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Current status of transmissible spongiform encephalopathies in ruminants

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Transmissible spongiform encephalopathies (TSE) encompass subacute neurological degenerative diseases for which the prototypes are scrapie in sheep and some forms of Creutzfeldt-Jakob disease in man. The emergence of a new form of TSE in cattle in United Kingdom (UK) since 1986, namely bovine spongiform encephalopathy (BSE), sharply increased the interest for these diseases, especially because of the epidemic nature of BSE in UK, its subsequent spread in continental Europe and the later discovery of its zoonotic character. The number of measures of veterinary public health taken to control the disease and to prevent its spread to animals and human beings increased in time and culminated by the total feed ban. Indeed, since the beginning of 2001, feed containing proteins of animal origin is prohibited for the feeding of production animals, including ruminants and monogastric species. The effect of this total ban of mammalian meat and bone meal needs to be evaluated. The incidence of BSE has a trend to decrease in UK and in most of the other European member states. However, as BSE is a rare event distributed in a large bovine population, it is difficult to state unambiguously whether this trend is significant. Furthermore, the evaluation of this measure will be only effective at least five years after its introduction, since this period is the mean incubation time of BSE. The main concern is currently the eradication of BSE in the infected countries. Additionally, the control of scrapie is also carried out due to the possible contamination of sheep with the BSE agent. These actions must take into account several new facts: the recent discovery of BSE cases in countries with a low geographical BSE risk level as Japan, Canada and the United States of America (USA); the growing incidence of chronic wasting disease, a spongiform encephalopathy observed in deer in USA; the characterization of a new pattern of bovine amyloidotic spongiform encephalopathy in Italy, atypical scrapie cases in sheep and atypical BSE cases in cattle in Europe and the efficacy of sheep selection based on scrapie resistant genotypes.
Keywords. Transmissible spongiform encephalopathy, prion, scrapie, bovine, sheep.

1. INTRODUCTION

Transmissible spongiform encephalopathies (TSE) are a group of invariably fatal transmissible neurodegenerative disorders, due to the accumulation of an abnormal isoform of the host-encoded prion protein (PrP) causing spongiform vacuolation and neuronal loss in the central nervous system accompanied by proliferation of astrocytes and in some cases deposition of amyloid plaques. They have long incubation times and are transmissible within and between species (Lasmézas, 2003; Trevitt, Singh, 2003). However the clinical picture is not pathognomonic of BSE and a careful differential diagnosis is always needed (Saegerman *et al.*, 2003; 2004).

They differ from sporadic spongiform encephalopathies by their transmissibility (Lasmézas, 2003). Nevertheless, the diagnostic methods cannot differentiate between the two categories. These methods rely on the identification of the prion protein, or more precisely to its resistant form PrP^{res}, which is associated with infectivity. The abnormal protein is indeed resistant to protease activity and heat treatment. These properties are used for the diagnosis because of the degradation of the physiological form of the prion protein, a glycoprotein present on the membranes of neurons and other cell types, in the samples submitted to the diagnosis. New diagnostic methods rely on the use of antibodies specific of the conformation of the abnormal protein.

Although some forms of TSE are known for a long time, as scrapie in sheep and Creutzfeldt-Jakob disease (CJD) in man, bovine spongiform encephalopathy (BSE) is a newly emerged disease. Its origin is still in debate: either the adaptation of a strain of scrapie or the passage from a sporadic bovine case into a TSE by recycling the agent via meat and bone meal.

BSE is the most likely cause of variant CJD in man (Bruce *et al.*, 1997; Hill *et al.*, 1997; Hill, Collinge, 2003). The identification of BSE as a potential zoonosis in the nineties led to the development of a large set of measures in order to further prevent the spread of the agent in cattle and to enhance human food safety. Fortunately, the incidence of the variant CJD did not increase too much since the identification of the first cases in 1996. However, due to the long duration of the incubation period of this disease, new human cases are still expected.

This paper intends to review the current epidemiological situation of BSE in Europe and in the world with emphasis to the evolution of its profile over the last years. It also reviews the last developments in the recognition of unusual disease patterns, known as atypical BSE and scrapie forms, and the growing importance of chronic wasting disease in deer in North America.

