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Review on Bovine Spongiform Encephalopathy

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Abstract: The principal objective of this seminar paper is to highlight the public health and economic significance of Bovine Spongiform Encephalopathy (BSE). Bovine spongiform encephalopathy is zoonotic and fatal neurological disease of adult cattle caused by unique infectious proteins known as prions associated with ingestion of infectious material in meat and bone meal made from BSE-infected animals. The disease has both an economic and public health importance. The disease results in economic loss through death of cattle and ban of trade. The consumption of food of bovine origin contaminated with the agent of BSE has been strongly linked to the occurrence of Variant Creutzfeldt-Jakob disease in humans. It is a fatal disease that can infect a person for many years before making them sick by destroying brain cells. The disease can be diagnosed using histopathology, immunohistochemistry and rapid test techniques. Banning of infected meat and bone meals are the main prevention and control measure of the disease. In conclusion, the disease remains challenging because the disease is chronic and fatal, lack of cost- effective diagnostic tools in live animals and humans and lack of effective vaccine and treatment. In light of this conclusion, reinforcing banning on the use of infected meat and bone meal, establishing surveillance system and policy and collaborative research toward developing vaccine and treatment are recommended to prevent and control the disease.

Key words: BSE · Jakob Disease · Prion

INTRODUCTION

Bovine spongiform encephalopathy commonly known as mad cow disease is a transmissible, neurodegenerative of cattle. The disease has a long incubation period ranging from thirty months to eight years, with the infectious agent thought to be a specific type of mis-folded protein, called prion. These malformed prions cause other native prion proteins in the brain to mis-fold and aggregate, leading to a spongy degeneration of the brain and spinal cord. Transmission between cattle occurs via the consumption of contaminated meat and bone meal in cattle feed and BSE is fatal, with no known cure or treatment [1].

Apart from the obvious impact on animal and human health, BSE has also had a significant impact on consumer confidence in the meat industry and its worldwide trade, government regulatory practices and animal feed manufacturing processes. It is also dangerous to neglect those individuals infected but without significant clinical symptoms, because the time window from being infected to manifestation of the disease is takes log time [2].

It is believed that BSE may be transmitted to humans who consume infected beef or been exposed to other products derived from the nervous tissues of infected cattle. In humans, the disease is known as a new variant Creutzfeldt–Jakob disease (vCJD or nvCJD) [3].

With the presence of clinical signs, post-mortem identification can be carried out for BSE diagnosis. Post-mortem examinations can reveal microscopic vacuolations of the brain's grey matter and general neuronal degeneration [4]. It can be diagnosed using the Western blotting technique to detect the abnormal prion. There is no diagnostic test available whilst the animal is still alive [5]. Therefore, this seminar paper aims to highlight the public health and economic significance of BSE and to review the diagnosis, prevention and control of the disease.

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Etiology: BSE is caused by a mis-folded isoform of the prion protein (PrP), a widely expressed glycoprotein. PrP is a normal constituent of cell membranes in vertebrates and is encoded by the prion protein. The mis-folded pathogenic isoform protein is often referred to as a 'prion', a term made up from the contraction of the words 'proteinaceous' and 'infectious'. By convention, the normal cellular isoform of PrP is represented as PrPC. The C superscript refers to the cellular form. The prion form has the same amino acid sequence as the normal form and is represented as PrPSc. The Sc superscript is a reference to scrapie, a disease of sheep that is the prototypical animal prion disease. The prions replicate themselves by binding to the normal PrPC protein and acting as a template that coerces the PrPC molecule to refold into the abnormal PrPSc form [6, 7].

There are three strains of BSE that have been identified, although Only one strain, classical BSE, was responsible for the BSE epidemic which started in the United Kingdom (UK) and spread to other countries and the associated epidemic of vCJD in humans [8]. The typical strains, known as H (high) and L (low) type, are diagnosed rarely, typically in cattle of 8-20 years of age and appear to be sporadic and arise spontaneously [9, 10]. Prions are notoriously resistant to inactivation with conventional sterilization procedures used for preparation of surgical instruments and materials. PrPSc is resistant to UV irradiation at 254 nm, 70% alcohol treatment, gamma irradiation and conventional autoclaving (121°C for 20 minutes). PrPSc can be inactivated by severe autoclaving conditions (134°C for 8-18 minutes) in conjunction with detergents and hydrogen peroxide gas plasma sterilization. A number of procedures that modify or hydrolyze proteins can reduce the infectivity of prions. However, while PrPC is protease-sensitive and soluble in non-denaturing detergents, PrPSc is insoluble in detergents and contains a protease-resistant core.

