

# Zoonosis Update

## Bovine spongiform encephalopathy

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**B**ovine spongiform encephalopathy was described as a new disease of cattle in 1987.<sup>1</sup> From its first recorded appearance in England, more than 184,500 cases have been confirmed throughout the United Kingdom, an epizootic that peaked in 1992.<sup>2</sup> However, because the incubation period of this disease exceeds the usual slaughter age for cattle, these recorded case numbers represent only a fraction of the infected animals; it has been calculated that since 1980, approximately 2 million cattle have developed BSE in the United Kingdom.<sup>3</sup> The disease has now been confirmed in native-born cattle in 25 countries throughout the world, including Asia and North America.<sup>2</sup>

Bovine spongiform encephalopathy belongs to a class of diseases known as TSEs, so named because of the vacuolization often observed in the brains of TSE-affected animals. This group of uniformly fatal diseases causes a slow-onset encephalopathy that is notable by the distinct lack of any immune response and by aggregations of characteristic protein deposits that are detectable primarily in the CNS.<sup>1,4</sup> These unusual proteins, called prions,<sup>5</sup> are the infectious agent of all TSEs,<sup>6</sup> and inoculation with these prions or sometimes merely the ingestion of tissue containing prions transmits the disease to a new host.<sup>7-15</sup>

Other well-known TSEs are scrapie of sheep,<sup>16</sup> transmissible mink encephalopathy,<sup>17</sup> chronic wasting disease of cervids,<sup>18</sup> and kuru<sup>12,19</sup> and sporadic Creutzfeldt-Jakob disease of humans.<sup>20,21</sup> Bovine spongiform encephalopathy is the only TSE known to be transmissible from animals to humans.<sup>22,23</sup>

### Clinical Signs and Histopathologic Changes in Cattle With BSE

Cattle with BSE develop signs of CNS disease that can be vague and nonspecific and that are often accompanied by a decrease in milk yield and loss of body weight.<sup>24-26</sup> The most common neurologic signs in cattle with BSE are apprehension, pelvic limb ataxia, and hyperesthesia to auditory, visual, or tactile stimuli.<sup>1,24,27,28</sup> Bradycardia and reduced rumination are often observed, indicating disturbance of autonomic innervation.<sup>26,29</sup>

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### ABBREVIATIONS

BSE	Bovine spongiform encephalopathy
CJD	Creutzfeldt-Jakob disease
TSE	Transmissible spongiform encephalopathy
vCJD	Variant form of Creutzfeldt-Jakob disease

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The disease must be differentiated from other causes of CNS disease.<sup>27</sup> Rabies generally follows a shorter clinical course (< 8 days) than the prolonged disease seen in cattle with BSE.<sup>26</sup> In cattle with BSE, analysis of the CSF reveals no abnormalities, and there is no inflammatory response.<sup>1,30</sup> Because a diagnosis of BSE can be confirmed only by results of analyses of brain tissue collected after death, BSE in cattle is a diagnosis of exclusion.<sup>26,27</sup>

Examination of the brains of cattle clinically affected with BSE during the UK epizootic revealed a consistent pattern of pathologic changes.<sup>31,32</sup> The typical spongiform vacuolated changes were most noticeable in the medulla oblongata at the level of the obex.<sup>33</sup> Examination of brain tissue slices treated with antibody against prions revealed prion-positive accumulations that generally reflect the same distribution as that of vacuolation in the brain. Details of neuropathologic changes of cattle with BSE were well described by veterinary pathologists working in the United Kingdom during the epizootic<sup>34,35</sup> and have been recently reviewed.<sup>33</sup>

### Prions as the Infectious Agent of BSE

More than 25 years ago, Stanley Prusiner and colleagues identified the etiologic agent of TSEs as an unprecedented pathogenic entity: proteins that are able to propagate without the need for nucleic acid. These proteins were named prions.<sup>5,6</sup> For his work, Prusiner was awarded the Nobel Prize in 1997, and in recent years, the protein-only theory of prion infectivity has been validated by the work of many others.

