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Review

Pathogenesis and Transmission of Classical and Atypical BSE in Cattle

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Many mammalian species can be affected by prion diseases, also known as transmissible spongiform encephalopathies (TSEs). "Classical" bovine spongiform encephalopathy (C-BSE) was the first prion disease recognized in cattle and it is the only known zoonotic prion disease, having caused variant Creutzfeldt-Jakob disease (vCJD) in humans. Based on the biochemical signatures of disease-associated prion protein (PrP^{Sc}), two distinct forms of atypical bovine spongiform encephalopathies (H-BSE and L-BSE) have been distinguished from C-BSE since 2004. To date there is no comprehensive information about the origin of atypical BSEs (sporadic vs. acquired) and this has an influence on the interpretation of the knowledge gathered from experimental studies, regarding how well such models may represent the real distribution of the agent in the body of naturally affected animals. Moreover, there are only very limited data available concerning the pathogenesis of both atypical BSE forms, as compared to C-BSE. Thus, precautions that are presently taken to minimize the risk of prion contamination of the food supply might not be as effective at preventing the spread of these recently recognized strains. In the last few years a wide range of experimental transmission studies of atypical strains in different animal hosts have been performed. The most recent data on classical and atypical BSE studies concerning characteristics, pathogenesis and transmissions in cattle will be summarized in this review.

Key words: BSE, prions, cattle

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Abbreviations: BASE: bovine amyloidotic spongiform encephalopathy; BSE: bovine spongiform encephalopathies; C-BSE: Classical bovine spongiform encephalopathy; CNS: central nervous system; ENS: enteric nervous system; FDC: follicular dendritic cells; GALT: gut-associated lymphoid tissue; IHC: immunohistochemistry; mpi: months post infection; PrP^{sc}: disease-associated prion protein; PrP^{C}: cellular prion protein; TBM: Tingible body macrophages; TSEs: transmissible spongiform encephalopathies; vCJD: variant Creutzfeldt-Jakob disease; WB: western blotting

Bovine Spongiform Encephalopathy (BSE)

Bovine spongiform encephalopathy (BSE) is a fatal neurodegenerative disorder of cattle that belongs to a group of diseases known as transmissible spongiform encephalopathies (TSEs) or prion diseases. BSE was first identified in the United Kingdom in 1986 and then spread to at least other 28 countries, mostly in Europe, with occasional cases also confirmed in Asia (Japan), the Middle East (Israel) and North America. To date, more than 112.000.000 animals have been examined in Europe, and more than 184.500 cases of BSE have been confirmed in the United Kingdom, 5.500 in Europe and 60 cases in the rest of the world (Brasil, Canada, Israel and Japan). OIE data showed just 7 cases of BSE worldwide in 2015, of which 5 were found in Europe and 2 in Canada; in the current year only one BSE case has been discovered in France. These data confirm that the trend of cases is decreasing worldwide after the implementation of feed bans.

BSE is characterized by the accumulation of a disease-associated abnormal form of prion protein (PrP^{Sc}) in the central nervous system (CNS). PrP^{Sc} is commonly accepted as the pathological agent of TSEs and may be a post-translationally modified form of a normal cellular prion protein (PrP^{C})[1]. It is now believed that BSE may be transmitted to humans who consume infected beef or come into contact with other products derived from the nervous tissues of infected cattle causing the variant form of Creutzfeldt-Jakob disease (vCJD)[2]. BSE has a long incubation period, about 2.5 to 8 years, with clinical disease usually affecting adult cattle at a peak age onset of four to five years, and with all breeds being equally susceptible. Neuronal vacuolation and non-inflammatory spongiform changes in the grey matter are characteristics of the disease in cattle. These lesions are usually, but not always, bilaterally symmetrical. Currently, there are no diagnostic tests to identify TSEs *ante mortem*; the disease is diagnosed *post mortem* by detecting PrP^{Sc} in the CNS by immunodetection methods such as ELISA, western blotting (WB) and by immunohistochemistry (IHC).

