

Detection of Atypical BSE Cases

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Since the recognition of bovine spongiform encephalopathy (BSE) in Great Britain in the mid-1980s more than 180 000 cattle have developed the disease. To date, only a single strain of BSE has been found and is thought to have spread to humans by dietary exposure causing variant Creutzfeldt-Jakob disease. Recent findings of atypical BSE cases in Italy, France and Japan suggest that BSE is a more heterogeneous disease than originally thought. Furthermore, the atypical BSE case found in Italy shows molecular similarity to that encountered in a distinct subtype of sporadic Creutzfeldt-Jakob disease.

Transmissible spongiform encephalopathies (TSEs) encompass a group of fatal neurodegenerative diseases in animals and man. The etiology of naturally occurring TSEs seems to com-

prise horizontal and vertical transmission as well as genetic predisposition, yet for the majority of cases the etiology is unclear. The onset of clinical illness is preceded by a long incubation period of months to decades. Clinical symptoms of TSEs include dementia and loss of movement coordination. In the 1980s it was established that a common hallmark of TSEs was the accumulation of an abnormal protease resistant isoform (PrPres or PrPSc) of the host-encoded prion protein (PrPC) in the brains of affected animals and humans. Because PrPSc is the only reliable molecular marker for prion diseases, immunological detection in brain tissue of the protease resistant part of PrPSc is the basis for rapid diagnostic tests.

While the prototype of all prion diseases, scrapie in sheep and goats, has been known for more than two centuries, a new form of animal prion disease designated bovine spongiform encephalopathy (BSE) has since its first recognition in the UK in 1986 developed into an epizoonosis.

To what extent BSE-infected cattle have entered the human food chain is still a matter of debate and has been the subject of a number of studies in the past. Earlier estimates indicated that from a total of 1 mil BSE-infected cattle in the UK between 1980 and 1990 about 870 000 animals could have entered the human food chain [1]. A more recent study published by the Imperial College in London suggested that based on new epidemiological data the numbers might have been

twice as high with up to 1.9 mil BSE-infected cattle of which 1.6 mil could have ended up on the plate of British consumers [2].



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The human prion diseases comprise Creutzfeldt-Jakob disease (CJD), Gerstmann-Straeussler-Scheinker disease (GSS), fatal familial insomnia (FFI) and kuru. These diseases illustrate the three manifestations of prion diseases in general, namely, the sporadic forms of the disease (80–90% of all CJD cases), the inherited forms linked to mutations in the human PrP gene (familial GSS, familial CJD and FFI) and the infectious forms which are acquired by transplantation, injection or ingestion of prion-contaminated tissue-derived products (iatrogenic CJD, vCJD and kuru). With the emergence of a new form of CJD in the UK in 1996, a new episode in the battle against human diseases caused by food-borne pathogens has been initiated. To date, 146 cases of variant CJD (vCJD) have been reported in the UK, and compelling scientific evidence argues for a causal relationship between BSE and vCJD [3, 4]. With no obvious risk factors identified in these patients, a causal link to the exposure of BSE-contaminated food products seems very likely.

