Wen-Quan Zou · Pierluigi Gambetti
Editors

Prions and Diseases

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Chapter 1
Bovine Spongiform Encephalopathy

Gianluigi Zanusso and Salvatore Monaco

Abstract Bovine spongiform encephalopathy (BSE) is a fatal neurodegenerative disease of cattle caused by foodborne exposure to prions. First described in 1986, this novel disorder was clinically characterized by altered behavior, sensory changes, and locomotor signs. For almost two decades, BSE, now named classical BSE (C-type BSE), has been regarded as the only and exclusive prion disorder of cattle. The introduction of an active surveillance system for BSE in 2001 allowed the identification of two additional atypical forms of BSE, named H-type and L-type BSEs, because of distinct conformations of the pathological prion protein, or PrP^{Sc}, with higher (H-type) or lower (L-type) electrophoretic mobility of the unglycosylated protease-resistant PrP^{Sc} fragment. To date, a total of 34 L-type BSE and 27 H-type BSE have been detected worldwide by routine BSE testing in older cattle. The clinical phenotypes of atypical BSE forms are still undefined in field animals, although information has been obtained from intraspecies transmission studies. Transmission studies to mice show that C-type, H-type, and L-type BSE forms display distinct molecular properties, consistent with the occurrence of three different prion strains. Intriguingly, upon serial passages, H-type and L-type BSEs may acquire C-type properties, hence suggesting a possible role in the origin of BSE epidemics. Further, the evidence that atypical BSEs are transmissible to mammals, including nonhuman primates, are issues that raise public health concerns.

Keywords Amyloid • Atypical BSE • Bovine amyloidotic spongiform encephalopathy • Bovine spongiform encephalopathy • Creutzfeldt–Jakob disease • H-type BSE • L-type BSE • Prion strains

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1.1 Introduction

The story of bovine spongiform encephalopathy (BSE) began in December 1984, when a UK farmer called a veterinary surgeon to look at "a cow that was behaving unusually". Seven weeks later, the cow died. Early in 1985, more cows from the same herd developed similar clinical signs (O'Brien 2000). Afterward, BSE outbreak started.

1.2 BSE Epidemics in the UK

BSE epidemics originated from the exposure of cattle to a dietary protein-rich supplement, Meat and Bone Meal (MBM), prepared from rendered carcasses of livestock. This intensive practice of nutrition was introduced since 1940s to increase protein content in animal diet, particularly in dairy herds. The preparation of protein-rich supplement followed a rendering process, whereby the slaughterhouse refuse (offal) were separated into tallow and a defatted mixture of concentrated proteins, following sequential boiling, milling, and fat extraction with hydrocarbon organic compounds. Changes in the rendering process, in particular the omission of the use of organic hot solvent extraction and solvent recovery steps, resulted in an increase of the fat content in MBM, and inefficient inactivation of the infectious agent.

Between November 1986 and November 2000, confirmed cases of BSE in the UK were more than 180,000, but if included the asymptomatic cattle over 30 months, preemptively slaughtered and destroyed, the number of animals was nearly four and a half million (Brown et al. 2001).

To forefront the outbreak, UK Government issued a series of preventive measures. In July 1988, started the prohibition to use and/or supply with ruminant-derived proteins ruminant feed, in addition to compulsory slaughtering and destruction of animals suspected of having BSE. In November 1989, specified bovine offal (SBO), the most infective parts, including brain, spinal cord, tonsil, thymus, spleen, and intestines, were excluded from the animal and human food chains. Aim of this relevant public health measure was also focused to manage the risk of exposure to potentially infected tissues from clinically healthy animals, given the evidence that 1 g of BSE-infected brain material was an effective pathogenic oral dose (Wells et al. 1998). Moreover, BSE was successfully transmitted by parenteral route to pigs challenged with brain material from a clinically affected cattle (Dawson et al. 1990), although subsequent experiments showed that pigs are not susceptible to BSE following high doses of BSE by oral exposure (Wells et al. 2003). The positive effects of these measures of prevention were observed in 1993, when the BSE curve of epidemics downturned.

In April 1996, concurrently with the first report of variant Creutzfeldt–Jakob disease (vCJD) in ten young adults (Will et al. 1996), mammalian MBM preparations were definitely banned from feeding all farm animal species, horses, and fish

(Collee and Bradley 1997). In addition, to reduce the risk of human exposure to the BSE agent, the UK Government decided that no British cattle over 30 months (OTM) should be consumed, and from 1996 to 2000, 4.5 million of cattle were incinerated. In 2005, the OTM rule was replaced by mandatory BSE screening test of OTM cattle slaughtered for human consumption.

1.3 BSE in Europe

BSE spread to the Continent through the exportation of BSE affected livestock and of contaminated foodstuff. In the critical period after 1985, more than 50,000 pure bred breeding cattle, as well as large quantities of contaminated MBM, were exported worldwide. In European countries, a total of 34 BSE cases were ascertained in UK imported cattle, while cases of BSE in native-born cattle, assumedly exposed to MBM meal of UK origin, were first reported in 1989 in Ireland, and thereafter in Switzerland, Portugal, France, Belgium, Luxemburg, Netherlands, Lichtenstein, Denmark, Germany, and Spain (Cachin et al. 1991; Coles 1991; Smith and Bradley 2003). By the beginning of 2000, only 9 European countries reported new BSE cases in the native cattle population (Ducrot et al. 2008); however, 16 additional countries reported BSE cases during the following years, after the introduction of an active surveillance system.