Prusiner's identification of prions as infective agents was soon followed by the discovery of a native prion protein that is a normal constituent of cell membranes in vertebrates, expressed at particularly high concentrations in nervous tissue.<sup>36-45</sup> Assessment of the function of the normal cellular prion protein is an area of active research. In mammals, native prion proteins may protect cells from oxidative damage, regulate circadian rhythms and sleep, and aid in the creation of memories.<sup>45</sup> It is intriguing that a rare, inherited human prion disease (fatal familial insomnia) prevents its victims from sleeping, leading to dementia and death.<sup>46</sup>

Disease-causing prions and the normal native prion proteins have identical amino acid sequences and differ only in their folding pattern or conformation (ie, they are isoforms).<sup>47-49</sup> During development of a prion disease, an exogenous infectious prion isoform converts a native prion protein into a replicate of the infectious prion isoform. This newly formed isoform can, in turn, convert other native prion proteins in a kind of chain reaction<sup>50-52</sup>; thus, the infectious prion isoform replicates and propagates the infection.<sup>50-52</sup> The aberrant disease-associated isoforms accumulate as the prion deposits identified in the brains of infected animals, which cause generalized signs of CNS disease that leads inexorably to death.

One enigma of TSEs is the existence of different strains of prion disease within the same animal species; the strain differences cannot be attributed to differences in amino acid sequences because those are determined by a host's genome.<sup>53-55</sup> However, there is more than 1 folding pattern (prion isoform) that can develop from the same amino acid sequence. These alternate folding patterns are believed to be the basis for multiple strains of prion diseases.<sup>55-57</sup> Each TSE disease strain causes a distinct disease pattern, with predictable and reproducible incubation time and disease course, as determined by the experimental transmission of TSEs in laboratory animals.<sup>53,54,58,59</sup> Each strain converts the host's native prion protein into a strain-specific disease-associated prion with distinctive molecular properties (eg, differing resistance to denaturation in the presence of guanidine salts).<sup>55-57</sup>

Prion diseases have a natural cross-species transmission barrier: an exogenous prion is most infective when it closely matches the amino acid sequence of the host's own normal prion proteins so that the incoming prion can readily serve as a template for the transformation of native prion protein to the disease-associated isoform. If the exogenous infectious prion arises from a different species, the lack of homology between the incoming infectious prion and the host's own native prion protein protects the host's prion proteins from transformation. In the latter situation, there is a lower chance of infection by the exogenous prion, and if infection does develop, the incubation period is generally lengthened by this so-called species barrier.<sup>59,60</sup>

Although the concept of species barriers for prion diseases has been validated, these barriers are not as complete as once believed. It is now recognized that many apparently nonsusceptible animals exposed to infectious prions from another species (or prions from another animal of the same species that has a different native prion genotype) do, in fact, support the replication of the infectious prions in the absence of clinical disease. The tissues of these subclinical carrier hosts can be infective if introduced back into a host of the susceptible genotype.<sup>61-64</sup> From experiments involving susceptible and nonsusceptible transgenic (humanized) mice, the evidence suggests that infective prions in nonsusceptible carriers without clinical signs retain the capability to infect a susceptible host if tissue is exchanged through surgery or transplantation.<sup>65</sup>

Prion diseases originating in a different species can become host-adapted upon passage through the recipi-

ent host species.<sup>66</sup> As the infection progresses, more of the infectious prion contains the host sequence so that when it is transmitted to another animal of the same species, the prion is composed entirely of host sequence and is now compatible with the host native prion protein.<sup>67</sup>

## **Origins of BSE**

The origins of BSE remain a mystery. There has been conjecture that the UK epizootic might have originated as a bovine-adapted sheep scrapie strain; it has been suggested that through the rendering of sheep and cattle carcasses into cattle feed, and following multiple cycles of ingestion and infection, a natural field strain of scrapie may have become host adapted to cattle.<sup>68,69</sup> If this is true, the resulting diseases have differentiated themselves in their respective hosts since this cross-species transformation occurred: sheep scrapie samples from before 1975 and after 1990 induce different clinical signs in recipient cattle, but neither of these experimental diseases resembles BSE.<sup>70</sup> The typical BSE strain identified in the UK epizootic can infect sheep if injected intracranially or administered orally, but the resulting disease can be distinguished from scrapie.<sup>33,71-74</sup> Analogously, cattle have been experimentally infected with the sheep scrapie agent via intracranial inoculation,<sup>70,75,76</sup> but the resulting TSE (cattle-passaged scrapie) is different from BSE.<sup>33,75,76</sup> If cattle are orally administered the sheep scrapie agent, they do not become infected.<sup>77</sup>

