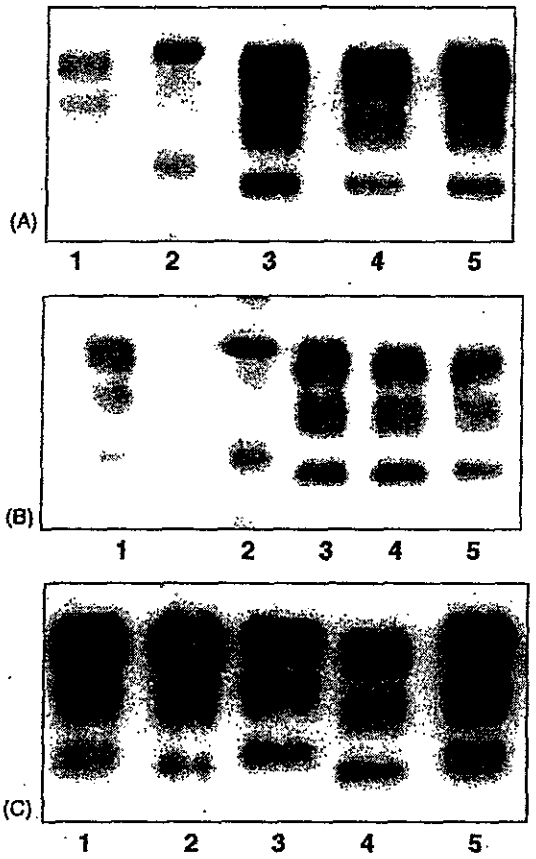

Fig. 2. Western blot detection of PrP^{res} in ovine transgenic mice (TgOvPrP4 mouse line) infected with a natural BSE-in-goat sample (CH636)(panel A, lanes 3–5) [28], CH1641 scrapie experimental isolate (panel B, lanes 3–5) or O104 field scrapie TSE isolate (panel C, lanes 3–5) [27]. Controls included experimental scrapie strain C506M3 in lane 1 and BSE in panel C, lane 2 as described in Ref. [22]. Molecular weight markers in panels A and B, lane 2, with the 29.0 and 20.1 kDa markers. Note the lower apparent molecular mass of unglycosylated PrP^{res} in all three mice infected with BSE or CH1641, by comparison to that from a C506M3-infected mouse, as well as the very intense labelling of the upper, diglycosylated, band in BSE-infected mice. In mice infected with the O104 isolate, two distinct PrP^{res} types, with different apparent molecular masses of unglycosylated PrP^{res}, can be detected according to the mouse brain analysed.

duce the distinct PrP^{res} molecular features from different scrapie and BSE sources [22] (Fig. 2). They indeed revealed that, contrary to what was previously observed with usual scrapie cases or with ovine BSE, PrP^{res} molecular features in mice were variable between individual mice, with two PrP^{res} forms differing by the apparent molecular masses of the unglycosylated form. Although biological characterisation of the infectious agent still remains to be achieved from such unusual TSE cases, such results could suggest the possible presence of distinct strains in such isolates.

3. Atypical scrapie/Nor98 in small ruminants

In EU countries, active surveillance of TSEs in small ruminants implemented since 2002 has allowed to identify rapidly a number of unusual isolates, notably in France, Germany and Great Britain [32,33], in sheep and in goats. These "atypical" isolates were characterised by (i) discrepancies in results obtained by different rapid diagnostic tests based on PrP^{res} detection and (ii) difficulties in confirmation by OIE recommended diagnostic methods. Overall results of biochemical studies suggested a lower protease resistance of a PrP^{res} form that might explain such discrepancies between diagnosis tests.