Pathogenesis of BSE

The pathogenesis of C-BSE in cattle has been extensively studied using time-kill experimental challenges, although there are still a number of knowledge gaps. After oral exposure to infective material, how BSE agent crosses the epithelium is not exactly defined, but the most likely mechanism is via M-cells, a cell type present in the follicle-associated epithelium of the gut and tonsil which specializes in the transport of macromolecules across the epithelium[3]. These cells are capable of transcytosing the prion protein from the lumen of the gut into the epithelium. During the first 8 months post infection (mpi), the earliest PrP^{Sc} accumulation is displayed by Tingible body macrophages (TBM) in gut-associated lymphoid tissue (GALT) of the ileocaecal junction and the jejunum, and in Peyer's patches of the ileum[4]. Moreover, at 6–10 mpi the infectivity is also located in palatine tonsils[5]. At 12 mpi, a peak of infectivity in the distal ileum is related to the number of follicles involved and the amount of PrP^{Sc} detectable in the follicular dendritic cells (FDC) and TBM, indicating an increased clearance activity of these cells. There is a second peak of infectivity at 24 mpi, where PrP^{Sc} is mainly located in TBM and FDC of jejunum and ileum, and later, a third peak of PrP^{Sc} accumulation between 32 and 40 mpi[6]. During the infection of the gut, the TSE agent can come into contact with the fine nerve fibers of the mucosal plexus of the enteric nervous system (ENS)[7]. Then, through mesenteric nerves, prion proteins accumulate in the cranial coeliaco-mesenteric ganglion complex, and then ascend to the thoracic spinal cord via the sympathetic nervous system (e.g. splanchnic nerves) and to the brainstem and the brain via the parasympathetic nervous system (e.g. vagus nerve) and nodose ganglion. From the thoracic spinal cord, PrP^{Sc} spreads rostrally to the cranial medulla and caudally to the cauda equina[8]. From the spinal cord, PrP^{Sc} then accumulates in the dorsal root ganglia, trigeminal and cervical ganglia[9], and the adrenal glands and sciatic nerve have also been described as positive tissues with demonstrable prion protein accumulation[10]. Between 42 and 84 mpi PrP^{Sc} spreads to the spindles of various muscles such as the masseter, the triceps brachii, intercostal muscles and the semitendinosus[11].

Atypical BSE

Two different atypical BSE strains in cattle were discovered in 2004 in Italy[12] and in France[13]. However, these strains have been also identified in others European countries[14], Japan[15] and the Americas[16]. They were designated L-type and H-type due to the molecular weight of PrP^{Sc} after protease degradation and Western blot analysis. The unglycosylated PrP fraction migrates 1–2 kDa higher in H-type BSE[14] and slightly lower in L-type BSE[12] as compared to

classical BSE (C-BSE). The L-type is also known as bovine amyloidotic spongiform encephalopathy (BASE) because of the presence of PrP-positive amyloid plaques in the brain.

The origins of atypical BSEs remain obscure, and it has therefore been postulated that they represent a spontaneous TSE in cattle, comparable to the majority of sporadic CJDs cases in man[17]. They are mainly detected in cattle that are 8 years of age or older.

Data on atypical BSE cases reported in the EU BSE databases since 2001 show that a total of 44 cases of L-type and 60 of H-type BSE have been identified in Europe. The prevalence of atypical BSE cases in the rest of the world is unknown because there are no official surveillance requirements or systematic reports from different countries.

Five cases of BASE were found in Italy and a detailed study has been conducted on a 15 year-old fallen stock cow of the Piedmontese breed, identified by the active surveillance system. Several tissues have been sampled and the PrP^{Sc} distribution and pathological and molecular features have been investigated by IHC and WB in both neural and extraneural tissues. The presence of PrP^{Sc} was detected in CNS by different diagnostic methods but no PrP^{Sc} was observed in any of the analysed peripheral tissues. Immunobiochemical and immunohistochemical studies in all brain areas showed molecular features and a PrP^{Sc} deposition pattern consistent with a BASE case. Neither clinical signs nor any history of the consumption of feed containing meat and bone meal were recorded, strengthening the hypothesis of a sporadic origin for this disorder. PrP^{Sc} in the trapezius, biceps femoris, semitendinosus and peroneus muscles was identified by IHC for the first time and, in order to test their infectivity, bioassays in transgenic mice overexpressing bovine PrP (Tgbov XV) were conducted, that confirmed infectivity in a variety of skeletal muscles from cattle with natural and experimental BASE[18].