Pathogenesis: After oral exposure of calves to infective material, PrPSc is first observed in Payer's patches of the ileum and detected in gut-associated lymphoid tissue of the ileo-caecal junction and the jejunum. The infectivity is located in macrophages and follicular dendrites cells. Later, infectivity can be identified in the enteric nervous system [11]. It is possible that after crossing the mucosal barrier of the intestine, prions infect the nervous tissue when they come into contact with the fine nerve fibers directly under the intestinal mucosa [12].

Once the nervous system is infected, infectivity then ascends to the brain via both the sympathetic and parasympathetic nervous systems. It has been proposed that orally acquired prion diseases can also reach the brain through the bloodstream, but infectivity is not detectable in the blood of BSE affected cattle. The spread of infection to adjacent cells may occur by transfer of PrPSc-containing membrane micro particles. It could also be transferred between adjacent cells is via tunneling nanotubes, thin membranous bridges that can form between cells and allow the transfer of organelles, plasma membrane components, cytoplasm molecules and pathogen [13, 14].

The propagation within the nervous system also include axonal transport, sequential infection of Schwann cells and via the flow of lymph in the vicinity of neurons [15]. The molecular pathways leading to cerebral damage are largely unknown, although various theories have been advanced. Depletion of PrPC does not appear to be a cause, as mice that have been genetically engineered to lack PrPC altogether and those in which PrPC expression is turned off in adulthood, do not develop clinical signs of BSE. In fact, depletion of PrPC in mice with established prion infection has been shown to reverse early spongiform degeneration and prevent progression to clinical disease. These findings suggest that the toxicity of PrPSc depends on some PrPC-dependent process. It has been suggested that PrPC is neuroprotective and its conversion to PrPSc interferes with this function and allows neurodegeneration [16].

Binding of PrPSc to PrPC triggers a signal transduction pathway leading to neuronal damage Based invitro observations, include impairment of breakdown of cellular waste by lysosomes, up-regulation of genes involved in endoplasmic reticulum function and reduced degradation of proteins by the proteasome system. The accumulation of PrPSc significantly damages cells, causing gliosis and neuronal loss. Furthermore, PrPSc aggregates make plaques, known as amyloids and form vacuoles in the neurons, which cause the typical spongiform structure in infected brains [17].

Clinical Signs: The clinical features of BSE are characterized by apprehension, behavioral changes, fear, increased startle response, or depression, hyper reactivity or hyperreflexia to touch, to sound and to light, ataxia of gait, including hypermetric and paresis, resulting in falling, adventitial movements such as muscle fasciculation's, tremor and myoclonus. Autonomic

dysfunction including, reduced rumination, bradycardia and cardiac arrhythmia and loss of body weight and deterioration in general health condition and reduction in milk yield are also signs of the disease [18-21].

Mode of Transmission: The epidemic of BSE, first recognized in 1986 in the UK, was propagated by the rendering of dead cattle infected with BSE to produce meat and bone meal, which was then included in feed for cattle**.** Ingestion of infectious materials made from BSE-infected animals was the only known route of transmission of the agent between cattle. The main vector of BSE in cattle is meat and bone meal, which, at the time BSE was first recognized, was commonly fed to cattle as a recycled protein source. This animal feed may have become contaminated through the incorporation of carcasses of TSE-affected animals, such as sheep with scrapie or cattle with BSE [22].

There is no evidence that BSE can be transmitted between cattle by routes other than consumption of feed contaminated with certain tissues from BSE infected cattle. It appears that ingestion of less than 1 mg of infected brain material may be sufficient to transmit infection between cattle. Studies have not revealed evidence of risk from semen, milk or embryos. There is a risk of the disease being transmissible between species. For example, it has been identified that humans who consume neural tissue from infected cattle contract the fatal variant Creutzfeldt-Jakob disease [23].

