikely) to GBR IV(BSE confirmed at more than 100 cases per million adult cattle). This has become redundant after EC decided to follow the OIE classification given below.

The OIE has categorized countries, zones or compartments into three levels according to the likelihood of the presence of BSE-infected animals in the domestic cattle population.

⇒ Negligible BSE risk
⇒ Controlled BSE risk
⇒ Undetermined BSE risk

OIE approves and publishes the list of countries with negligible and controlled BSE risk every year during the annual general session based on the documentary evidence submitted by each country on the risk assessment as per OIE guidelines. The list as on May'08 is given in the table below. This list is reviewed and updated every year. As per OIE database, India has never recorded any case of BSE.

OIE Category	List of Countries
Negligible BSE risk countries	Australia, Argentina, Finland, Iceland, New Zealand, Norway, Paraguay, Singapore, Sweden, Uruguay
Controlled BSE risk countries	Austria, Belgium, Brazil, Canada, Chile, Taipei, Cyprus, Czech Republic, Denmark, Estonia, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lichtenstein, Lithuania, Luxembourg, Malta, Mexico, Netherlands, Poland, Portugal, Slovak Republic, Slovenia, Spain, Switzerland, United Kingdom, USA.

OIE has also recommended that the trading of following commodities can be carried out regardless of the BSE risk status of the cattle population in the exporting country:

- Milk and milk products
- Semen and in vivo derived cattle embryos collected and handled as per International Embryo Transfer Society recommendations
- Hides and skins
- Gelatine and collagen prepared exclusively from hide and skins
- Protein-free tallow and its derivatives
- Dicalcium phosphate (with no trace of protein or fat)
- Deboned skeletal muscle meat (excluding mechanically separated meat) , blood and blood by-products from cattle 30 months of age or less which were not subjected to a stunning process prior to slaughter

Transmission

Ingestion of meat and bone meal (MBM)

MBM from TSE infected ruminants included in cattle feed appears to be the main source of infection, since meat and bone meal are manufactured from tissues discarded in the slaughter houses and also from dead and down livestock.

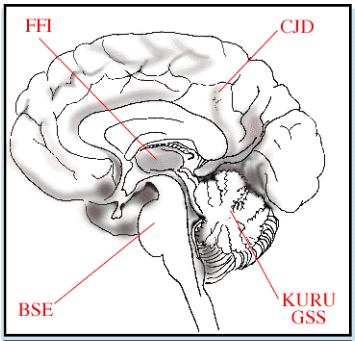
India and many other countries have placed prohibition on feeding cattle with products of ruminant origin.

There is an enhanced risk for disease in calves born to infected cows and this is higher in calves born after the onset of clinical disease in the cow. However, no detectable infectivity has been found in placenta from cows with the disease.

Zoonotic implications

Human TSEs, namely Kuru, CJD, FFI and GSS syndrome are not considered zoonotic. However, there is strong epidemiological evidence that the agent associated with vCJD is zoonotic and similar to that associated with BSE and feline spongiform encephalopathies. In contrast to the traditional forms of CJD, vCJD affects younger patients (average age 29 years, as opposed to 65 years in CJD) and has a relatively longer duration of illness (14 months as opposed to 4.5 months in CJD) and is strongly linked to exposure, probably through food, to BSE infected cattle.

Parts of cattle at high risk of harbouring the infectious agent for BSE include the skull, brain, eyes, vertebral column, and



Schematic representation of areas damaged by different TSE

Source: www.physiol.net

spinal cord. The tonsils and portions of intestine (containing Peyer's patches) also may contain the agent. Public health agencies have prohibited the use of these parts of cattle in the production of human foods to reduce the risk of infection.

While study of possible transmission of vCJD through blood continues, some countries like UK no longer source plasma from its inhabitants as a precautionary measure. Some other countries have prohibited blood donations from persons who have resided in countries with higher risk of BSE.

BSE agent may transmit via conjunctiva, nasal mucosa and cutaneous abrasions. Therefore, veterinarians and animal handlers should take appropriate precautions when handling the tissues of infected animals.

Clinical findings

The disease is insidious in onset and the clinical course progresses over several weeks to 6 months. There is a constellation of clinical signs with alterations in behaviour, temperament, posture, sensorium and movement, but the clinical signs are variable from day to day although they are progressive over time.

The predominant neurological signs are apprehensive behaviour, hyperaesthesia and ataxia; a high proportion of cases lose body condition and have a diminishing milk yield during the clinical course of the disease.