After the earliest reports of BSE outside UK, only in 1990 the European Commission stopped the importation of live cattle and MBM/SBO preparations for ruminant feeding, thus allowing for almost 2 years the importation of MBM from the UK (Butler 1996). This in contrast with the French ban prohibiting UK meal for ruminant feed in August 1989. Notwithstanding, UK exports continued to grow through increased sales of MBM to communities outside the EU. In 1991, Israel imported 10,000 tons and Thailand 62,000 tons of UK feed (Butler 1996).

Since July 1994, EU prohibited the use of proteins derived from mammalians in ruminant feed in the whole community, although some member states had implemented such a ban before that date. However, the persistence of BSE cases in native-born animals suggested a large cross-contamination of ruminant feed, still authorized in other species such as pigs or poultry. Therefore, in January 2001, mandatory measures were implemented by prohibiting processed animal proteins to all farmed animals, birds, and fishes.

1.4 The Impact of BSE Surveillance System and the Emergence of Atypical BSE Forms

The identification of BSE-affected cattle by a passive surveillance system was one of the first measures setup in the UK and in European countries. The real effectiveness of this measure, based on the mandatory reporting of clinically suspected BSE cases

by veterinarians, was questionable, since it depended on the appropriateness of the case definition, the variability of clinical signs, the disease awareness of the veterinarian or the cattle owner, and the quality of *ante mortem* slaughter inspection; this, in addition to the paved loss of the entire herd as a consequence of BSE reporting, the inadequate compensation and the stigmatization of the cattle owner (Ducrot et al. 2008; Doherr et al. 2001).

The true efficacy of mandatory reporting of clinical BSE suspects was unknown until diagnostic confirmatory tests were available. The BSE test was a reliable control measure for estimating the number of BSE positive cases among clinically affected cattle or cattle subpopulations with a higher BSE incidence, as well as asymptomatic animals. Finally, the analysis of the active surveillance results showed that BSE positive cases were eight times higher in at risk cattle population (downer cattle and at emergency slaughter) than at routine slaughter, indicating that if correctly pursued passive surveillance would be a safe measure of prevention.

1.5 The Active Surveillance

In 1999, Switzerland was the first country to introduce the measure of an active surveillance system for the ascertainment of BSE in adult cattle. While maintaining a passive surveillance system, the entire population of cattle over 24 months "at risk", including dead on farm animals, euthanized cases, emergency-slaughter or downer cattle were tested (Doherr et al. 2001). Moreover, 3% of adult cattle sent to routine slaughter were randomly sampled and tested.

In 2000, also France initiated BSE active surveillance of at risk stocks in its three most affected regions, including Basse-Normandie, Bretagne, and Pays de Loire (Morignat et al. 2002).

In January 2001, the European Union implemented BSE surveillance by statutory active surveillance program based on systematic testing of all slaughtered bovines over 24 months of age in France, Germany, and Italy, and over 30 months in other countries; Austria, Finland, and Sweden randomly tested 10,000 cattle per year, since they were classified by the "Office International des Epizooties" at level II risk, i.e., "unlikely, but not excluded" (Bird 2003). Portugal, Greece, and Belgium had the lowest rate of surveillance on routinely slaughtered bovines. Non-EU countries, including Canada and the USA maintained a passive surveillance. In Japan, active surveillance began in April 2001 on all clinical BSE suspects and fallen stock (Yamanouchi and Yoshikawa 2007).

After the establishment of active surveillance, several countries, including Italy, that did not report BSE cases in native-born cattle before the 2001, found BSE cases. Accordingly, Italy reported 48 cases, whereas Spain reported an increase of 41 times, Belgium 20, France 17, Germany 18, The Netherlands 10, and Switzerland 1.2 times.

In 2001, the active surveillance in Europe system snapshot the real occurrence of foodborne BSE, consistent with exposure during the period 1995–1996, in accordance with the estimated incubation period of 5 years. The reduction of BSE cases in several European countries during the following years, suggest that the 2001–2002 period corresponds to the peak of BSE epidemics in Europe.

Thereafter, the number of BSE cases progressively declined, and in 2011 only 15 cases were reported. Based on these results, in 2009, EU member states increased the age limit for testing from 30 to 48 months for healthy slaughtered cattle and from 24 to 48 months for at risk bovines. Since July 2011, the active surveillance system has been restricted to healthy slaughtered animals over 72 months and to at risk cattle over 48 months. Additional relaxation measures have been prospected for 2013, including the testing of at risk cattle population and randomly healthy slaughtered cattle.

1.6 The Detection of Atypical BSE Forms by Routine Testing

Since 1999, EU validated three BSE screening tests, which were based on the detection of protease-resistant PrP^{Sc} by ELISA (Platelia® and Enfer test®) or by Western blot analysis (Prionics-Check®), thus allowing a rapid and large-scale analysis of BSE cases (Schaller et al. 1999). Testing was carried out on brainstem samples obtained at the slaughtered house, and all brain samples testing positive were further investigated by additional confirmatory Western blot test and immunohistochemistry.

While the aforementioned validated tests shed light on the dimension of underreported BSE cases by assessing the presence of PrP^{Sc}, the use of the confirmatory Western blot provided a qualitative analysis of PrP^{Sc} conformation, hence allowing the detection of variant PrP^{Sc} conformers. In 2003, two novel forms of BSE were found in France and Italy, which were characterized by a pathological prion protein differing in gel mobility and glycotype from C-type BSE. The three French cattle showed a PrP^{Sc} migrating "Higher" as compared to C-BSE PrP^{Sc} (H-type BSE), whereas the two Italian cattle had a PrP^{Sc} migrating "Lower" than C-BSE (L-type BSE) (Biacabe et al. 2004; Casalone et al. 2004) (Fig. 1.1a). L-type BSE was originally named "bovine amyloidotic spongiform encephalopathy" (BASE) to highlight the unprecedented neuropathological phenotype, characterized by the abundance of amyloid–PrP plaques in brain tissues.