In fact, discrepancies between diagnosis tests and a lower protease resistance of PrP^{res} had already been reported in the first description of some unusual scrapie isolates, which had been identified as early as 1998 in Norway as Nor98 scrapie isolates [34]. In these Nor98 isolates, PrP^{d}, although never present at very high levels, was found to be more abundant in the cerebellum and in the cortex than in the brain stem, in contrast with classical scrapie or ovine BSE. Also, Western blot analyses, when positive, showed a complex electrophoretic pattern including an unusual band of low apparent molecular mass (≈10–12 kDa), while in classical scrapie PrP^{res} is detected as three bands between 18 and 29 kDa corresponding to the un-, mono- and di-glycosylated PrP^{res} forms (Fig. 3) [35]. This PrP^{res} fragment was recently found to be cleaved at both N- and C-terminal ends of the protein, in contrast with the only N-terminal cleavage found in classical scrapie and BSE [36]. The hypothesis that such atypical cases could only be artefacts and not authentic TSEs was discussed for a long time, especially for those cases found in France, Germany and Great Britain which were first identified by discrepancies between different rapid tests and/or confirmatory diagnosis methods [32]. Importantly all these last cases were identified following attempts to identify PrP^{res} in the brain stem, which is the most appropriate sample for classical scrapie or BSE.

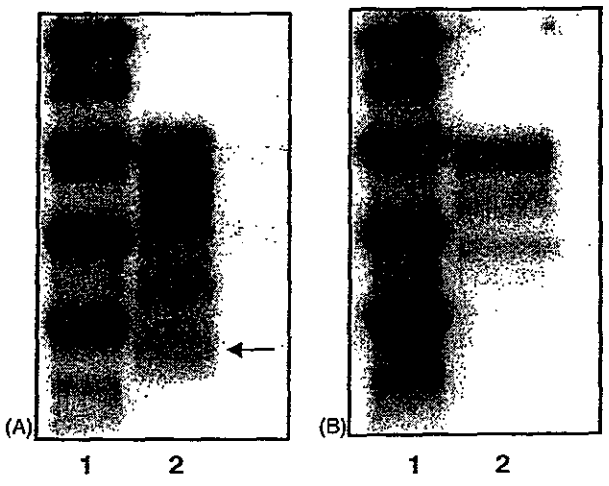

Fig. 3. Western blot detection of PrP^{res} in sheep with atypical (A) or classical (B) scrapie. Molecular weight markers in lane 1 showing the 58.1, 39.8, 29.0, 20.1 and 14.3 kDa markers. Note the complex electrophoretic pattern in atypical scrapie with at least five major bands, including a band at ≈10–12 kDa (arrow), while three bands between 18 and 30 kDa are present in classical scrapie.

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Furthermore, a number of these cases were identified in sheep with genotypes of the prion gene associated with high resistance to classical scrapie or BSE, such as those with the A136 H154 Q171 or A136 R154 R171 [32,33]. Some cases were also found in sheep homozygous for the A136 R154 R171 allele [33,37], which are considered as the most resistant to classical scrapie. Interestingly, Nor98 isolates had also been described in sheep with such "resistant" genotypes [34,35]. More recently, a strong association of Nor98 isolates with a leucine/phenylalanine polymorphism at codon 141 was also recognised in sheep with the A136 R154 Q171 allele [38].

The possibility that such cases were not genuine TSEs was definitively ruled out by the successful transmission of 10 French atypical isolates and three Norwegian Nor98 cases to transgenic mice over-expressing the ovine PrP (V_{136} R_{154} Q_{171} allele) [39]. These successful transmissions also revealed striking similarities between French atypical TSE cases and Nor98, which all showed consistent incubation periods, distribution of brain lesions and PrP^{res} molecular features in mice. Remarkably, by Western blot, PrP^{res} in both atypical scrapie and Nor98 infected mice showed a unique electrophoretic pattern from all the analysed sources, with notably a small PrP^{res} fragment of 10–12 kDa, quite distinct from the three bands (18–29 kDa) PrP^{res} pattern observed in classical scrapie and in BSE infected mice. Similar results regarding the successful transmission and PrP^{res} Western blot features were more recently obtained in a transgenic mouse line (TgOvPrP4) [31] expressing the ovine A136 R154 Q171 PrP protein.