Clinical signs in BSE affected animals

Source : www.halat.pl & www.afip.org

Behavioural changes are gradual at onset and affected cattle are disoriented and may stare, presumably at imaginary objects, for long periods. Many throw their head sideways and show head shaking when the head or neck is touched. Other changes in temperament include the avoidance of other cows in loose housing. Affected animals may kick during milking and show resistance to handling. Some cows also show excessive licking.

Relatively early in the course of the disease, there is hindlimb ataxia with a shortened stride, swaying gait, and difficulty in negotiating turns. Knuckling, stumbling and falling with subsequent difficulty in rising is common in the later stages of the disease.

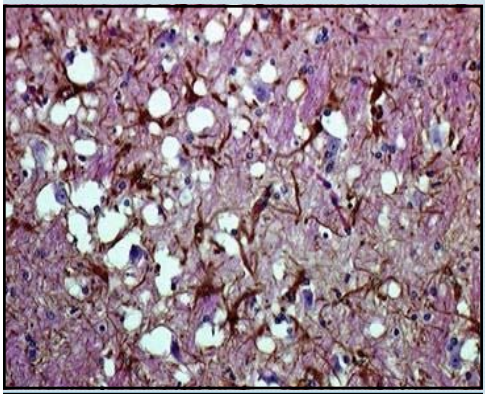
Clinical pathology

Currently there are no diagnostic tests available for confirmation of the disease in live animals . Since immune responses have not been detected in TSE and, in the absence of TSE specific antibodies there is no basis for serological tests. No vaccines are available against BSE.

Diagnosis

OIE has prescribed neuro-histological examination of brain as the confirmatory test for BSE. The correlation between the clinical diagnosis and the neuro-histological diagnosis can, with appropriate experience, be greater than 90%. The pathognomonic lesions are bilaterally symmetrical spongiform change in grey matter neuropil# and neuronal vacuolation of certain brain stem nuclei.

 # - It is the unmyelinated neuronal processes (axonal and dendritic) within the gray matter of the central nervous system.



Prions and vacuolations seen in stained brain section of BSE affected cattle

Source: www.brynmawr.edu

A western blot kit (Prionics® Check WESTERN) manufactured by Prionics® has been certified by OIE as "validated and fit for purpose" in May '08 for post-mortem diagnosis of BSE in cattle.

In addition to the above, immunochemical methods like immunoblotting for detection of accumulation of abnormal Prion Protein (PrPSc) in the CNS of affected animals from unfixed brain extracts and also by immunohistochemical methods in formalin fixed affected brain are methods that are being widely used as confirmatory diagnostic methods and are recommended as adjuncts to histological examination.

A couple of companies also manufacture BSE test kits which are ELISA based for the detection of the abnormal prion protein (PrPSc) in bovine postmortem tissues.

There are only four OIE reference laboratories worldwide as of today which are located in United Kingdom, Switzerland, Canada and Japan.

Differential Diagnosis

Hypomagnesaemia, Nervous form of ketosis, Rabies, Lead poisoning, Listeriosis, Polioencephalomalacia, Tremorogenic toxins (certain mycotoxins) and intra - cranial tumours.

WHO conclusions and recommendations to reduce exposure to BSE agent

- No infectivity has yet been detected in skeletal muscle tissue.
- Milk and milk products are considered safe for consumption.
- Tallow and gelatin are also considered safe if prepared as per guidelines.
- Human and veterinary vaccines prepared from bovine materials may carry risk of TSE transmission.
- Pharmaceutical and cosmetic industry should ideally avoid use of materials from animal species in which TSE naturally occur.
- All countries must prohibit the use of ruminant tissues in ruminant feed and must exclude tissues that are likely to contain BSE agent from any animal or human food chain.
- All countries should ideally conduct risk assessments to determine if they are at risk for BSE.

Sources :

1. Veterinary Medicine,9th Edition , (Radostits et al 2000). A textbook of the diseases of cattle, sheep, pigs, goats and horses. W.B. Saunders Company Ltd.
2. www.rkm.com.au
3. www.fao.org
4. www.who.int
5. Christine Hoffmann, Ute Ziegler, Anne Buschmann, Artur Weber, Leila Kupfer, Ajna Oelschlegel, Baerbel Hammerschmidt and Martin H Groschup. Prions spread via the autonomic nervous system in cattle incubating bovine spongiform encephalopathy. Journal of Virology (2007), 88:1048-1055.