Since the first cases of BSE appeared in the UK, the question of the origin of

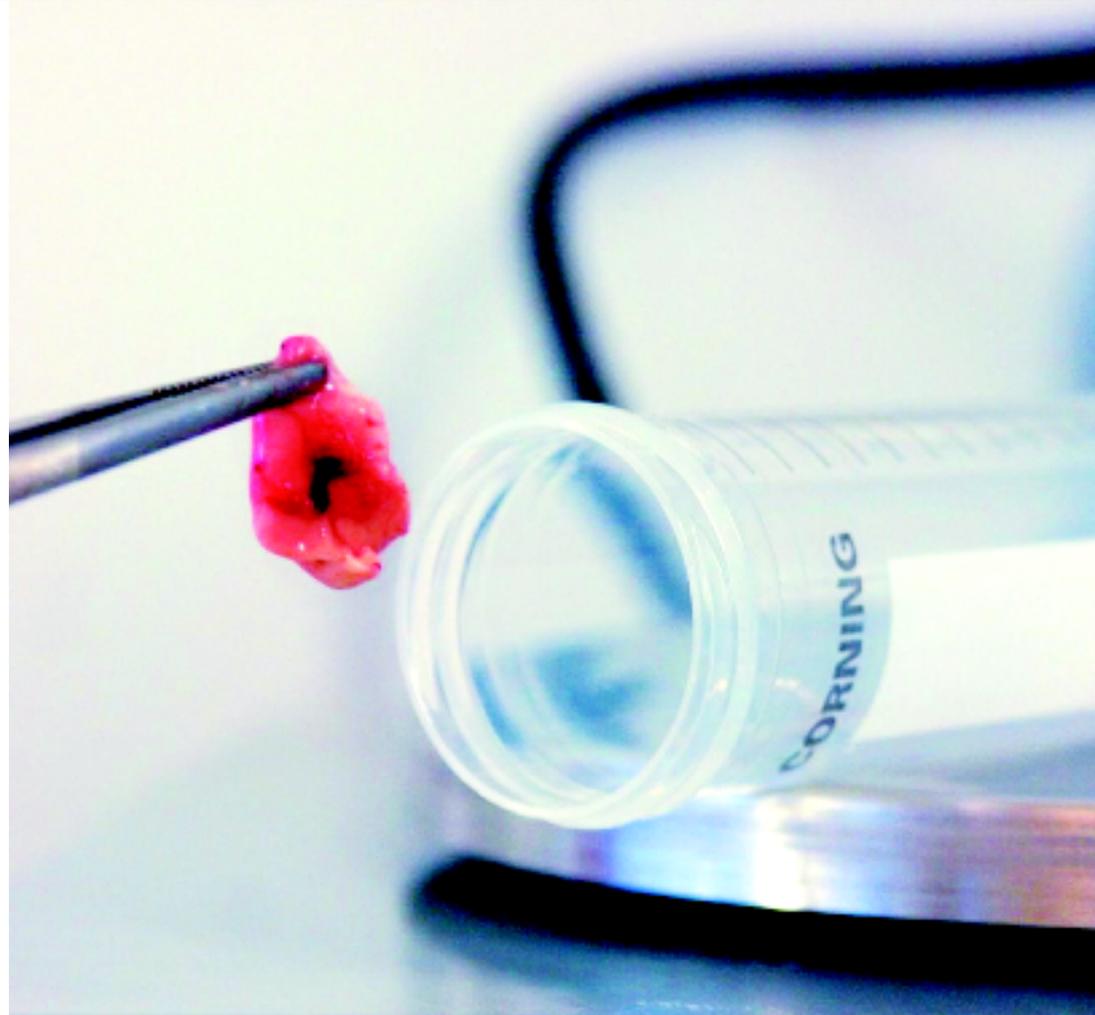
Key Points

- Atypical BSE bovine spongiform encephalitis cases were identified in apparently healthy cattle diagnosed in routine surveillance programs in Italy, Japan and France
- Prions in the atypical Italian BSE cases show similar molecular properties as prions from sporadic CJD
- French atypical BSE cases harbour prions which show similarities to sheep scrapie prions

BSE has posed a major challenge to the scientific community. CJD and sheep scrapie appear as many different strains, while the BSE epidemic in the UK seemed to be caused by a single strain of BSE giving a unique lesion profile in the brain of infected animals [5]. Generally, it is believed that a change in the rendering process for feeding offal to cattle leads to the adaptation and amplification of a previously unrecognized cattle TSE agent and the emergence of a BSE strain with different pathogenic properties [6]. This suggests that the new rendering methods resulted in the amplification of a disease in cattle that had already existed.