Other speculations regarding the origin of BSE have been made. One hypothesis posits that BSE may have originated from an atypical strain of BSE in cattle.<sup>78,79</sup> A rare prion isoform may have appeared spontaneously and subsequently propagated through the feeding of meat-and-bone meal prepared from an original host animal. It has even been speculated that BSE may have originated as a bovine-adapted human TSE, and that meat-and-bone meal imported into the United Kingdom from the Indian subcontinent might have inadvertently contained some human remains.<sup>80</sup> However, this theory is contradicted by results of experiments involving bovinized transgenic mice (ie, mice that express the bovine native prion protein and are therefore highly susceptible to BSE); these mice are not susceptible to human transmissible spongiform encephalopathies other than vCJD, the human disease linked to BSE.<sup>81</sup>

Presently, there is no evidence to suggest that BSE can arise from a natural transmission of cervid chronic wasting disease or transmissible mink encephalopathy.<sup>82</sup> Cattle are only susceptible to chronic wasting disease if they are inoculated intracranially, and the resulting disease is distinct from BSE.<sup>83-86</sup> In a detailed pathologic analysis, cattle living and grazing on land presumed to be heavily infected with the agent of chronic wasting disease developed no related pathologic changes after 8 years.<sup>87</sup> Transmissible mink encephalopathy has been experimentally transmitted to cattle via intracranial inoculation,<sup>76,88</sup> but not via SC inoculation.<sup>88</sup>

## **Transmission of BSE Among Cattle**

Ingestion of infectious tissue from BSE-infected animals via meat-and-bone meal is the only route of

transmission that has withstood epidemiologic and experimental scrutiny and that is considered sufficient to initiate and sustain an epizootic among cattle.<sup>24,89</sup> Among domestic cattle during the UK epizootic, there was no indication that the agent of BSE was being transmitted directly from animal to animal; even during the most intense years of that epizootic, most farms had only a few cases.<sup>24,89,90</sup> Soon after identifying meat-and-bone meal as the transmission vehicle, UK authorities banned the feeding of any ruminant-derived protein to other ruminants,<sup>91</sup> an act that resulted in an 80% decrease in infection rate among cattle born during the following year.<sup>89</sup>

Although the number of clinical cases declined dramatically among cattle born after the 1988 meat-and-bone meal feed ban in the United Kingdom, more than 40,000 cases of BSE developed in cattle born during the years that followed.<sup>92</sup> These cattle were most likely infected by the cross-contamination of cattle rations by feed intended for nonruminant farm animals.<sup>90,93</sup> Therefore, in 1996, it became illegal in the United Kingdom to prepare feed containing any mammalian protein for any farm animal. Furthermore, it became illegal even to store such feed on farm premises; all farmers were notified to clean their feed storage areas to remove any trace of contaminated feed.<sup>94</sup> In the interval since inception of the 1996 feed ban, the annual number of cases of BSE in the United Kingdom has declined steadily each year.<sup>2,95–97</sup> Only 163 cases of BSE have been identified in animals born after 1996.<sup>97</sup>

In the United Kingdom, a thorough review of the first 93 cases of BSE in cattle born after the 1996 feed ban revealed that BSE can occasionally be transmitted to cattle from lingering traces of contaminated feed.<sup>98,99</sup> The extraordinary susceptibility of cattle to BSE has been borne out by results of oral administration of progressively smaller quantities of BSE-infected brain homogenate to cattle. The experimental ingestion of 1 mg of brain slurry from clinically affected cattle has transmitted BSE to 1 of 15 calves, albeit after an extended incubation period.<sup>100,101</sup> It has been estimated that for cattle, the dose of this brain preparation that is sufficient to achieve transmission to 50% of animals via oral ingestion is approximately 200 mg (95% confidence interval, 40 to 1,000 mg).<sup>101</sup> The threshold for an infective oral dose must be assumed to be < 1 mg of infected brain material.<sup>101</sup>

Because of the etiologic and pathologic similarity of this disease to scrapie,<sup>16</sup> the possibility of maternal transmission of BSE was of real concern. There did appear to be some small risk (0% to 2.8%) of maternal transmission to calves born in the last 6 months of the dam's incubation period.<sup>3,102,103</sup> However, infectivity has not been found in any male or female reproductive tissue, including mammary glands. Neither bovine milk nor placental tissue from BSE prion-infected cattle has been found to be infective.<sup>104–109</sup> Transmission via embryo transfer has also been investigated, but neither infected donor bulls nor infected donor cows were capable of transmitting BSE to recipient heifers or to the offspring.<sup>110</sup>