Overall, these data, showing major differences with classical scrapie and BSE in a number of features of the disease, raise important questions about the origin of such cases, that have now been recognised with a high incidence and in an increasing number of countries [40–44]. Major uncertainties remain especially regarding the pathogenesis of the disease in these cases, as well as concerning their potential transmissibility within the affected species and to other species.

4. Atypical TSEs recognised in cattle

Molecular characterisation of PrP^{res} has also allowed to recognize atypical cases of TSEs in cattle. BSE in cattle was indeed considered to be a disease with unique features, in relation with its origin as a food borne epidemic [45,46]. The origin of the infectious agent involved in BSE remains unknown, but infectious meat-and-bone meal in which a TSE agent would have been recycled is considered as the origin of the epidemic. However, it was found that a few cattle showed by Western blot molecular features of PrP^{res} distinct from the majority of cattle with typical BSE (Fig. 4).

In a study of three French cattle, PrP^{res} with a higher apparent molecular mass of its unglycosylated form was reported (H-type) [47]. This was associated with strong labelling by a particular antibody, P4, contrary to typical BSE, showing that in these atypical H-type isolates, the PrP region recognised by P4 antibody was preserved following proteinase K digestion. Such cases were also recognised in other countries such as Germany [48]. Recently, transmission of a disease in wild-type (C57Bl/6) mice following intra-cerebral inoculation from such cases has been reported, which showed that the distinct molecular features were maintained in mice [49]. First histopathological data also showed a different distribution of spongiform lesions, and the presence of amyloid plaques, which are not found in this mouse model with typical BSE.

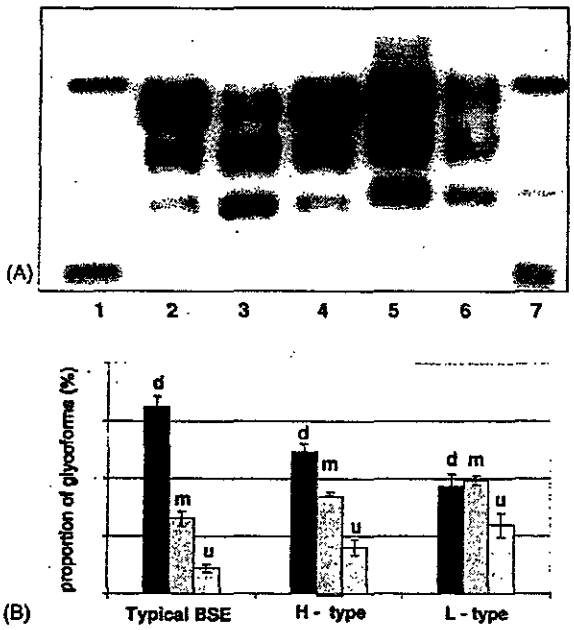

Fig. 4. (A) Western blot detection of PrP^{res} in cattle with atypical forms of BSE, including the H-type (lanes 5–6) and L-type (lane 3), in comparison with typical BSE (panel A, lanes 2 and 4). Molecular weight markers in lanes 1 and 7 showing the 29.0, 20.1 and 14.3 kDa markers. (B) Proportions of the three PrPres glycoforms, diglycosylated (d), monoglycosylated (m) or unglycosylated (u), showing the decreased levels of diglycosylated PrP in atypical cases of BSE, especially in the L-type, compared to typical BSE.

Another study described two cattle in Italy in which PrP^{res} showed a lower apparent molecular mass of the unglycosylated PrP^{res} (L-type isolates) but the most striking difference with BSE was that of the strongly decreased levels of the diglycosylated PrP^{res} form which is prominent in typical BSE [50]. Study of these two cases in Italy also reported significant differences in the distribution of brain lesions, but also in the nature of lesions, with the presence of amyloid plaques; this led to propose the term of bovine amyloidotic spongiform encephalopathy (BASE) for this novel pathology. Such cases have also been reported in some other countries, such as Poland, Germany and France [48,51,52]. Interestingly, such a L-type case in Germany could be transmitted by intra-cerebral inoculation in a bovine transgenic mouse model, in which incubation period of the disease was significantly shorter than that found with typical BSE [48]. Molecular analyses showed similarly decreased proportions of diglycosylated PrP^{res} to those initially described in cat-

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tle, but also distinct distribution of brain lesions compared to mice infected with typical BSE.