But was BSE present in cattle before the 1980s and was simply not recognized due to lack of awareness? In any case, the origin of BSE must have been either a sporadic case of BSE in cattle, similarly to sporadic CJD in humans, or alternatively the epidemic could have started with a special case of scrapie in sheep that could have crossed the species barrier and subsequently led to the adaptation of the agent to cattle during the rendering process. However, since scrapie has been endemic in many countries such as Iceland where no BSE or CJD has ever been reported, the scrapie agent is unlikely to play an important role as a common factor in the etiology of BSE. In addition, the strain of TSE agent causing BSE is phenotypically distinct from any of the many strains of TSE agent causing scrapie.

New evidence has now been published suggesting the existence of more than one strain of BSE and supporting the



notion that the BSE epidemic might have its origin in a sporadic form of BSE occurring at very low frequency in cattle. As the numbers of BSE cases are gradually declining in most countries where BSE incidence is monitored using mandatory surveillance programs, it is likely that atypical BSE cases are now being detected after having been obscured by the presence of typical BSE. The atypical BSE strains identified in Italy [7], France

[8] and Japan [9] exhibited novel molecular and pathological phenotypes and were detected among routinely diagnosed BSE cases following active surveillance of the disease using rapid TSE test for the detection of PrPres in brain tissue of cattle. Table 1 summarizes the atypical cases and their phenotypic presentations. All atypical BSE cases identified in Italy, France and Japan were found in apparently healthy cattle with no clini-

Table 1

Frequency of animals presenting with previously unknown strains of bovine spongiform encephalopathy prions					
Country	Frequency ¹	Age, yr	Unglycosylated PrPSc	Histology	Test used for initial diagnosis
Italy	2/8	15	Low Mr	Plaques	Prionics®-Check WESTERN
		11	Low Mr	Plaques	Prionics®-Check WESTERN
France	3/58	10	High Mr	n.d.	Platelia
		15	High Mr	n.d.	Prionics®-Check WESTERN
		8	High Mr	n.d.	Prionics®-Check WESTERN
Japan	1/9	2	Low Mr	negative	Platelia

¹Frequency refers to the number of atypical cases identified per total BSE-positive animals analyzed in the reported study.

n.d. not done

cal signs suggestive of BSE. Whereas the cases in Italy and France were found in old cattle, the Japanese case was the first BSE case confirmed in an animal less than two years old. This underscores the importance of continued active surveillance of cattle at the slaughterhouse and demonstrates that current postmortem rapid tests are capable of detecting BSE in presymptomatic animals. Characterization of the atypical BSE cases was performed by strain typing using Western blot analysis of the protease-treated PrP^{Sc}. Classically, TSE strains have been characterized through inoculation of brain homogenates into a series of mouse lines. Distinct strains are then differentiated based on incubation time and lesion profile within the same mouse line. These are the most important criteria for differentiation of strains. More recently and with the availability of immunochemical diagnostic methods, a more detailed analysis was made possible based on glycosylation patterns of protease-treated

PrP^{Sc}. On Western blots, Proteinase K-treated PrP^{Sc} is resolved into three bands that correspond to the di-, mono- and unglycosylated prion protein. These bands can exhibit different mobility patterns, presumably reflecting different conformations of PrP^{Sc} associated with different prion strains (figure 1). To date, all BSE cases that were analyzed showed the same PrP glycopattern with the highest intensity of the diglycosylated form of PrP suggesting that a single strain of agent is responsible for BSE [3, 10].