2. PATHOGENESIS OF BSE

In experimental conditions, oral transmission of BSE agent is 10^5 less efficient than the intracerebral route (European Commission, 2000). Only a few inoculation experiments have been performed. Therefore the knowledge of the pathogenesis is still very scarce. The currently available data can be summarized as follows. In this paper, PrP^{sc} refers to the abnormal, pathologic prion protein; PrP^{res} refers to the fraction of PrP^{sc} which is resistant to heat or protease treatment and which therefore remains in the sample after this treatment.

Oral inoculation of calves aged 4–6 months was performed with 100 g of pooled cerebral tissues from 75 clinically affected bovines. PrP^{res} protein can be detected:

– from 6 to 18 months post infection (PI): in the distal part of the ileum, especially in Peyer's patches;
– from 10 months PI in the tonsils;
– from 32 months PI in trigeminal ganglia;
– from 36 months PI in the enteric nervous system;
– at 36–40 months of age: in the central nervous system of clinically affected animals.

These studies suffer from a severe bias: indeed, from 18 to 32 months of age, the stage of neuroinvasion is unknown (Wells *et al.*, 1998; Terry *et al.*, 2003).

The hypothetic pathogenesis of BSE is as follows. The primary site of multiplication and the route of dissemination are unknown. The distal ileum seems to be the portal of entry. BSE agents then replicate in cells of the reticulo-lymphocytic system at an early stage. The infection of these cells is proven in sheep affected by scrapie and deer affected by chronic wasting disease. The peripheral autonomous nervous system is susceptible to be the route of BSE towards the central nervous system (Prince *et al.*, 2003; Wells, 2003), as also evidenced in mouse scrapie (Kimberlin, Walcker, 1980). Although myocytes have been found to accumulate prion protein in scrapie-infected sheep several months before the onset of clinical disease, muscle infection has not been observed in BSE infected cattle (Andreoletti *et al.*, 2004).

3. EPIDEMIOLOGICAL SITUATION IN EUROPE

The epidemiological situation must be envisaged taking into account three pivotal years: the first notification of BSE in United Kingdom (UK) in 1986, the first diagnosis of BSE in continental Europe in 1990 in Switzerland, and the introduction of compulsory testing of bovine carcasses in 2001 in European member states belonging to level III of geographical BSE risk (GBR) (**Table 1**).

UK experienced the emergence of BSE in 1986. The peak incidences were recorded in 1992 and 1993. Indeed the role of meat and bone meal was early suspected in the transmission of BSE and the first measures of prevention were enforced as early as 1988. The effects of these measures were visible in 1994 by a first decrease of the incidence. Retrospectively, the analysis of the epidemic curve allowed to estimate at five years the mean incubation period of BSE in cattle. Therefore the shape of the epidemic curve in UK was not the reflect of a natural evolution of the disease but was the result of the stringent control measures. The Over Thirty Months Rule (OTMS), which means that all animals over 30 months of age slaughtered are proscribed for human consumption, was introduced in UK in 1996 (Arnold, Wilesmith, 2003). In 2003, 612 new cases of BSE were still diagnosed. Several hypotheses are raised about the origin of these cases: insufficient respect of the control measures, other routes of transmission than feeding, maternal transmission or sporadic cases, e.g. (**Figure 1**).

In most of the other European member states BSE was firstly diagnosed in the nineties. The first cases recognized in Ireland in 1989 are explained by the close vicinity with UK. Furthermore, the cases diagnosed the same year in the Falkland Islands and the Oman Sultanate are imported cases. Several European countries declared positive cases only from 2001 (**Figure 2**). It is surprising that most of these countries, although belonging to the same GBR level as France or Belgium, for example, did not identify cases earlier. One can speculate about the quality of the epidemiosurveillance network which was greatly

Table 1. Countries are classified in four groups following the evaluation of their geographical risk of exposure to BSE (GBR) (European Commission, 2004b).

GBR level	GBR definition	European member states belonging to the level	Non European countries belonging to the level
I	Highly unlikely		Argentina, Australia, Botswana, Brazil, Chile, El Salvador, Iceland, Namibia, New Caledonia, New Zealand, Nicaragua, Norway, Panama, Paraguay, Singapore, Swaziland, Uruguay, Vanuatu
II	Unlikely but not excluded	Sweden (in revision)	Canada (in revision), Colombia, Costa Rica, India, Kenya, Mauritius, Nigeria, Pakistan, USA (in revision)
III	Likely but not confirmed or confirmed, at a lower level	Austria, Belgium, Cyprus, Czech Republic, Denmark, Estonia, Finland France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Poland, Slovakia, Slovenia, Spain	Albania, Belarus, Former Yugoslavia, Principality of Andorra, Bulgaria, Croatia, Israel, Japan, Macedonia, Romania, San Marino, Switzerland, Turkey
IV	Confirmed at a higher level	Portugal, United Kingdom	