Host Range: Transmission of BSE to other species is possible. This has been documented in experimental infection of several species including domestic cats. In ruminants and large cats kept in British zoos and by the epidemic of the new variant of Creutzfeldt-Jakob disease in humans with over 100 cases in the UK and 4 cases reported from France so far.BSE can be orally transmitted to sheep and goats where it results in a TSE very similar to scrapie. Pigs are highly resistant to oral infection with the BSE prion, but may be infected by parenteral challenge [24, 25].

Epidemiology: The origin of BSE, as a cattle disease, is an issue of controversial debate. The most widely accepted hypothesis is that cattle BSE originated from sheep scrapie i.e. that one of the British sheep scrapie strains was recycled with MBM to cattle and was or became infectious for cattle during recycling. After this adaptation, on-going intra-species recycling caused the BSE epidemic in British cattle. The feeding of MBM to cattle was banned in the UK in 1988 and in 1996; it became illegal in the UK to prepare any feed containing any mammalian protein for any farm animal. However, because of the long incubation period of BSE cases continued to occur, peaking in 1992. It became very the incidence of new cases has steadily declined since then and the disease rare. The infection was spread elsewhere in Europe, Asia (Japan), the Middle East (Israel) and North America by exports of infected cattle and MBM from the UK. Currently, BSE surveillance in European Union countries targets all downer (non-ambulatory) cattle, fallen stock (cattle who die of nonspecific causes), cattle >24 months of age slaughtered on an emergency basis and all slaughtered cattle >30 months of age [26].

Public Importance: BSE captured worldwide attention because of strong evidence indicated its transmission to humans, causing a variant form of CJD. The unusually young age of the patients and their clinicopathologic homogeneity led UK researchers to suspect that the cases may represent an emergence of a new form of CJD resulting from BSE transmission to humans. The occurrence of this variant form of CJD (vCJD) was announced in 1996, approximately nine years after the identification of BSE in the United Kingdom. Absence of similar cases in other countries with comparable surveillance programs, their continued occurrence almost exclusively in the United Kingdom and additional laboratory studies further strengthened the causal link between vCJD and BSE [27].

Consumption of beef contaminated with infected bovine central nervous system tissue led to an epidemic of vCJD in humans. Although the majority of vCJD cases have been attributed to consumption of such contaminated beef, four cases of person-to-person vCJD transmission by blood or plasma transfusion have been reported in the UK [28].Similar transmission of vCJD remains a concern because retrospective analysis of tonsil and appendix specimens led to the estimation that up to 1 in 4,000 persons exposed during the UK epidemic may be a sub-clinical carrier(2).However, the infectious agent, although most highly concentrated in nervous tissue, can be found in virtually all tissues throughout the body including blood. Between 460,000 and 482,000 BSE-infected, animals had entered the human food chain before controls on high-risk offal were introduced in 1989 [29].

The first 10 human patients with vCJD were reported in April 1996 in the UK [30]. As of November 1, 2004, a total of 151 vCJD cases had been reported from the United Kingdom. In addition, three cases (one each from Canada, Ireland and the United States) among persons with potential BSE exposure in the United Kingdom because of their past U.K. residence, 8 vCJD cases from France and 1 case from Italy have been identified. As of December 2012, 227 vCJD cases have been reported in total. The majority of cases (176) occurred in the UK [31].

Economic Importance: The outbreak of BSE has crippled beef industries throughout the world. Not only are nations with confirmed BSE unable to export beef, beef products, or live ruminants, they are also suffering financial loss from the destruction of cattle infected or thought to be infected with BSE. In 1995, before the announcement that there was a probable link between BSE and vCJD, the United Kingdom exported 77,000 metric tons of beef and veal around the world. Immediately after the 1996 announcement, domestic sales of beef products in the United Kingdom fell by 40 percent and within a month, household consumption of beef fell 26 percent from the previous year's level. In the first year of the crisis (1996), the total economic loss from BSE to the United Kingdom was estimated to range between £740 million and £980 million. In the year 2000, the UK was forecast to export less than 2,000 metric tons as new cases of BSE are reported, exports will likely cease [32].