During the following years, atypical BSE cases were found in almost all European countries, in the USA, Canada, and in Japan (Table 1.1). Eight years after their identification, a common phenotypic characteristic of atypical BSE forms is the relatively old age of affected cattle, as compared to cattle with classical BSE, and the apparent absence of clinical signs, with a few exceptions (Brown et al. 2006; Jacobs et al. 2007; Dudas et al. 2010).

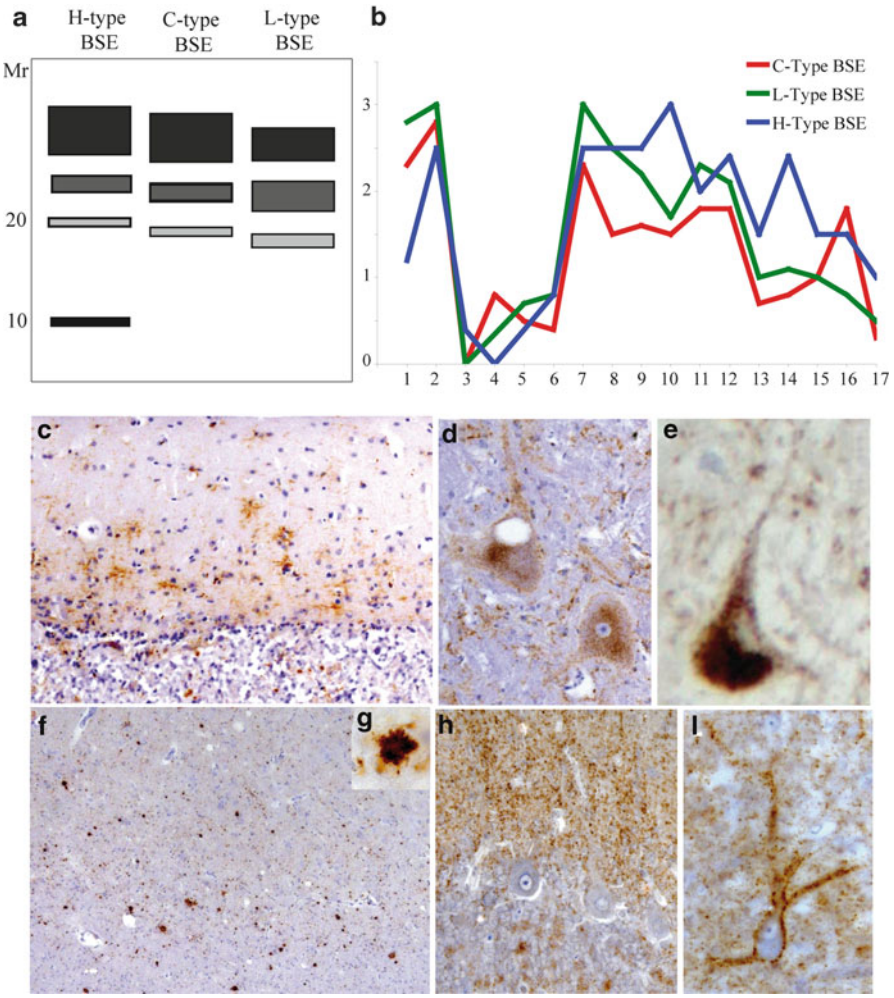

Fig. 1.1 *Biochemical features, lesion profile, and pathological phenotypes of classical and atypical BSE forms*. (**a**) Electrophoretic patterns of protease-resistant PrP in C-type and atypical BSE forms (H-type and L-type); (**b**) Histograms of the lesion load in H-type, C-type, and L-type BSEs; numbers in abscissa denote brain areas (1, Nucleus of the solitary tract; 2, Nucleus of the spinal tract of the trigeminal nerve; 3, Hypoglossal nucleus; 4, Vestibular nuclear complex; 5, Cochlear nucleus; 6, Cerebellar vermis; 7, Central gray matter; 8, Rostral colliculus; 9, Medial geniculate nucleus; 10, Hypothalamus; 11, Nucleus dorsomedialis thalami; 12, Nucleus ventralis lateralis thalami; 13, Frontal cortex; 14, Septal nuclei; 15, Caudate; 16, Putamen; and 17, Claustrum); numbers in ordinate denote vacuolation score (1 mild, 2 moderate, and 3 severe); (**c–i**) Patterns of PrP deposition in different BSE forms. Typical stellate PrP pattern in the molecular layer of the cerebellum (**c**), and intraneuronal PrP staining in C-type BSE (**d**); intraneuronal PrP staining in H-type BSE (**e**); immunohistochemistry in L-type BSE showing PrP–amyloid plaques in the frontal cortex (**f** and **g**), granular and axonal PrP deposition in the cerebellum (**h**), and perineuronal PrP deposition (**i**)