The origin of such deviant features in cattle TSEs remains unexplained yet. However, several hypotheses can be considered including (i) a major change of the BSE agent, (ii) infection of such animals with another source of TSE agent, such as scrapie from sheep and goats, (iii) the existence of a previously unrecognised form of TSE in this species. Each of these hypotheses can lead to some comments: (i) a phenotypic change of the BSE is unlikely, since a number of experimental studies have shown the remarkable stability of the BSE agent, including following cross-species transmission; such an phenomenon can however not be fully excluded since such a phenotypic switch has already been documented in a study of BSE transmission in a particular wild-type mouse line (SJL)([53], (ii) contamination by scrapie has been considered as a major hypothesis for the origin of the BSE epidemic in cattle, which could have been triggered initially by the recycling via meat-and-bone meal of such an agent after transmission in cattle, (iii) the situation in cattle, with two distinct phenotypes of TSEs beside the typical form of BSE, is reminiscent of the situation in human with distinct forms of "sporadic" Creutzfeldt-Jakob disease in addition to the so-called variant form of Creutzfeldt-Jakob disease which is caused by the BSE agent [14,18].

5. Conclusion

The precise role of the prion protein in the transmissibility of TSEs remains debated, especially regarding the possibility that this protein might be the sole constituent of the infectious agent ("protein-only" hypothesis). The existence of "strains" with distinct and specific transmissible biological properties, at least in mouse experimental models, has largely contributed to this controversy. Recent experimental data have, however, reinforced the possibility that strain properties might be associated with the disease-associated prion protein. Molecular studies of this protein have recently allowed to identify several forms of TSEs which were previously unrecognised, in cattle and in small ruminants. These findings were also a consequence of the substantial increase of TSE surveillance, especially in Europe. While the significance of these forms of diseases remains to be more precisely established, they already question about their possible occurrence, in other parts of the world, including in regions such as New Zealand or Australia, which were considered to be free of animal TSEs so far. It is possible that some of these newly recognised forms of TSEs are similar to "sporadic" forms of neuro-degenerative diseases, including most of the cases of Creutzfeldt-Jakob disease in humans and, in all these situations, the occurrence of such "sporadic" cases would raise the question of the origin of such diseases.

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一般的名称			研究報告の公表状況	Bovine spongiform encephalopathy and the safety of milk from Canadian dairy cattle M. G. Tyshenko, Veterinary Record, 160, 215-218 (2007)	公表国 カナダ	
販売名(企業名)						

研究報告の概要	使用上の注意記載状況・その他参考事項等
1980 年代後半に英国で乳牛におけるウシ海綿状脳症(BSE)が発生して以来，牛乳及び乳製品の安全性は重大な懸念事項であった。予防として，専門家らは罹患ウシ及びそのミルクがヒトの食物連鎖の中に入るべきではないということを提言してきた。本文献では，種々の動物のミルク及び初乳中のプリオンに関する研究から，ミルクの安全性に対する証拠を検討している。スクレイピー(ヒツジのプリオン病)の母子伝播がヒツジ及びヤギにおいて認められているが，スクレイピーに感染しているヒツジから得られたミルク，初乳又は乳腺組織を異なる経路を介して実験的にマウスに注射したが，症状を誘導することはできなかった。ウシにおける BSE の母子伝播に関する大規模試験によって，母子伝播は感染動物の 10％を占めることが立証された。しかしながら，マウスで実施された実験から，感染ウシからその仔牛への BSE 伝播にミルクが関与している可能性は否定された。1997 年に，ミルク，初乳及び乳腺組織は，BSE 感染性が検出されない組織として分類された。ヒトにおいて，母乳による伝播を含む vCJD の母子伝播に関する疫学的な証拠は存在しない。結論として，潜在的 BSE 感染ウシから得られたミルクを摂取することによって vCJD に罹患するリスクは実際にはごく僅かであることが示唆された。	BYL-2007-0274