In 1996, the EU banned the use of bovine material in non-food products. Many of these products use processed bovine byproducts such as collagen, elastin, gelatin and tallow derivatives Dietary supplements and medicines have historically used byproducts including components of bovine lung, heart, kidney, spleen and brain. More than eight hundred (800) medicines throughout the world have been suspected to carry a risk of vCJD. In fall of 2000, an oral polio vaccine had to be recalled in England after it was determined that the vaccine used a serum that could have potentially been infected [33].

Food industries have been struggling with losses induced by BSE. Restaurants throughout Europe are decreasing the number of beef dishes on their menus or eliminating beef altogether. European meat wholesalers, who often supply restaurants, have seen demand fall off by as much as 40%. The impact of BSE has affected nearly every channel of the food industry in Europe [34].

Diagnosis: Currently, there is no available test to diagnose BSE in the live animal. BSE can be confirmed by histological examination of brain tissue or by detecting the abnormal form of prion protein (PrPsc) in brain samples. The latter can be done by electron microscopy or immunological methods. When properly prepared samples from brains affected with BSE are examined under the electron microscope, PrPsc will show up as rods named SAFs (scrapie-associated fibrils). Immunological methods include the detection of PrPsc by immunohistochemistry or Western immunoblotting and by the rapid tests based on ELISA or immunoblotting [35, 36].

Histopathology: The microscopic changes in BSE are highly specific and considered pathognomonic [37]. They are degenerative, symmetrical and bilateral lesions distributed to certain regions of the brain stem gray matter. Neuronal vacuolization occurs as two presentations. In the neuropil, 20-ìm vacuoles are observed in neurites; this change is known as spongiform encephalopathy. The other presentation included larger (30-40-ìm), single or multiple vacuoles in the neuronal perykaryon; these vacuoles distend the perykaryon resulting in ballooning neurons that maintain only a thin rim of cytoplasm. The presences of vacuoles in the gray matter neuropil and in the neuronal perykaria are the main criteria for a positive histological diagnosis of BSE [38].

Immunohistochemistry: The IHC examination to detect PrPSc accumulation is applied to sections cut from the same formalin-fixed paraffin-embedded material of medulla at the level of the obex as that used for the histopathological diagnosis [39]. It is possible (but not desirable) to undertake immunohistochemistry for PrP on material that has been frozen prior to fixation. Freezing prior to fixation will not compromise the immune reactivity of a sample, but it may compromise the proper identification of target sites. A positive case will have disease-specific immune labelling in at least one of the diagnostic target areas. For a case to be diagnosed as negative it must be possible to identify the presence of the target areas and to demonstrate that the IHC 'run' was technically successful through appropriate controls. If there is no disease-specific immunolabelling and target areas cannot be identified, the case should be classified as 'unconfirmed' as opposed to negative. Both H and L-type variants demonstrate accumulation of PrPSc in the medulla at the level of the obex [40].

Western Blots: Immunoblotting techniques, are carried out on fresh (unfixed) tissue and can be applied successfully even when tissue is autolyzed [41]. The SAF-immunoblot was the first such method for use in BSE diagnosis. It has similar diagnostic sensitivity to the IHC techniques and remains the method of choice, along with immunohistochemistry, for the confirmation or dismissal of a BSE suspicion. In the last decade, alternative methods have been developed that are less time-consuming and less costly. Most of these techniques use a precipitation of PrPSc using phosphor tungstic acid (PTA) or by other chemicals and some are commercially available. While Western blot methodology is now in general use around the world, analytical sensitivity when used to detect PrPSc varies significantly between methods and laboratories [42].