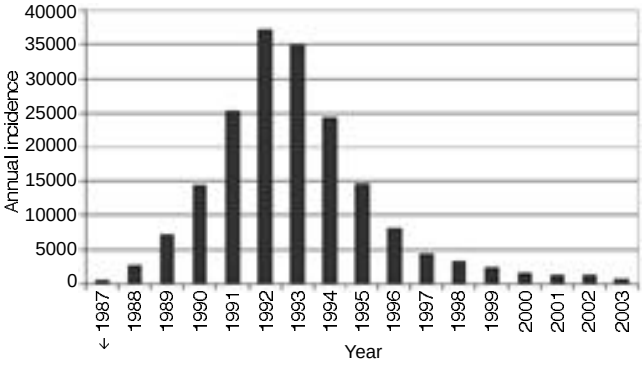

Figure 1. Annual incidence of BSE cases in United Kingdom since the emergence in 1986 (Office international des Epizooties, 2004).

improved in late 2000 and mostly in 2001 by the start of the compulsory testing of carcasses at slaughterhouses by the use of rapid tests (Pastoret *et al.*, 2001) (**Table 2**).

In non European countries, where imported cases are excluded, the disease was identified from 2001 in Japan and in 2003 in Canada. One case imported from Canada was found in USA in 2004 (**Table 2**).

In Europe, the evolution of the annual incidence is difficult to interpret because the cases have been recruited from different epidemiosurveillance networks. The Belgian situation can be taken as an example for the other European countries. The first case was reported in 1997. This does not preclude that BSE affected cows could have been present in Belgium before but not diagnosed. Until 2001, all the cases were obtained by the clinical epidemio-surveillance network. The two complementary programs, testing at slaughterhouse and at rendering plants, added cases to the incidence (Deslys *et al.*, 2001). It is therefore impossible to simply compare the incidences before and after 2001 (**Figure 3**). The introduction of these two programs was simultaneous to a sharp decrease of the identification of cases by clinical investigation on living animals.

A decrease in incidence was observed in Belgium in 2002 and 2003. The same evolution was observed in France and several other countries. It can be the result of the measures taken to ban the meat and bone meals in ruminants and other food-producing animals. However, as BSE is a rare event distributed in a large bovine population, it is difficult to state unambiguously whether this trend is significant. It must be confirmed by a steady decrease of the incidence until 2006, i.e. five years after the start of the total ban of mammalian meat and bone meal, because this period is the mean incubation time of BSE in cattle. Moreover for healthy slaughtered animals the lower limit of age is not the same for all countries: 24 months in France, Germany, Spain and Italy, and 30 months in the other European countries. This situation induces a bias in epidemiological indicators because BSE is rarely confirmed in animals younger than 30 months (Saegerman *et al.*, 2003).

A difficult epidemiological situation is encountered in countries which recently showed an increase in incidence: Ireland, Spain and Portugal (**Figure 2**). This feature suggests that BSE appeared