OIE - Significant animal diseases reported to OIE during Apr – Jun'08

Sl.No	Disease Outbreak	Countries reporting
1	Foot and Mouth Disease	Mozambique (not typed), Bahrain (o), Ecuador (o), Columbia (o)
2	Brucellosis (suis and melitensis)	Ukraine & Croatia respectively
3	Highly Pathogenic Avian Influenza	Japan, UK, China, Pakistan
4	Low Pathogenic Avian Influenza	South Korea, Denmark, USA, Haiti
5	Blue Tongue	Australia
6	Trypanosomiasis	Costa Rica
7	Rift Valley Fever	Madagascar
8	Scrapie	Portugal
9	Classical Swine Fever	Bulgaria, Slovakia
10	West Nile Fever	Unite Arab Emirates
11	Enzootic Bovine Leukosis	Finland
12	Malignant Catarrhal Fever	USA
13	Rabies	France
14	New Castle Disease	Germany

Source: www.oie.int

Artificial viral drug delivery system developed

The potential for utilising viruses to deliver drugs and in gene therapy has been recognised for some time but has been held back as the viruses created were the wrong size and shape for drug delivery.

By adopting a different technique for viral creation, researchers from Yonsei University in Seoul, Korea claim to have got round the difficulties encountered previously and created a filamentous artificial virus.

The virus' binding and transfection (process of introducing foreign material into cells) capacity was tested by using small interfering RNA (siRNA), a double stranded RNA with potential in gene therapy.

By using transmission electron microscopy the team established that the siRNA formed a complex with the virus. The transfection capability of the complex was then tested on human cervical cells and was found to be effective.

In addition the structure of the virus enables it to encapsulate hydrophobic molecules and deliver them to cells. The virus was found to be capable of delivering both a hydrophobic molecule, a dye in this case, and siRNA into a cell.

This dual delivery is useful when there are multiple causes of the cancer, as simultaneous delivery of anticancer drug (e.g. doxorubicin) and anticancer gene should improve the potency of anticancer therapy by targeting more than one oncogene.

Source :www.in-pharmatechnologist.com

A new class of anthelmintics - the Amino– Acetonitrile Derivatives (AADs)

Anthelmintic resistance in helminths has been spreading in prevalence and severity to a point where multidrug resistance of nematodes against the three major classes of anthelmintics; the benzimidazoles, imidazothiazoles and macrocyclic lactones, has become a global phenomenon in farm animals. There is therefore an urgent need for an antihelmintic with a new mode of action. A new chemical class of synthetic anthelmintics called amino-acetonitrile derivatives (AADs) is reported in the March'08 edition of *Nature* that are efficacious against various species of nematodes. The AADs are well tolerated and have low toxicity in mammals.

The AADs seem to have a novel mode of action involving a unique, nematode-specific group of acetylcholine receptor subunits.

Source: www.ncbi.nlm.nih.gov

New multi-disease screening chip for livestock

A microarray chip has been developed by scientists at the UK Institute of Animal Health (IAH), which is said to be able to detect up to 300 different viruses that infect animals and humans, including farm livestock and birds.

The microarray chip has already been used to detect infectious bronchitis virus that infects poultry and, foot and mouth disease (FMD) virus.

The microarray chip contains specific small regions of virus genes that react with any viruses in the samples being tested, showing up as coloured spots on glass slides. The method can also be employed to ascertain if a sample contains a number of viruses.

This system that can be used by almost anyone, quickly and accurately to identify the particular virus early-on, which is vital to control these diseases before they spread.

The biggest advantage of the microarray based diagnosis is that disease investigators do not have to know which virus they are looking for. It can be used in the early stages of a disease outbreak to quickly identify the threat to animals.

The chip consists of over 2,800 stretches of genes from over 300 viruses from 36 different virus families.

The cost of the chip is currently high , however the IAH hopes to make some available soon to members of the 'Epizone' project which aims to improve research on preparedness, prevention, detection, and control of epizootic diseases within Europe to reduce the economic and social impact of future outbreaks of foot-and-mouth disease, classical swine fever, avian influenza, and other relevant epizootic diseases.

Source: www.foodqualitynews.com

Clostridium difficile - A new threat ?

Clostridium difficile (called C-diff)), is found in the colon and can cause diarrhea and a more serious intestinal condition known as colitis. It is spread by spores in faeces which are difficult to kill. It has grown resistant to certain antibiotics that work against other colon bacteria, resulting in its flaring up when those antibiotics are given. This virulent strain of C-diff was rarely seen before 2000. The number of persons hopitalised with C-diff infection has been growing by more than 10,000 cases annually in the USA.

Source : health.yahoo.com

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