Environmental contamination is not an important source of infection, nor is transmission by birds, ro-

dents, or other vectors.<sup>111,112</sup> An epidemiologic review of the BSE prion-infected cattle born after the strict feed ban rules implemented in 1996 revealed that these cases did not have an increased likelihood of originating from UK herds with a high incidence of BSE in the past.<sup>111</sup> Sewage sludge from abattoirs was deemed unable to sustain an epizootic, but could pose a small risk to animals grazing on pastures contaminated by such sludge.<sup>113</sup>

## **Transmission of BSE to Humans**

Bovine spongiform encephalopathy is the only known zoonotic TSE, but its zoonotic nature was not known until years after the outbreak began in cattle.<sup>22,23</sup> In 1996, UK physicians recognized a previously unseen fatal neurodegenerative disease that resembled CJD, a known human prion disease. Creutzfeldt-Jakob disease is rare in persons < 50 years old, but by 1995 there was an emerging pattern of unusually young patients with clinical, electroencephalographic, and neurodegenerative signs that differed from the usual form of CJD (now called sporadic CJD).<sup>22,114–116</sup> The newly recognized vCJD was distinguishable from sporadic CJD, even in the rare instance when sporadic CJD occurred in an adolescent.<sup>117</sup>

Analysis of the prion isoform deposits in vCJD patients revealed the same biochemical properties (eg, electrophoretic banding patterns and antibody staining affinities) as those associated with prion deposits in BSE prion-infected cattle; the properties were distinct from those of prions associated with any other known spongiform encephalopathy, including prions in patients with sporadic CJD.<sup>118,119</sup> Inoculation of nonhuman primates with brain material from vCJD patients induced a disease indistinguishable from that caused by inoculation with brain material from cattle with clinically evident BSE.<sup>23</sup> The human cases had presumably contracted vCJD via the consumption of infective tissue from cattle with BSE.<sup>100</sup>

In contrast to vCJD, there is no reason to suspect that sporadic CJD is a human-adapted animal TSE because this disease develops even among lifelong vegetarians.<sup>120</sup> Concurrent with the BSE epizootic, there has been no significant increase in the background incidence of sporadic CJD in the United Kingdom or Europe.<sup>121,122</sup>

Unlike BSE in cattle, vCJD, the human manifestation of this disease, involves widespread tissue infectivity beyond that of brain and other nervous tissue. Four cases of vCJD have been strongly linked to transfusions of blood from donors who developed clinically evident vCJD after the time of their donation.<sup>123–127</sup> There is evidence that young persons are more susceptible to vCJD; after controlling for age-related beef consumption patterns, susceptibility appears highest for adolescents and young adults.<sup>128,129</sup> A competent immune system may increase the susceptibility of young animals to prion disease; the lymphoid follicular dendritic cells that escort prions through the immune system to the nervous system lose functional capability with age.<sup>130</sup>

Humans appear to have substantial differences in their susceptibility to BSE, based on the genetic poly-

morphism of the human prion genome.<sup>131</sup> The most common polymorphism is methionine/valine (M/V) at position 129 of the native prion protein; persons who are homozygous for methionine (ie, M/M) at this site appear to be more susceptible to developing prion disease. All known clinical cases of vCJD, including 3 of the 4 transfusion-acquired cases, have occurred in M/M homozygotes, despite the fact that such individuals comprise only 40% of European populations.<sup>123,124,126</sup> Among persons with kuru (a historical human TSE transmitted via ritual ingestion of human brains), M/M homozygotes developed the disease much sooner after exposure than did heterozygotes, in some of whom clinical disease developed > 30 years from the time of exposure.<sup>12,132,133</sup>

Bovine spongiform encephalopathy, like other prion diseases, can be transmitted not only via ingestion, but also via the iatrogenic transfer of tissue from a subclinical carrier. The human medical community has known for decades that sporadic CJD and other prion diseases can be iatrogenically transmitted through tissue exchange or transplantation<sup>134–139</sup> or through use of conventionally sterilized surgical instruments.<sup>136,140</sup> In BSE prion-infected cattle, the infectious prions are mostly confined to the CNS. However, in patients with vCJD, the infectious prions are detected in multiple tissues throughout the body, conferring a risk of iatrogenic transmission, even through routine surgical procedures, if adequate prion decontamination procedures are not followed.<sup>124,141–143</sup>