Table 1.1 Typical and atypical BSE cases detected worldwide from 1989 to date

	C-type			
Country	1989–2000	2001–2011	L-type	H-type
Austria	–	8	2	–
Belgium	19	114	–	–
Canada	–	18	1	1
Czech Republic	–	30	–	–
Denmark	1	15	1	–
Finland	–	1	–	–
France	95	775	13	14
Germany	7	406	1	1
Ireland	507	1,057	–	1
Israel	–	1	–	–
Italy	–	142	4	–
Japan	–	36	2	–
Luxembourg	1	2	–	–
Netherlands	8	79	2	1
Poland	–	69	8	2
Portugal	522	546	–	–
Slovakia	–	25	–	–
Slovenia	–	9	–	–
Spain	2	771	–	–
Sweden	–	1	–	1
Switzerland	366	98	–	1
UK (GB)	179,087	2,568	–	3
USA	–	2	–	2
Total	*180,615*	*5,457*	*34*	*27*

1.7 Disease Phenotypes of Classical BSE and Atypical Forms of BSE

After its original description, the clinical phenotype of classical BSE has been largely reported, being characterized by an insidious onset of altered behavior, with nervousness or apprehension, followed by sensory changes, including overreactivity to external stimuli, spontaneous or evoked startle responses, hypersensitivity to external stimuli, and by locomotor signs such as tremor, hypermetria, ataxia, and recumbency (Wells et al. 1987). The neuropathological profile of BSE was clearly defined in over 600 cases at the beginning of epidemic in the UK. The distribution and the score of vacuolar changes in different brain areas was examined by Scott and coworkers, who showed the highest lesion load in the medulla, midbrain, and thalamus, while cerebellum, hippocampus, cerebral cortex, and basal ganglia were relatively less involved (Scott et al. 1990). Spongiform degeneration was invariably observed in two medulla oblongata nuclei, i.e., the solitary tract nucleus and the spinal tract nucleus of trigeminal nerve, allowing a 100% diagnostic specificity (Wells et al. 1989), in addition to central gray matter of the midbrain. Spongiform changes were located in the neuropil, albeit intracellular vacuoles, either in neuronal

perikarya or in their axonal extensions, were observed in addition to astrocytic proliferation. Exclusive intraneuronal vacuolation, but not neuropil spongiosis, was considered not diagnostic.

In contrast to BSE, the clinical phenotype of atypical BSE forms is not clearly defined in field cases, albeit H-type and L-type BSE cases have been reported among fallen stock, and these animals might have displayed unreported clinical abnormalities; an exception is the Japanese L-type case which exhibited dystocia at abattoir (Dudas et al. 2010; Masujin et al. 2008).

Available data on the clinical features of atypical BSEs have been obtained by experimental transmission studies. We firstly reported that the clinical phenotype in BASE-affected cattle was characterized by dullness, hypersensitivity to facial stimuli, and weight loss, followed by fasciculations and amyotrophy, in the absence of cerebellar signs (Lombardi et al. 2008); conversely, H-type BSE was characterized by loss of weight, deteriorating body condition, low head carriage, high sensitivity to acoustic and visual stimuli, and slight hind limb ataxia (Balkema-Buschmann et al. 2011a; Okada et al. 2011). Hence, the prevalence of behavioral changes and constitutional signs in atypical BSEs may in part explain the lack of the recognition of these forms at slaughter inspection.

The neuropathological lesion profile of atypical BSE forms differed from C-BSE (Fig. 1.1b), and also immunohistochemical analysis showed patterns of PrP deposition, distinct from PrP deposits of granular type (in the neuronal cytoplasm or in gray matter neuropil), linear type (thick, thread-like profiles), and glial type, observed in C-BSE (Fig. 1.1c, d). In H-type BSE, PrP immunohistochemistry disclosed a prevailing intraneuronal and intraglial pattern of deposition (Fig. 1.1e), whereas in L-type BSE, or BASE, perineuronal synaptic staining, accompanied by abundant amyloid–PrP deposition, was observed in deep gray nuclei and in the white matter (Fig. 1.1f–i) (Fukuda et al. 2009; Balkema-Buschmann et al. 2011a, b; Buschmann et al. 2006; Richt et al. 2007; Gavier-Widén et al. 2008). Interestingly, PrP^{Sc}-positive plaques, but not amyloid deposits, have been reported in H-type BSE (Okada et al. 2011).

1.8 Prion Strain Properties in Typical and Atypical BSEs

In addition to providing valuable information on the disease phenotype in its natural host, intraspecies transmission studies showed that atypical BSE forms displayed biological properties diverging from C-type BSE. Accordingly, cattle exposed to atypical BSEs, either H-type or L-type, had disease duration significantly shorter than C-type BSE, while the incubation period was longer (Lombardi et al. 2008; Balkema-Buschmann et al. 2011a).

Moreover, experimental studies in transgenic bovinized mice (Tgbov), challenged with H-type, L-type, and C-type BSEs, showed an incubation period significantly

shorter in animals inoculated with L-type BSE as compared to mice exposed to C-type BSE; conversely, Tgbov mice inoculated with H-type BSE showed the longest incubation period, findings which favor the occurrence of different strains of the BSE agent (Buschmann et al. 2006).

Further, experimental transmission studies of atypical BSEs and C-type BSE to wild-type mice have provided intriguing results. At the first passage, the L-type isolate failed to transmit the disease to wild-type mice (C57Bl/6 or SJL), while H-type BSE transmitted to C57Bl/6, although with features differing from C-type BSE. Intriguingly, after serial passages in inbred mice or in a transgenic mouse model overexpressing ovine PrP (tg338), the L-type BSE strain acquired biological properties and phenotypic characteristics of the C-type BSE strain. Similar results were observed in C57Bl/6 mice serially challenged with H-type BSE, in which C-type BSE properties were observed in some of the infected mice, while others maintained the H-type BSE properties (Capobianco et al. 2007; Béringue et al. 2008; Baron et al. 2011).