報告企業の意見	今後の対応
これまでに報告されていたミルクやウシ初乳の摂取によるプリオン感染リスクはごく僅かであることが，本研究結果によって再確認された。	現時点で新たな安全対策上の措置を講じる必要はないと考える。

Bovine spongiform encephalopathy and the safety of milk from Canadian dairy cattle

M. G. TYSHENKO

The detection of bovine spongiform encephalopathy (BSE) in beef cattle closed Canadian beef export markets to 30 countries, including the USA, with devastating financial losses. The detection and confirmation of the fifth and seventh BSE-infected animals but first infected dairy cows extended the problem of risk management to Canadian dairy farmers. As the public are concerned about the safety not only of beef but also of milk and milk products that may contain disease-causing prions, this review examines the evidence for the safety of milk from studies on prions in milk or colostrum and their vertical and lateral transmission in various animal systems. The evidence indicates that the risk of contracting new variant Creutzfeldt-Jakob disease through the consumption of milk is negligible.

THE outbreaks of bovine spongiform encephalopathy (BSE) in cattle, and new variant Creutzfeldt-Jakob disease (vCJD) in human beings were spread by the practice in many countries of feeding ruminant-derived materials to other ruminants, and the movement of infected live cattle, meat products and contaminated feeds. The outbreak in the UK raised questions about whether various animal-derived food products, including dairy products, posed a risk to the health of human beings. For cattle entering the human food chain, restrictions were placed on high-risk materials, including the brain, spinal column and certain organs that have the highest titres of infective material. In Canada, beef consumers became aware of prion disease in domestic beef cattle after the detection of the first BSE-positive cow in 2003 (Coulthart and others 2003). The focus of research on BSE and the safety of human health have centred around beef, as many Canadians consume large quantities of meat in their diet. However, the detection of one imported and two domestic dairy cows infected with BSE have raised public concerns about the safety of milk and milk products in North America (Centers for Disease Control and Prevention 2004).

Although BSE-infected dairy cattle have been detected only recently in North America, the question of the safety of milk from dairy cattle is not new. During the outbreaks in the UK from the late 1980s to 2005, many dairy cattle became infected with BSE. Dairy cattle were used for milk production and for processing into foods such as ground beef, sausages and cuts of meat. The concern over prions in milk resulted in it being one of the products first targeted for a risk analysis. The Southwood Working Party was the first expert panel convened in the UK to assess the level of risk. On the basis of previous experience with scrapie, a similar spongiform encephalopathy affecting sheep, the risk of transmission to human beings was believed to be remote. However, owing to the degree of uncertainty, the expert panel recommended that affected animals and their milk should not enter the human food chain (Smith 2004).

The British government enforced the recommendation to ban milk from BSE-infected cattle for both human consumption (Pratt 1991). In 1991, the Ministry of Agriculture, Fisheries and Food was given powers under section 35(1) to seize, destroy and dispose of animal carcases suspected of having died from BSE. The powers under sections 1 and 8 were also used to protect human health by ordering the disposal of milk from cows affected by BSE (BSE Inquiry 2000a).

Milk is produced by a lymphatic-type tissue, and as prions can be detected in lymphatic tissue there is the possibility that milk may carry disease-inducing prions (Aguzzi 2006). Infected dairy cows may yield milk before they show clinical signs of BSE, and it is known that prions accumulate for years in infected animals before clinical signs appear. When infected but apparently healthy cows are milked, their milk is mixed in a collection tank with milk from other healthy cows; if the disease-causing prion is present in this milk it would contaminate the entire batch, and could repeatedly contaminate milk from each subsequent batch. Thus, an infected dairy cow's milk may enter the human food chain and be consumed over many months or years.