Transmission Studies of Atypical BSE in Cattle−first Passage

Experimental transmission studies in animals with both atypical BSE forms have been performed at different institutes and many papers have been published in these years. Most of the transmission experiments involved mice (57%) and cattle (19%), and a small minority macaques, lemurs, hamsters and sheep. Regarding cattle, there are only a few articles about the first passage of experimental transmission studies of atypical BSE in cattle. The first work done by Lombardi described the intracerebral (i.c.) challenge of Friesian and Alpine brown cattle with Italian BSE and BASE isolates. The BASE-infected cattle developed amyotrophic changes accompanied by fasciculations, dullness, and hypersensitivity to facial and tactile stimuli. The molecular and neuropathological profiles closely matched those observed in the original cases[19]. Another study was performed in Japan, where three Holstein calves aged 2–3 months were inoculated i.c. with medulla oblongata from a BSE case (BSE/JP24). Experimental cattle developed an ataxic gait, inactivity and showed little aggression. This experiment demonstrated the successful transmission of L-type BSE, and confirmed that the characteristics of BSE/JP24 prion closely resemble those of BASE detected in Italy and they are different from C-BSE[20].

In another study, eleven calves were challenged i.c., five with German H-type and six with German L-type BSE cases. The incubation time was 15 months for both the atypical BSE types, and the main characteristic in the early stages of the diseases was a loss of condition and depression. Immunoblot analysis revealed that the atypical banding patterns of the respective inoculum were maintained after this passage in cattle[8].

In the study by Konold et al, groups of four calves were inoculated i.c. with either L-BSE or H-BSE brain homogenate. All the animals presented a nervous disease with some similarities to C-BSE, which progressed to a more dull form in one animal from each group, but difficulty rising was a consistent feature of both disease forms. Both the atypical types were readily detectable by confirmatory methods using the medulla brain region at the level of the obex[21].

Pathogenesis of Atypical BSE

A limited time-kill study of intraspecies transmission of a Japanese case of L-type BSE showed that, using WB, PrP^{Sc} was first detected in peripheral tissues in the nerve roots and subsequently in the peripheral nerves. This study demonstrates that, following involvement of the brain, almost all the peripheral nerve tissues tested become positive in a time dependent manner, whereas no PrP^{Sc} is detectable in lymphoid tissues. These result suggested the possibility that, L-type BSE prions propagated in the central nervous system and were spread centrifugally by nerve pathways[22].

Currently, it is very difficult to hypothesize about the pathogenesis of atypical BSE based only on data from the literature, because information on the tissue distribution of PrP^{Sc} in cattle affected by atypical BSE is limited, and largely

confined to animals at clinical end-point. According to experimental transmission studies, PrP^{Sc} has been reported in CNS tissues, peripheral ganglia and nerves, muscles (muscle spindles), adrenal glands and retina for both H-BSE and L-BSE. No lymphoid tissues or gastrointestinal tissues have tested positive in atypical cases[4–6,8,11,20,23–29].

Conclusions

Many fundamental questions regarding atypical cases remain unanswered. In particular, it is speculated that the origin of these atypical cases is sporadic and the possibility of the spread of disease is still unclear. The spread of C-BSE has been confirmed as a common source epidemic linked to feed, but without any definitive resolution of its origins. Furthermore, we have limited information about the clinical signs and pathology of atypical cases; data are more detailed in studies of C-BSE whereas for atypical BSE we only have a small amount of experimental data. Regarding PrP^{Sc} tissue distribution, data are very limited, with samples available from only a few field cases of L-type BSE, and none from H-BSE cases. The i.c. challenge experimental models could be an appropriate proxy for studying the distribution of the agent if the origin of the disease is spontaneous and originating in the brain, but may have limited relevance for foodborne risk assessment.

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