Whereas the Italian and Japanese cases showed a PrP^{Sc} type with predominance and a lower apparent molecular mass of the unglycosylated PrP, the French animals presented with a higher apparent molecular mass of the unglycosylated PrP (figure 1). Based on these results, it was speculated that the Italian and Japa-

nese cases share similarities whereas the French cases represent a distinct BSE subtype. However, further investigations on the pathology of the atypical BSE cattle revealed striking differences between the Japanese and Italian cases. In contrast to the Japanese case which showed no BSE-typical pathology the Italian cases showed PrP-positive amyloid plaques, a feature which has not been associated with typical BSE. These findings prompted the authors to name this disease BASE, for bovine amyloidotic spongiform encephalopathy. Surprisingly, the authors then show that the PrP^{Sc} glycoforms associated with BASE are very similar to the glycoforms found in some types of sporadic CJD (sCJD). This raises the question whether a similar link between atypical BSE cases and sCJD exists as between typical BSE and vCJD. However, it might be too early for such far-reaching conclusions based on molecular characterization of a small number of novel BSE phenotypes. More solid support for a common etiology between BASE and sCJD has to await characterization of these TSE agents by inoculation into mice. Further characterization of the French atypical cases revealed a striking similarity to cattle that developed BSE following experimental infection with sheep

scrapie. Thus, the question was raised whether this represents a scrapie strain that was transmitted from sheep to cattle by contact or through environmental contamination. From the low numbers of cases analyzed so far, it is difficult to predict how frequent these atypical BSE cases in the cattle population are. Nevertheless, assuming a frequency of 1/4 to 1/20 as reported in the three independent studies (table 1), it will be crucial to apply the most stringent testing criteria in order to reliably detect atypical BSE cases in the future. The Italian cases and two of the French cases were identified with the Prionics®-Check WESTERN blot. One of the French cases and the Japanese case were found by the Platelia ELISA test, and the strain characterization was later performed by WESTERN blot analysis. Thus, WESTERN blot-based BSE tests have the advantage that they allow the identification of new BSE strains because detection of PrP^{Sc} is based on several molecular criteria such as immunological reactivity, molecular weight and glycosylation pattern.

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Key References

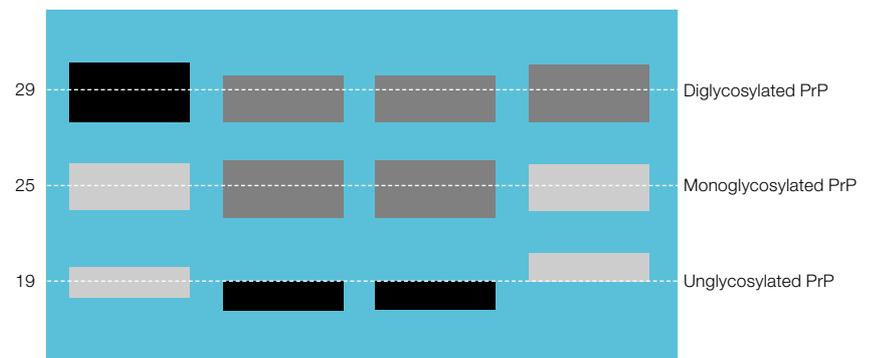
Casalone, C., et al. Identification of a second bovine amyloidotic spongiform encephalopathy: Molecular similarities with sporadic Creutzfeldt-Jakob disease. Proc. Natl. Acad. Sci. U. S. A. 2004. Mar. 2;101. (9.):3065. -70. Epub. 2004. Feb. 17. 101, 3065-3070 (2004).

Biacabe, A. G., Laplanche, J. L., Ryder, S. & Baron, T. Distinct molecular phenotypes in bovine prion diseases. EMBO Rep 2004. Jan.;5(1): 110. -5. 5, 110-115 (2004).

Yamakawa, Y., et al. Atypical proteinase K-resistant prion protein (PrPres) observed in an apparently healthy 23-month-old Holstein steer. Jpn. J Infect

Reference List
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Figure 1



Schematic representation of a WESTERN blot of protease-treated PrP^{Sc} from brain tissue of a typical BSE case and from atypical cases as reported in Italy, Japan and France. Gray scales of the bands represent the intensity of the PrP band with black corresponding to highest and light gray to lowest intensity. Apparent molecular weight markers are depicted on the left in kDa.