Atypical H-Type Bovine Spongiform Encephalopathy in a Cow Born after the Reinforced Feed Ban on Meat-and-Bone Meal in Europe

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The significance of atypical bovine spongiform encephalopathies (BSE) in cattle for controlling the BSE epidemic is poorly understood. Here we report a case of atypical H-type BSE in a cow born after the implementation of the reinforced feed ban in Europe. This supports an etiology of H-type BSE unrelated to that of classical BSE.

CASE REPORT

A 6.5-year-old Red Angus cow presented downer cow syndrome after the birth of a dead calf in Switzerland in February 2012. The animal was born in Germany in 2005 and imported into Switzerland at the age of 17 months. According to the owner, there were no signs of illness of the cow prior to giving birth. After emergency slaughter, medulla oblongata samples were taken in compliance with the Swiss statutory bovine spongiform encephalopathy (BSE) surveillance. The initial BSE rapid test (Check Western; Prionics) (21) performed by a regional laboratory was positive. Consequently, the medulla oblongata sample was sent, together with the remaining brain, which was still available at the slaughterhouse, to the Swiss BSE Reference Laboratory. There, the animal was confirmed BSE positive with the TeSeE Western blot (Bio-Rad) (2), using limited proteinase K digestion and immunodetection with two prion protein-specific monoclonal antibodies (MAbs), Sha31 (11) and 12B2 (16). Molecular masses of proteinase K-resistant prion protein peptides (PrPres) in the Western blot were determined with Quantity One software version 4.6.2 (Bio-Rad). In comparison to a classical (C-type) BSE control sample, the PrPres bands seen in this case showed ~1.3- to ~1.4-kDa higher molecular masses as well as an additional band at ~7.2 kDa. Also, the sample reacted with MAb 12B2 (Fig. 1). This is consistent with the molecular phenotype of H-type BSE (14). The distribution of the disease-associated prion protein (PrP^d) throughout the brain was determined by enzyme-linked immunosorbent assay (ELISA) (BSE-scrapie antigen test kit; Idexx). PrP^d was detected mainly in the thalamus and the obex and, to a lesser extent, in the cerebellar cortex, hippocampus, lobus pyriformis, and basal nuclei (Fig. 2). Histopathological analysis was performed on hematoxylin-and-eosin (H&E)-stained paraffin sections of the same brain regions as those analyzed in the ELISA. Minimal spongiform lesions were present in the obex region (Fig. 3a) and in the midbrain, but not in other brain structures. By immunohistochemistry (using MAb F99) (17), mild PrP^d deposits were observed in the dorsal motor nucleus of the vagus nerve, the caudal olivary nucleus (Fig. 3b), the cuneate nucleus (Fig. 3c), the hypoglossal nucleus, the spinal tract nucleus of the trigeminal nerve, and the solitary tract nucleus (Fig. 3d), as well as in the midbrain and thalamus. These deposits were of the coarse particulate, intraneuronal, and intraglial type. There was no PrP^d labeling in the cerebellum, hippocampus, basal nuclei, and cerebral cortex. The entire open reading frame of the bovine prion protein was sequenced and revealed no DNA variant in comparison to the reference sequence (GenBank accession no. AJ298878.1). In particular the E211K mutation thought to cause a genetic variant of H-type BSE (19) was not present. After laboratory confirmation of the disease, the carcass of the animal, including all by-products, was destroyed, and no material entered the food chain.