1.9 On the Origin of BSE

The enigma of BSE epidemic is still unsolved. Although it is clear that infected tissues had been included in MBM fed to cattle, several possibilities have been proposed as to the ancestral culprit of foodstuff contamination, including scrapie or genetic BSE. The hypothesis of an origin from scrapie is the more circumstanced. In the UK, sheep is the only recognized natural reservoir of the scrapie agent in the ovine population, with a prevalence of about two cases per 1,000 (Morgan et al. 1990). Further, cattle have been shown to be susceptible to scrapie infection (Gibbs et al. 1990; Konold et al. 2006) and it might be reasonable to assume that BSE epidemic started when the scrapie agent entered in the food chain crossing the sheep–cow species barrier (Fig. 1.2a).

Another possibility remains the unapparent endemic presence of cattle BSE, or the occurrence of spontaneous cases of BSE in the cattle population (Kimberlin 1993; Brown 1998) (Fig. 1.2b). Several lines of evidence indicate that atypical BSE forms might be sporadic forms of BSE due to strict analogies with sporadic Creutzfeldt–Jakob disease (sCJD) in humans (Brown et al. 2006). These include the incidence of 1.9 case per million of atypical BSEs in healthy slaughter cattle, the late age of disease onset, the occurrence of two distinct biochemical PrP^{Sc} types, and the presence of distinct patterns of PrP deposition (synaptic-type in H-type and amyloid-forming plaques in L-type) (Biacabe et al. 2008).

Recently, a pathogenic E211K mutation has been reported in a cattle with H-type BSE, but the biological relevance of this finding is still unclear (Fig. 1.2c) (Richt and Hall 2008).

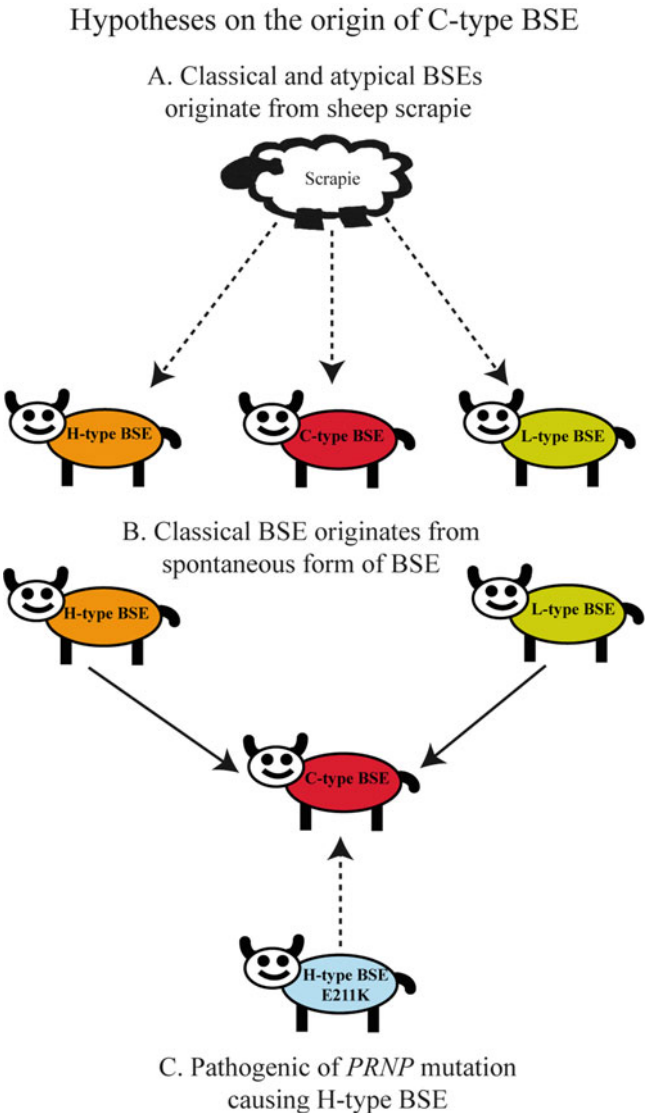

Fig. 1.2 Hypotheses on the origin of C-type BSE

1.10 Cattle BSE and Human Prion Diseases

In the original description of BASE (Casalone et al. 2004), we argued that BASE had molecular and pathological features similar to the MV2 molecular subtype of sCJD, since both conditions shared the biochemical type of PrP^{Sc} and were characterized by PrP–amyloid plaques in the nervous tissues.

The potential link between sCJD and BASE had been partially addressed in in vivo experimental models by challenging transgenic humanized mice (TgHu) and nonhuman primates. Kong et al. (2008) showed that BASE was transmitted to TgHu mice overexpressing human PrP Met/Met at codon 129 (Tg40 mice), with an attack rate of 60%. The biochemical type of PrP^{Sc} observed in Tg40 mice was "monoglycosylated dominant", as observed in sCJD. In another study, TgHu mice (*tg650*) were intracerebrally inoculated with C-type, H-type, and L-type BSE. At first passage, all mice exposed to L-type BSE developed the disease, while mice inoculated with C-type BSE had an attack rate of 100% only at the second passage; in contrast, BSE H-type agent failed to transmit the disease (Béringue et al. 2008). The above studies indicate that the C-type BSE is less efficiently transmissible as compared to L-type BSE. Therefore, a zoonotic risk is potentially higher for BASE than for classical BSE and H-type BSE, as a likely effect of different species barrier properties (Béringue et al. 2008).