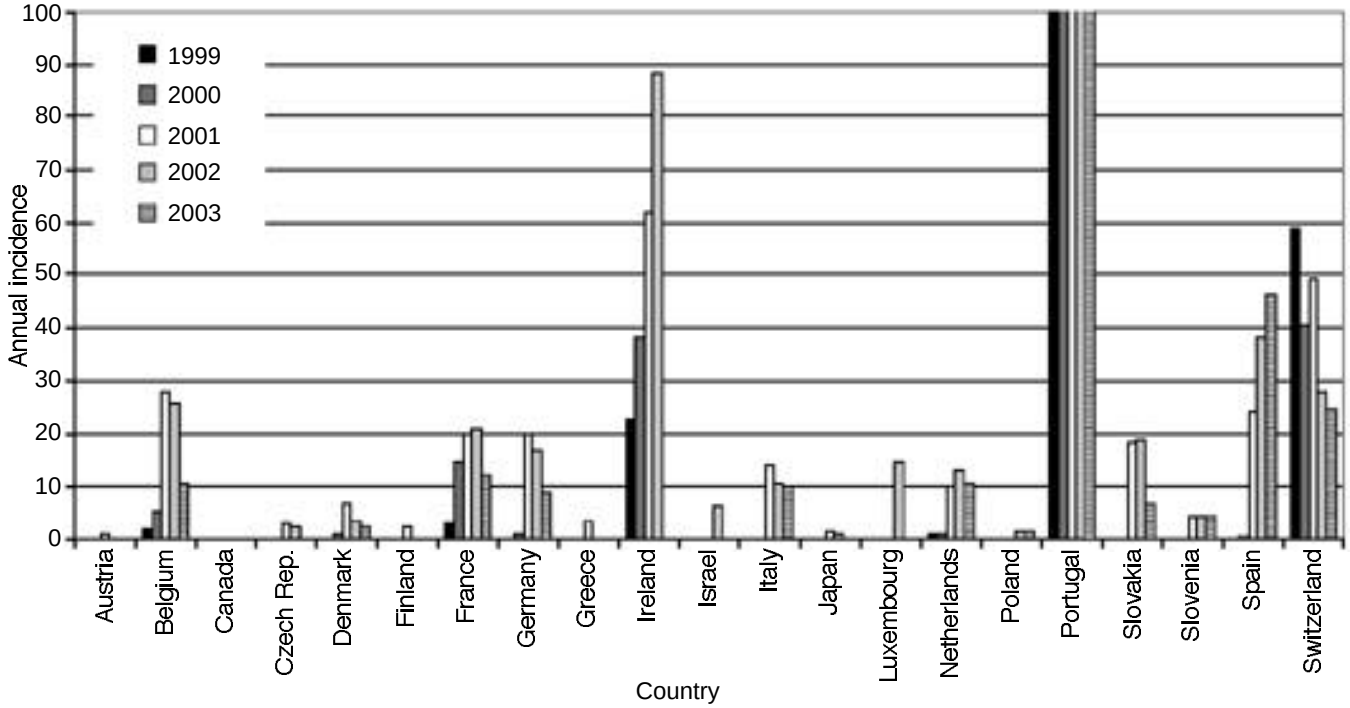

Figure 2. Annual incidence rates of BSE per million bovines of over 24 months of age in all countries which reported cases from 1999 to 2003 except United Kingdom. Some figures are so low that they are not or hardly visible in the diagram. It is the case for Austria (0.96 in 2001), for Canada (0.16 in 2003), for Denmark (1.14 in 2000), for Germany (1.07 in 2000), for Japan (1.44 in 2001, 0.97 in 2002), for the Netherlands (1.03 in 1999, 1.07 in 2000), for Poland (1.28 in 2002, 1.49 in 2003) and for Spain (0.59 in 2000). On the other hand, Portugal experienced high BSE incidences reaching figures that fall outside the scale of the graph: 199.50 in 1999, 186.95 in 2000, 137.88 in 2001, 107.80 in 2002 and 137.19 in 2003. The reported data are raw rates. Indeed, depending on the country, the minimum age of tested carcasses at slaughterhouse may vary: Germany performs a voluntary testing under 24 months of age and a compulsory testing over 24 months; France, Italy and Spain have a compulsory testing over 24 months and the other European member states over 30 months. These variations must be taken into account if the epidemiological situations are compared (European Commission, 2004a; Office International des Epizooties, 2004).

Table 2. Year of first diagnosis of bovine spongiform encephalopathy (Office International des Épizooties, 2004).

Year	Country
1986	United Kingdom
1989	Ireland, Oman Sultanate (2 imported cases) Falkland Islands (imported case)
1990	Switzerland
1991	France
1994	Portugal
1997	Belgium
1998	Netherlands
2000	Germany, Denmark, Spain
2001	Austria, Czech Republic, Finland, Greece, Italy Japan, Slovakia, Slovenia
2002	Israel, Luxemburg, Poland
2003	Canada
2004	USA (imported case)

later in these countries and makes therefore impossible to speculate about an effective control of BSE in these countries.