BSE is a transmissible and neurodegenerative disease that emerged in the United Kingdom in the mid-1980s and later in continental Europe, Japan, and North America (26). It is caused by prions, which are misfolded cellular prion proteins (PrP^d) that accumulate in the brain of affected cattle. Prion diseases may either be acquired, (i.e., transmitted by infection), have a genetic basis, or develop spontaneously as sporadic cases (9). Three types of BSE are currently differentiated: the C-, L-, and H-types. While C-type BSE has been by far the most frequent form of the disease, L- and H-type BSEs, also referred to as atypical BSEs, are rare conditions that present biochemically and biologically distinct characteristics from C-type BSE (6, 8). C-type BSE is acquired and prion transmission occurs by the ingestion of infected tissues—in ruminants notably of meat-and-bone meal (MBM) being used as a feed supplement (27). Due to an incubation period of several years, the average age of BSE-affected cattle was 5 to 6 years during the epidemic, but cases have also been identified in much younger and older animals. H-type BSE has only been diagnosed in about 30 cattle worldwide, all over 8 years of age (24). Detection was mostly by active surveillance schemes (i.e., by laboratory testing of large numbers of adult cattle not suspected of having clinical BSE). Biological strain typing by experimental transmission to rodent models and cattle indicated that H-type BSE is caused by a prion agent distinct from those of C- and L-type BSEs (4, 5, 15, 18). Down to the present day, the pathology and etiology of H-type BSE remain poorly understood (24). Especially the question of whether the disease is acquired, genetic, or sporadic leaves a major
gap in our knowledge. The case presented here extends the understanding of H-type BSE in several aspects.

Whole brain pathology of H-type BSE has only been reported in a bull of the miniature zebu breed (23, 25) and in experimentally intracerebrally inoculated cattle (15, 18). For all other natural H-type BSE cases, only brain stem samples were available, and it is not known which brain structures are the best suited diagnostic target. The zebu was 19 years old and showed prominent neurological signs, and spongiform lesions as well as PrPd deposits were severe and widespread in the brain. In contrast, the animal described here was 6.5 years old, central nervous system (CNS)-specific neurological signs were not observed, and spongiform lesions as well as PrPd deposits in the brain were minimal. All of these findings support that the cow was in an early, preclinical stage of the disease. In this regard, it is important to point out that these minimal lesions and PrPd deposits were found in the gray matter structures of the obex region of the medulla oblongata, the midbrain, and the thalamus. These findings are essentially similar to those in preclinical C-type BSE (1, 12, 13, 22) and support that sampling of the obex region in surveillance schemes implemented for C-type BSE might be similarly suitable for detection of naturally occurring H-type BSE.

The main disease control measure of C-type BSE is the ban on mammalian MBM in ruminant feed. This feed ban was enforced in Switzerland and the European Union in the early 1990s and considerably reduced the number of newly infected cattle. However, the recycling of the C-type BSE agent in the cattle population was not blocked until the MBM feed ban was reinforced in 2001, now excluding the use of animal proteins in feed of all farmed animals (10). Whether H-type BSE similarly is transmitted orally in the cattle population with MBM as a vehicle remains unknown. If oral transmission occurs and is the sole etiology, the reinforced MBM feed ban should be an appropriate measure to prevent the spread of H-type BSE as well, and H-type BSE should not be detected in animals born after its implementation, i.e., after 2001 in Switzerland and Germany. To our knowledge, this is the first report of an H-type BSE-affected animal being born after the reinforced MBM feed ban in the respective country. Therefore, this case provides further evidence that the etiology of H-type BSE may be unrelated to the ingestion of prion-contaminated meat-and-bone meal. Taken together, this supports the widely expressed postulate that H-type BSE originates from a spontaneous misfolding of cellular PrP with a pathophysiology similar to that of sporadic Creutzfeld-Jakob disease in humans (7, 20). Alternatively, other yet unknown routes of transmission or genetic determinants must be considered. This said, H-type BSE might persist after eradication of C-type BSE. What are the implications of this scenario? Studies with mice provided experimental evidence that H-type BSE may shift its disease phenotype to that of C-type BSE (3) upon transmission. It has therefore been hypothesized that the C-type BSE epidemic originated from spontaneously occurring H-type BSE cases. If this was the case, there would be a constant risk that C-type BSE would reemerge in the cattle population once the feed ban is discontinued. Consequently, some measures of disease control would need to be maintained indefinitely. Since the standards for the determination of a country’s BSE risk status currently do not differentiate between BSE subtypes (28), BSE risk assessments will certainly need to take such considerations into account. This highlights the need for continuing research into the relationship between classical and atypical BSE variants to provide the scientific basis for future disease surveillance and control policies.
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REFERENCES