Furthermore, it is known that the infectious prions are extremely resistant to heat and resist normal sterilisation processes (Brown and others 2000), so pasteurisation does not completely destroy them (Fernandez Garcia and others 2004). As a result, the public continue to be concerned about the safety of milk and other dairy products in relation to BSE infectivity (Trevitt and Singh 2003, Vetrugno 2004). This paper reviews the results of experiments that have been conducted in various animal systems to try to answer questions regarding milk safety.

STUDIES IN SHEEP

Maternal (vertical) transmission – evidence from sheep and scrapie

At the beginning of the BSE outbreak in the UK, it was hypothesised that the source of the infection was scrapie in sheep, and it was estimated that scrapie affected one-third of all British sheep flocks (Morgan and others 1990). It had also been assumed that the disease in sheep was transmitted genetically, but this could not explain the widespread prevalence of the disease (Dickinson and others 1965, 1966). Studies proving the occurrence of maternal transmission provided a partial explanation for the distribution of scrapie within sheep flocks. The studies of maternal transmission also tested the possibility that offspring might become infected from their mother's milk. Mammary gland tissue from two scrapie-affected ewes and colostrum samples taken at parturition from six high-risk ewes were used to prepare prion material for intracerebral injection into groups of 10 mice. No transmission of infectivity was detected in the mice (Hadlow and others 1982).

Mouse bioassay for scrapie

Although maternal transmission of scrapie had been observed in sheep and goats, it had not been induced in laboratory mice infected experimentally with scrapie. Various membranes and fluids were tested for infectivity, including fetal membranes, faeces, urine, saliva, milk, semen and colostrum, and different methods of infecting the mice were used, including intracerebral, subcutaneous and intravenous injections or oral dosing. None of the mice infected with any of these materials, by any of these routes, developed scrapie (Hourrigan and others 1979).

Veterinary Record (2007) 160, 215-218

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TABLE 1: Evidence in various animal systems and known transmission of disease-causing prions by detection in milk or colostrum, vertical transmission or lateral transmission

Animal system	Prion detection in milk or colostrum	Vertical transmission (mother to offspring)	Lateral transmission (iatrogenic, placental, ingestion, other tissue contamination)
Human (kuru)	No* (Ridley 1995), untested but inferred	No* (Ridley 1995)	Yes* (Ridley 1995), previous practice of eating contaminated brain
Human (vCJD)	No (Vetrugno 2004) Yes† (Tamai and others 1992), results retracted	No (Baker and Ridley 1996)	Yes (Williams 2006), human by blood transfusion and iatrogenic transmission
Sheep and goat (BSE/scrapie)	No (Hadlow and others 1982), inferred by mouse bioassay Yes (Aguzzi 2006), detection in milk sediment in mammary tissue (Pattison and Millson 1962)	No‡ (Foster and others 2004), challenged with cattle prion Yes (Lacey and Dealler 1994, Caplazi and others 2004), challenged with sheep prion (Bellworthy and others 2005), natural BSE transmission in sheep flock	Yes (Ryder and others 2004), contaminated feed (Foster and others 1993, 2001)
Cattle (BSE)	No (Vetrugno 2004, Everest and others 2006)	Yes§ (Donelly and others 1997a)	Yes (Dealler and Lacey 1991), contaminated feed
Mouse bioassay (BSE)	No (Taylor and others 1995), orally infected with BSE cows' milk	No# (Taguchi and others 1993)	Yes (Maignien and others 1999), experimentally infected
Mouse bioassay (knockout transgenic expressing cattle BSE prion)	No (Castilla and others 2005a)	Yes (Castilla and others 2005b)	Yes (Castilla and others 2005b), experimentally infected

* Incidence of kuru decreased significantly in people born after cannibalism ceased in the mid-1950s; no children born after 1959 to any of the women who had or subsequently developed kuru have themselves developed the disease

† One case was reported in 1992 of a 38-year-old pregnant woman with sporadic Creutzfeldt-Jakob disease (CJD) whose colostrum was found to be infected when injected intracerebrally into mice. However, following further morphological examination of fixed mouse brain by immunohistochemistry for PrP^{Sc}, the Japanese authorities concluded that the published results were invalid because no spongiform change or PrP^{Sc} was found on first passage from human colostrum to mice. The mouse brain from the second passage (mouse to mouse) did show spongiform change and PrP^{Sc} but this was not attributed to transmission from the colostrum