Furthermore, experimental infection of a single nonhuman primate with the L-type BSE isolate showed an incubation period shorter than that observed in animals exposed to C-type BSE; moreover, L-type and C-type infected animals displayed distinct disease phenotypes and PrP^{Sc} conformations (Comoy et al. 2008).

It is still not possible to assess whether the BASE strain is more pathogenic than C-type BSE for primates (including humans). Likewise, data are still too incomplete to prove a link between BASE and sporadic human CJD. However, results so far obtained justify some concerns about a potential human health hazard from atypical forms of BSE. In this context, it would be of help to monitor epidemiological data of sCJD as well as the occurrence of atypical sCJD phenotypes.

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Chapter 2
Classical and Atypical Scrapie in Sheep and Goats

Christine Fast and Martin H. Groschup

Abstract Scrapie is a naturally occuring transmissible spongiform encephalopathy (TSE) in sheep, goat and mufflons almost world-wide and is known for about 250 years. It is characterized by the accumulation of an abnormal isoform (PrP^{Sc}) of host encoded prion protein (PrP^{C}) in the central nervous system which leads to progressive neurodegeneration and death. Scrapie represents the prototype of the so-called prion diseases. It is observed to date as two types, classical and atypical scrapie. The susceptibility to both types is modulated by polymorphisms of the prion gene. Whereas classical scrapie is clearly a naturally occurring transmissible disease, atypical scrapie may also be caused by the spontaneous misfolding of prion protein. This review gives an overview on the current knowledge of classical and atypical scrapie in sheep and goats with special emphasis on epidemiology, clinical and pathological signs, genetic susceptibilities, diagnosis and the characteristics of the most common scrapie strains.

Keywords Atypical scrapie • Classical scrapie • Pathological prion protein • Prions • Scrapie • TSE

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2.1 Overview

Scrapie is the most common name for the transmissible spongiform encephalopathy (TSE), which affects sheep, goats and moufflons almost worldwide. Like all other prion diseases, scrapie is a neurodegenerative progressive and eventually fatal disease. Scrapie is associated with a number of clinical signs ranging from subtle behavioural abnormalities to more obvious neurological signs. The clinical diagnosis needs to be confirmed by the demonstration of pathognomonic spongiform lesions and the immunodetection of pathological prion protein (PrP^{Sc}) depositions in the CNS primarily (OIE-Manual of Diagnostic Tests and Vaccines for Terrestrial Animals). PrP^{Sc} depositions can be revealed by immunohistochemical and biochemical methods (see Chap. 13). To date, two distinct scrapie types are known: classical and atypical scrapie.

2.2 History

Scrapie is not only the prototype of TSEs but also the prion disease with the longest history of publication. The first authentic report on scrapie was written in Germany and dates back to year 1750 (Leopoldt 1750). However, a later publication (Comber 1772) even mentions cases in England that occurred already in 1732. Several authors at later times even referred to much earlier time periods, spanning from Roman times up to the seventeenth century, but without giving corresponding references (for a detailed review see Schneider et al. 2008). Moreover in former times, many sheep diseases were confused with scrapie. Other difficulties were the various names that were used to describe this disease throughout Europe: “Goggles”, “Ricketts”, “Rubbing Disease” and “Trotting Disease” in England, “Scratchie” and “Yeukie pine” in Scotland, “Basqvilla Disease” in Spain, “La maladie convulsive”, “La Tremblante” and “Prurigo lumbaire” in France, “Rida” in Iceland, “Gnave-og travesjuke” in Norway and “Gnubberkrankheit”, “Petermännchen”, “Traber” or “Reiberkrankheit” in Germany. Altogether, at least 42 different names were used in Europe and India (Schneider et al. 2008) for this disease in small ruminants.

The infectious nature of scrapie was already reckoned in the eighteenth century (Leopoldt 1750). In the following decades and centuries, different transmission routes were discussed in which the sexual intercourse was the most suspected modus. However, among other causes like atmospheric disturbances, a few authors proposed a mere coexistence of infected and non-infected animals or a spontaneous origin of the diseases (Schneider et al. 2008). In addition, a broad consent existed already in the nineteenth century concerning the role of hereditary factors for scrapie. Initially, a hereditary predisposition and the transmission by asymptomatic animals were assumed (Thaer 1821; von Richthofen 1821) and even the existence of hereditary and non-hereditary scrapie forms was postulated (von Richthofen 1826).

A number of experimental transmission studies were subsequently carried out in order to clarify the origin and transmission routes of scrapie. These experiments

included contact studies with infected and non-infected sheep and subcutaneous and intravenous inoculation studies using different tissues and bodily fluids from infected animals. However, most of these studies were terminated prematurely and therefore failed due to the long incubation period of scrapie (for detailed review see Schneider et al. 2008). However, in 1936, the transmissibility of scrapie was first time proven by experimental inoculation of healthy animals with brain and spinal cord of diseased sheep. In this experiment, the inoculated animals were kept for longer periods of time and sheep could develop scrapie after incubation periods of up to 2 years (Cuille and Chelle 1936, 1938a, b).