Rapid Tests: A fast diagnosis of BSE is of importance for the veterinary authorities and the farmer when confronted with an animal thought to have BSE and for protective measures in the food chain during monitoring of the slaughter line process. While histopathology, immunohistochemistry and Western blotting/ SAF detection are reliable tests, they are too laborious to allow a rapid diagnosis. Alternative assays, which are more rapid, less cumbersome and thus adequate for slaughter line monitoring, are under development and are based on the detection of the modified, protease-resistant moiety of PrPSc. At ID-Lelystad, such an assay is now ready for testing under field conditions. It can be performed within 6 hours, needs only a limited number of manipulations and can be automated for mass screening. The method is an ELISA-like test system and is based on PrP-specific antibodies, partial resistance of PrPSc to protease and enhancement of immunoreactivity of PrPSc by denaturation. To make use of this latter property, a dual measurement is performed, increasing both specificity and sensitivity [43].

Prevention and Control Measures

Prevention: The best means of preventing the introduction of BSE is to control the import of certain BSE risk products from countries with BSE or countries that are at risk of having BSE. Introduction of the BSE agent into recipient countries has occurred by live animal trade and by direct or indirect trade between BSE affected and BSE free countries with MBM and other products potentially containing Bovine spongiform encephalopathy [44]. According to the EU's geographical BSE risk analysis for the risk control of BSE, more strict policies and actions recommended to prevent BSE from spreading to countries with poorer conditions [45].

In Animals: Measures to protect animal health include feed bans, proper rendering parameters and avoid specified risk materials**.** Recognition of MBM as a source of infection led to bans on feeding MBM to ruminants in order to break the cycle of cattle re-infection [46]. Rendering parameters indicates rendering of animal by-products (e.g. bovine tissues discarded at the slaughterhouse) and fallen stock into MBM, which is then fed to ruminants, can recycle the agent and allow amplification. When rendering processes are properly applied, the level of infectivity is reduced. It has been determined that batch (rather than continuous) rendering at 133 ºC and 3 bars of pressure for 20 minutes effectively reduces infectivity (providing that the particle size is less than 50 mm) although it does not completely inactivate the agent. Also specified risk materials (SRM) are tissues that have been shown (or are assumed) to contain BSE infectivity in infected animals and that should be removed from the food and feed chains [47, 48].

In Humans: Several important public health preventive measures were implemented before and after evidence of BSE transmission to humans surfaced in 1996. These measures included a 1989 specified risk material ban for human food, a 1996 prohibition of the processing of cattle ≥30 months old for human food and total ban on the feeding of mammalian protein to any farmed animals**.** Excluding SRM and mechanically recovered meat (MRM) from the human food chain effectively minimizes the risk of human exposure and is the most important measure taken to protect consumers. Measures for minimizing risks for human health require the identification and elimination of clinically affected animals before slaughter, which can only be achieved through an adequate surveillance programme. Because the SRM from clinically affected animals is known to contain infectivity, removal and destruction of these animals prior to entering the slaughterhouse have two clearly positive effects: The risk of infective material entering the food and feed chains is reduced and there is less contamination of the slaughterhouse and less potential for cross contamination of normal carcasses. In addition, most countries in Europe have been conducting laboratory testing of all slaughter cattle over 30 months of age (or even younger) for BSE since 2001[49].

Control: As BSE is solely transmissible through the ingestion of BSE-positive meat and bone meal, most countries have banned the use of ruminant-derived feed products. Due to the prion's resistance to destruction, all tissues from the infected animal must be incinerated. Classical control measures for infectious diseases (biosecurity, quarantine, vaccination) do not generally apply to BSE. Some on-farm strategies, primarily those that focus on feed as a source of infection and some culling programs do contribute to the control and eradication of BSE. Culling strategies vary among countries and often change over time. Some different culling strategies that have been applied include herd culling and cohort culling. While herd culling may be a politically expedient means of increasing consumer confidence and facilitating exports, it is unlikely to be an efficient risk management measure [50].

CONCLUSION

Bovine spongiform Encephalopathy is a transmissible neurodegenerative disease of cattle caused by mis-folded protein, prion. Ingestion of infectious material in meat and bone meal made from BSE-infected animals is the major known route of transmission of the agent between cattle. The disease has both public health and economic importance. Humans principally acquire the infection through consumption of beef contaminated with infected bovine central nervous system tissue. The disease is remain challenging due to the fact that the disease is chronic and fatal, lack of cost- effective diagnostic tools in live animals and humans and lack of effective vaccine and treatment.

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