‡ Cheviot ewes challenged orally with bovine spongiform encephalopathy (BSE) cattle brain produced lambs of various PrP genotypes; of 72 surviving to more than 30 months of age, 29 were of the most susceptible PrP genotype; none of the progeny showed any signs of disease

§ Data reveal a significantly higher risk of disease in calves born to BSE-affected dams, with the risk being greatest in calves born after the onset of clinical signs of disease in the dam

No transmission of CJD in mice was observed in 75 offspring born to CJD agent-inoculated females or to normal females mated with inoculated males, or in 19 normal offspring maintained by foster nursing with the inoculated mothers

The attempt to compare intracerebral, intravenous and oral doses complicated the studies. The infectious dose of prions injected intracerebrally was markedly smaller than the infectious doses of prions given parenterally, intravenously or orally. The dose of prions needed to cause an infection may also vary with the strain of prion, the physical composition of the aggregated prions, the source tissue, and the genetic susceptibility of the host animal (Erdtmann and Sivitz 2003).

STUDIES IN CATTLE

Maternal (vertical) transmission – evidence from cattle and BSE – the maternal cohort study in cattle, 1989 to 1997

The most important research undertaken to investigate maternal transmission in cattle was the cohort study carried out between 1989 and 1997 at the Central Veterinary Laboratory in the UK. In this study, 300 calves from affected dams were matched with 300 control calves from unaffected dams to follow the subsequent development of BSE. One weakness of the study was that calves in both the groups had already been exposed to infected cattle feed; a second weakness was that there could have been significant differences in genotypes between the subjects and controls that might have affected the outcome. However, it was believed that the size and design of the study, and the fact that both groups had had similar exposures to contaminated feed, would minimise the former weakness. The results showed that BSE occurred in 14 per cent of the offspring of the affected dams but in only 4·3 per cent of the offspring of the unaffected dams; the difference between the control and experimental groups was statistically significant (Wilesmith and others 1997).

A secondary analysis of the results of the cohort study by other groups independently confirmed that maternal transmission was responsible for up to 10 per cent of affected cattle, but that this rate would not have been adequate to maintain the BSE epidemic (Donnelly and others 1997a, b, Ferguson and others 1997, Gore and others 1997). The experimentally established value of 10 per cent remains the accepted contribution for the maternal transmission of BSE in cattle populations.

Mouse bioassay for BSE

The infectivity of BSE is usually demonstrated experimentally. A bioassay is used to show that BSE can be transmitted between animals, in which suspect tissue is inoculated into a target species or laboratory animal. Such research concluded that the transfer of BSE from affected cows to their calves in milk could be excluded as a route of transmission.

In one experiment, milk from BSE-affected cows was given orally to mice; a cohort of 275 mice consumed an average of 300 ml of milk per mouse over a period of 40 days. Groups of mice were also given either an intracerebral injection of 0·02 ml milk or an intraperitoneal injection of 0·1 ml milk. The mice were observed for two years during which none of them developed signs of disease and none had the characteristic neurological lesions postmortem (Taylor and others 1995). Although these results support the hypothesis that milk does not transmit infective prions, there are doubts about the relative transmissibility of the disease from cows to mice owing to the potential species barrier. The results of a similar experiment, in which calves were infected intracerebrally or orally with milk from infected cows, thus obviating the species barrier, have not been reported (Wells and others 1998).

Experiments to resolve the infectivity of cattle tissues in the mouse bioassay suggested that several non-neural tissues known to be infective for scrapie were not infective for BSE. The results supported the idea that the mouse bioassay was probably not sufficiently sensitive to detect BSE infectivity across the species barrier, and that the pathogenesis of BSE differed markedly from the pathogenesis of scrapie. The comparative bioassay has shown that the mouse bioassay is