Since the 1930s, scrapie research was intensified when substantial financial losses to the sheep industry were caused by increasing numbers of cases. These losses prompted also studies on the true nature of the infectious agent. Besides parasites (M'Gowan 1914) and bacteria (Bastian 1979) as causative agents, a virus infection was the most commonly proposed theory, already formulated in 1938 (Cuille and Chelle 1938a, b). In 1954, the term of a "slow virus infection" was first time introduced (Sigurdsson 1954). However, already in 1966, an alternative to the virus origin was postulated as the causative agent, i.e., polysaccharides (Alper et al. 1966, 1967; Field 1966) or lipids (Alper et al. 1978). In 1967, for the first time, a protein was assumed as infectious agent (Pattison and Jones 1967) and the first "protein-only-hypothesis" was enunciated (Griffith 1967) followed in the 1970s by the "virino" theory (Dickinson and Outram 1979). Finally, based on the resistance of the pathogen, in 1982, the term "proteinaceous infectious particle" (acronym: prion) was introduced (Prusiner 1982) and the conversion of a normal cellular protein (PrP^{c}) into a pathological isoform (PrP^{Sc}) as key event of TSE pathogenesis was postulated shortly after (Oesch et al. 1985). PrP^{Sc} is currently considered to be the biochemical marker and the causative agent of TSEs. However, the prion theory is still debated since PrP^{Sc} is not always infectious and the phenomenon of strains is still an enigma (Lasmezas et al. 1997; Piccardo et al. 2007).

In 1998, the atypical form of scrapie, termed Nor98, was first time discovered in Norwegian sheep (Benestad et al. 2003). However, retrospective studies revealed atypical scrapie cases in the UK already in the late 1980s. Therefore, this disease is not considered as new emerging form of TSE (Bruce et al. 2007). Atypical scrapie is distinguished from classical scrapie by clinical and epidemiological as well as by molecular and histopathological features. It is not rare compared to classical scrapie in most countries and found worldwide at a comparable incidence rate, which is indicative for a different, perhaps non-infectious aetiology (Fediaevsky et al. 2008).

Scrapie in goats was initially described after an experimental exposure in 1939 (Cuille and Chelle 1939) and the first natural case was reported a few years later (Chelle 1942). The first experimental challenge of goats with sheep scrapie showed 100% susceptibility suggesting that goats are highly susceptible (Pattison et al. 1959; Cuille and Chelle 1939). Like classical scrapie atypical scrapie cases were reported also in goats (Fediaevsky et al. 2008, for detailed review see Vaccari et al. 2009) but showed a lower prevalence as compared to sheep (EFSA 2010).

In Moufflons, only classical scrapie was reported in six natural cases so far (Wood et al. 1992a, b).

2.3 Geographical Distribution and Surveillance

Scrapie is endemic in almost all member states of the European Union (EU 27) as well as in Norway, Iceland and Switzerland. Brazil, Canada, Israel, Japan, Palestinian Autonomous Territories, Russia, Tajikistan and the USA reported scrapie cases (atypical and/or classical) in the last 6 years. Only individual atypical scrapie cases were documented on the Falkland Islands and New Zealand (Epstein et al. 2005, EU Commission, Kittelberger et al. 2010, World Animal Health Data Base (WAHID); http://web.oie.int/wahis/public.php?page=disease_timelines). According to the "World Livestock Disease Atlas 2011" (Anonymous 2011), scrapie ranks third worldwide as cause for sheep and goat losses.

An introduction of classical scrapie via imported sheep from the UK was suspected for countries like Australia and New Zealand (1952–1954), South Africa (1964–1972), Colombia (1968–1971) and Kenya (1970). After thorough eradication by slaughtering the imported sheep and their flock mates, Australia and New Zealand remained free of scrapie to date (Detwiler and Baylis 2003).

However, the true scrapie status of many countries remains unknown because there is usually only an inadequate passive surveillance system in place to detect infected animals. It is nearly impossible to establish freedom from infection without establishing an active surveillance system, which includes the examination of fallen stock and emergency slaughter (Detwiler and Baylis 2003, OIE Manual). This is exemplified by the introduction of a harmonised active surveillance program for scrapie in sheep and goats throughout the EU in 2003. In the context of this program, animals over 18 months of age (fallen stock, emergency slaughter, as well as healthy slaughtered animals) were examined for TSE.

2.4 Prion Protein Gene and Susceptibility

It has been shown in several epidemiological studies that the successful transmission of classical scrapie requires genetically susceptible sheep. In year 1968, the effect of a so-called *Sinc*-gene (scrapie incubation gene) on the length of the incubation period of experimentally infected mice and a synonymously so-called *Sip*-gene (scrapie incubation period gene) in sheep were proposed (Dickinson et al. 1968a, b). Eventually, different polymorphisms of the prion protein gene (*Prnp*) were matched in the 1980s and 1990s with the *Sip*-/*Sinc*-genes (Oesch et al. 1985; Westaway et al. 1987; Goldmann et al. 1991; Moore et al. 1998; Hunter et al. 1996).

The murine Prnp consists of two alleles, s7 and p7, which differ in their PrP amino acid sequence at codons 108 and 189 and are associated with short or prolonged incubation times after infection with particular (i.e., ME-7) experimental strains. However Infections with other strains (i.e., 22A) showed reversed results (Dickinson et al. 1968a). Similar results were obtained in sheep. The ovine Prnp consists of two alleles sA (short incubation period) and pA (prolonged incubation period), which are distinct primarily in the amino acid sequences encoded at codon

136 (Dickinson et al. 1968b; Hunter et al. 1996, 1997). Similar as in mice, the length of the incubation period is depending on the scrapie strain that is used (Foster and Dickinson 1988). Furthermore, in susceptible animals, effects on the incubation period can also result from polymorphisms at codons 154 and 171 (Hunter et al. 1996). Thus, the incubation period is determined at least by two factors: the genotype of the host and the agent strain.