4. SUBCLINICAL CASES

Both experimental and field data support the existence of subclinical forms of TSEs (Hill, Collinge, 2003). These subclinical cases can have various origins: crossing of the species barrier, resistant genotype, prolonged incubation period, e.g. The epidemio-surveillance in European countries reveals that most of the BSE cases reported since 2001 have been identified by the systematic sampling in slaughterhouses. Other animals were identified in the rendering plants, therefore suggesting a disease causing death. Therefore all these animals were not recruited by the clinical epidemiosurveillance network. Those reported positive at abattoirs can be considered as subclinically infected at the time of slaughter or it may be that they had very faint clinical signs. As shown in **figure 3**, most of the detected BSE cases are therefore animals in good condition, without clinical signs or only very few. It is indeed impossible to predict if these animals would have developed clinical BSE if they were kept alive. It cannot be excluded that they could constitute later on a cohort of

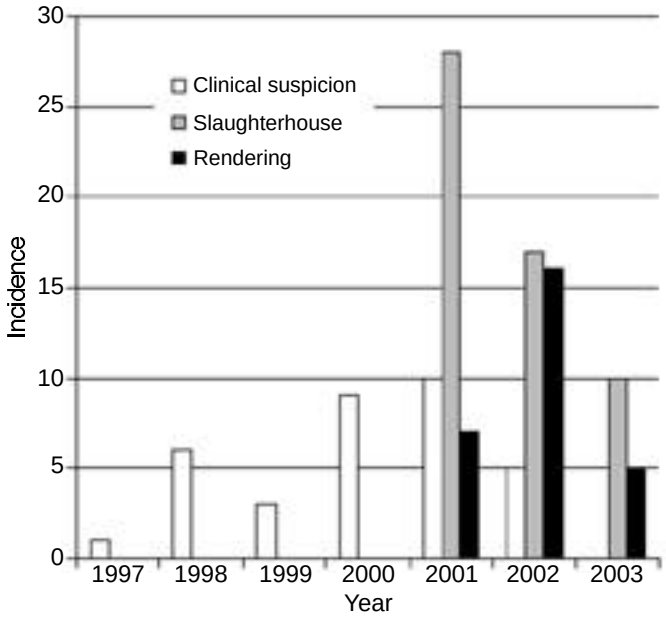

Figure 3. Incidence of BSE cases in Belgium, from the first case in 1997 till 2003, following the three networks of epidemiosurveillance: clinical suspicions identified in the field; carcasses over 30 months of age tested at slaughterhouse and over 24 months of age tested at rendering plants, which started in 2001. All cases were confirmed by the histopathological examination, immunohistochemistry and search for scrapie associated fibrils (Federal Agency for the Safety of the Food Chain, 2004).

animals which segregates from the typical BSE cases. Furthermore some of the atypical cases reported in the following sections could be also part of the subclinical cases.

5. ATYPICAL BOVINE SPONGIFORM ENCEPHALOPATHIES

The epidemiosurveillance network which was set up in Europe and several other non European countries identifies hundred of BSE and scrapie cases every year. Strain variation was already demonstrated for scrapie agent, but not for BSE so far. The recent identification of atypical cases could modify the epidemiology of BSE if these cases are attributed to new strains. Furthermore, these new data add to the complexity of the system and reveal that other situations can be encountered in natural conditions than those observed during the first phases of the BSE epidemic.

5.1. Atypical BSE cases in Japan

Since 2001, all cattle slaughtered at abattoirs in Japan are screened for BSE using a rapid test. A 23 months old steer was detected positive at the slaughterhouse. It was apparently healthy before slaughter. The histology showed no spongiform changes and immunohistochemistry revealed no accumulation of PrP^{res} typical of BSE. The analysis of PrP^{res} from the obex region revealed an electrophoretic profile different from the typical cases: the low content of the diglycosylated form, a faster migration of the non glycosylated form and less resistance against protease digestion than typical cases (Yamakawa *et al.*, 2003).

5.2. Atypical BSE cases in France

Three cases among those diagnosed at the slaughterhouse (two cases) and rendering plants (one case) in animals over 24 months old were confirmed BSE positive by Western blot detection of PrP^{res} extracted from the brain stem. These three samples showed relatively low levels of $PrPr^{es}$. They showed a atypical electrophoretic profile: it was mainly characterized by a higher molecular mass of the unglycosylated PrP^{res}. The same profile was identified previously in a cattle which had been experimentally inoculated intracerebrally with scrapie; however no genetic polymorphism was observed between atypical and typical BSE cases. The three apparently healthy cows were aged 8, 10 and 15 years, respectively.

The explanations of these atypical cases are still hypothetical: cattle may have been infected by another source of TSE agent like scrapie agent from sheep or goat; another possibility could rely on sporadic cases of BSE, as it has been already demonstrated in humans with CJD (Biacabe *et al.*, 2004).