The ovine Prnp is located on chromosome 13 (Iannuzzi et al. 1998) and the functional length of the PrP gene is approximately 21 kb and is composed of three exons, from which exon III contains the complete uninterrupted open reading frame. The length of the unprocessed precursor protein is 256 amino acids. After post-translational modifications, about 210 amino acids remain in the mature protein (for detailed review see Goldmann 2008).

Ovine PrP polymorphisms influence not only the susceptibility to the disease but also modulate the progression including the incubation period and clinical signs. The vast majority of polymorphisms are due to single nucleotide polymorphisms (SNP) in the DNA, which often cause single amino acid changes. Of particular interest are polymorphisms at codons 136, 154 and 171 within the ORF, which are clearly linked to scrapie susceptibility in sheep (Goldmann 2008). Standard abbreviations describe the alleles in reference to the three codons:

- A136V in which Alanine (A) is associated with resistance and Valine (V) is associated to susceptibility (Goldmann et al. 1991; Hunter et al. 1994).
- Q171R in which Arginine (R) is associated with resistance and Glutamine (Q) is associated with susceptibility (Westaway et al. 1994; Clouscard et al. 1995; O'Rourke et al. 1997).
- R154H in which Histidine (H) is associated with resistance (Goldmann et al. 1991; Laplanche et al. 1993).

The polymorphisms mentioned above result in five different alleles (ARQ, VRQ, AHQ, ARR and ARH), leading to 15 different genotypes, which are the only alleles with significant distribution worldwide (Goldmann 2008). Some further genotypes, ARK and TRQ among others, are known (Gombojav et al. 2003; Guo et al. 2003; Billinis et al. 2004), but due to their low frequencies they are not included into a TSE genotype classification system (Dawson et al. 1998). This five group risk classification (Table 2.1) is the basis for breeding and scrapie eradication programs applied in the EU. The highest risk to develop scrapie carry VRQ/VRQ animals, the highest genetic resistance is associated to ARR/ARR sheep (Belt et al. 1995; Hunter et al. 1996; Hunter 1997). However, this classification is subject to restriction as, for example two ARR/ARR sheep from different flocks in France and Germany have been shown to be subclinical carriers of classical scrapie (Groschup et al. 2007). Additionally, ARQ/ARQ animals, classified in R3, can be at highest risk in flocks where the VRQ allele is absent for example due to breed (Goldmann 2008).

Furthermore, several polymorphisms are described at other positions, for example 25% of all ARQ alleles revealed additional polymorphisms (Goldmann 2008). However, it is unclear whether such polymorphisms have a profound effect on the disease. Some studies refer to resistance and/or prolonged incubation times in sheep

Table 2.1 Ovine five group risk classification system

Risk group	Genotype	Susceptibility
1	ARR/ARR	Highest genetic resistance
2	ARR/AHQ	Genetic resistance
	ARR/ARH	
	ARR/ARQ	
3	AHQ/AHQ	Low genetic resistance
	AHQ/ARH	
	AHQ/ARQ	
	ARH/ARH	
	ARH/ARQ	
	ARQ/ARQ	
4	ARR/VRQ	Genetic susceptibility
5	AHQ/VRQ	Highest genetic susceptibility
	ARH/VRQ	
	ARQ/VRQ	
	VRQ/VRQ	

carrying for example AC151RQ, AT137RQ, or ARQK176 (Acin et al. 2004; Thorgeirsdottir et al. 1999).

The classification system described above and in Table 2.1 does not work for atypical scrapie. In contrast to classical scrapie in most of the atypical cases, animals of PrP genotype risk groups R1-3 (Benestad et al. 2008) are affected. Most frequently found in such cases are haplotypes such as AHQ/AHQ, AHQ/ARQ and ARR/ARR, respectively. It has been shown that polymorphisms at codons 141 and 154 are linked to susceptibility. Genotype AF141RQ encoded for a higher susceptibility than the AL141RQ allele or even the AHQ genotype (Goldmann 2008).

Although the wild-type amino acid sequence of goat and sheep PrP are similar, the PrP genetics in goats is much more variable, yet without polymorphisms at codons 136 and 171 surprisingly. In goats 29 other polymorphisms of the caprine Prnp, resulting in amino acid changes, have been found in different countries and breeds (Vaccari et al. 2009; Goldmann et al. 2011). At least five of them seem to be associated with TSE susceptibility (for detailed review see Vaccari et al. 2009):

- I142M haplotypes have a lengthened incubation period after experimental inoculations and are associated with increased resistance to classical scrapie under natural conditions (Goldmann et al. 1996; Barillet et al. 2009).
- R154H haplotypes are associated with some resistance to classical scrapie in different breeds and countries (Barillet et al. 2009; Billinis et al. 2002; Papasavva-Stylianou et al. 2007; Vaccari et al. 2006) but have a comparable high risk associated with atypical scrapie (Moum et al. 2005; Arsac et al. 2007; Seuberlich et al. 2007).
- N146S/D polymorphisms encode low risk (some resistance) for scrapie infection but this genotype is confined to Damascus/Damascus crossbreed goats on Cyprus primarily (Papasavva-Stylianou et al. 2007).
- R211Q haplotypes have shown an increased resistance to classical scrapie in French case–control studies (Barillet et al. 2009).