5.3. Atypical BSE cases in Italy

Two healthy cows aged 11 and 15 years were positive among other cattle by histopathology, Western blot and immunohistochemistry. They exhibited however distinct features as predominance of the low molecular weight glycoform and a PrP^{res} fragment of lower molecular weight, as identified by Western blot. Additionally, the pattern of PrP^{sc} deposits was different in the central nervous system: the two cases showed large PrP^{sc} aggregates and dense PrP^{sc} amyloid plaques, instead of the amyloid deposition in typical BSE cases. Furthermore, the localization of the lesions was different in the brain. Therefore these cases are called bovine amyloidotic spongiform encephalopathy (BASE). This phenotype was unrecognized previously in BSE positive cattle. BASE was observed in old cows and shows similarities with a distinct subtype of sporadic CJD in humans (Casalone *et al.*, 2004).

5.4. Atypical BSE case in Belgium

A slaughtered 64 months old cow was positive in the abattoir by the rapid test. Further testing was carried

out on this case. No spongiform changes were observed by histopathology, no PrP^{sc} accumulation was seen by immunohistochemistry and the search for scrapie-associated fibrils was negative. Western blot testing was however positive, but the electrophoretic profile of PrP^{sc} was different from typical BSE cases. This atypical case is very similar to those already identified in Japan (De Bosschere *et al.*, 2004b).

6. RESISTANCE TO SCRAPIE AND ATYPICAL CASES IN SHEEP

Scrapie is not transmitted from sheep to humans. The human risk is associated with a possible exposure of sheep to BSE-contaminated feed. Since 2002, European Union has initiated a scrapie program: among several measures, the genotyping of 1% of the sheep population must be performed. Furthermore, it is also possible to select in the sheep population animals which exhibit a genotype resistant to scrapie. This genotype relies on amino acid substitutions in the PrPsequence, in positions 136, 154 and 171. The most resistant sheep belong to the genotype ARR/ARR[1]. However, several studies revealed that their resistance is not absolute. A few percentage of these resistant animals can be effectively infected by the scrapie agent (Ikeda *et al.*, 1995). Furthermore, one ARR/ARR[1] sheep died after intracerebral inoculation of BSE agent (Houston *et al.*, 2003).

A positive effect of this new surveillance program is the increase of the number of analyses. Among the positive results obtained, new scrapie cases were identified from sheep possessing a highly resistant genotype ARR/ARR[1] (Agence française de sécurité sanitaire des aliments, 2004). However there is no indication of a link between these cases and BSE.

Several atypical cases of scrapie were identified in Norway since 1998 (Benestad *et al.*, 2003). Recently a similar case was diagnosed in Belgium (De Bosschere *et al.*, 2004a). These sheep possess genotypes which are rarely associated with scrapie (AHQ/AHQ[1]; AHQ/ARQ[1]). They also do not show the typical histopathological brain lesions. Furthermore, other possible atypical scrapie cases in France and Germany can be missed by the usual rapid tests (Buschmann *et al.*, 2004) and their identification requires therefore very sensitive methods.

7. CHRONIC WASTING DISEASE IN CERVIDS

A chronic wasting disease is described in the USA since 1967 in three deer species: mule deer (*Odocoileus hemionus*), white-tailed deer (*Odocoileus virginianus*) and Rocky Moutain elk (*Cervus elaphus nelsoni*). This disease is also a TSE (Miller, Williams, 2003). It remained for many years as a scientific curiosity but recently the interest in this disease grew due to the emergence of BSE and an increase of the incidence and the geographic distribution of the disease. The death of three hunters by CJD in 2003 was enough to produce a “mediatic” emergence in the USA although no link was evidenced with chronic wasting disease and a human TSE. The disease is not observed outside North America.

8. CONCLUSIONS

After the initial BSE epidemic in Europe, the current trend is a decrease in incidence in most European countries. Fortunately, the number of variant CJD cases is kept low although any human case is dramatic. However, the relatively high number of BSE positive animals in UK and the increase in incidence in a few European member states require to maintain a high level of epidemiosurveillance. The continuous surveillance of BSE and scrapie reveals now the existence of so called “atypical cases” which need a careful attention.

TSEs are still emerging diseases. Although the scientific knowledge is steadily increasing, many aspects of the pathogenesis and the epidemiology of these diseases remain to be elucidated. However, efficient control measures were enforced in most of the European member states. Every measure which can improve the detection of infected animals and the quality and the respect of the feed ban is a step towards eradication of BSE in cattle.

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[1] One letter IUPAC code for amino acids with A = alanine, H = histidine, R = arginine and Q = glutamine.

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