Despite the low incidence of clinical vCJD, a retrospective analysis of tonsil and appendix biopsy specimens estimated that as many as 1 in 4,000 persons exposed during the UK epizootic may be a subclinical carrier and may be capable of efficient iatrogenic transmission to others of more susceptible genotype.<sup>125,144,145</sup> Subclinical infection was evident even in persons with the resistant V/V genotype.<sup>145,146</sup> Postmortem examination of a person who was known to have received a blood transfusion from a donor who later died of vCJD revealed the presence of prions in the spleen, although the recipient had died of non-neurologic disease and had possessed a prion-resistant M/V genotype.<sup>125</sup>

### **Transmission of BSE to Other Species**

The species barrier is quite evident for BSE—cattle are more susceptible than most other animals. However, several other ruminants in zoologic collections became infected during the BSE epizootic in the United Kingdom and France; kudu are especially susceptible to widespread tissue infection.<sup>147–155</sup> Other agriculturally important animals were presumably exposed to feed infected with the BSE agent but did not develop BSE.<sup>156,157</sup> To our knowledge, there have been no confirmed cases of naturally acquired BSE in sheep<sup>158,159</sup> and only a single field case of BSE in a goat.<sup>160</sup> The BSE agent has been experimentally transmitted to sheep and goats via intracranial inoculation and via ingestion of infectious material from BSE prion-infected cattle.<sup>71,72</sup> These experimentally induced BSE prion infections in small ruminants were associated with characteristic patterns of neuropathologic changes in the brain and distinctive banding patterns in prion western blots; thus, BSE

prions in goats and sheep can be reliably differentiated from any known strain of the scrapie agent.<sup>161–163</sup>

During the BSE epizootic in the United Kingdom, no pigs were found to be affected with BSE; pigs are not susceptible to the BSE agent via oral ingestion. Pigs do not become subclinical carriers following oral ingestion of the BSE agent; the tissues of pigs that ingest the BSE agent do not become infective (as determined from results of rodent bioassays).<sup>157,164</sup> However, following experimental parenteral (intracranial, intraperitoneal, and IV) inoculation of infectious material, pigs can become nonclinical carriers of BSE.<sup>156,164,165</sup>

Recently, roe deer were experimentally infected with the BSE agent; although the deer were susceptible to infection, the infection was limited to the CNS and peripheral nerves and did not affect the lymphatic system. Distinction between such experimentally induced BSE and chronic wasting disease in these animals would be difficult.<sup>166</sup> Rabbit native prion protein appears unable to assume the form of infectious isoform, thereby protecting rabbits from prion diseases.<sup>167</sup> Fish and birds are unlikely to propagate the BSE agent because of the low sequence homology with mammalian prion protein.<sup>39,42</sup>

During the epizootic in the United Kingdom, domestic cats and dogs were presumably equally exposed to the BSE agent through consumption of infected meat. Many domestic cats and captive large felids developed a feline spongiform encephalopathy that was identified as being caused by the BSE-associated prion, but domestic dogs and wild canids did not.<sup>157,168–172</sup> Although feline and canine native prion molecules are highly similar, amino acid substitutions in critical areas appear to protect dogs, but not cats, from infection with the BSE agent.<sup>173</sup>

### **Strains of the BSE Agent**

Although most cattle with BSE appear to have been infected with 1 strain of the BSE agent, there have been rare, atypical strains of BSE prion identified in cattle in many countries.<sup>174–185</sup> Throughout the BSE epizootic in the United Kingdom, all cases appear to have been affected with the same prion strain,<sup>31,186</sup> although rare strains may not have been detected by use of the testing protocols available at that time.<sup>89,186,187</sup> A recent retrospective analysis of preserved samples verified that a rare strain was present at least occasionally during the UK epizootic.<sup>181</sup>

It now appears that there are at least 3 strains of the BSE agent in cattle: the classical BSE strain of the UK epizootic and 2 atypical strains (designated as L-type and H-type [denoting characteristic light and heavy molecular prion banding patterns revealed via western blot analysis]).<sup>176,177,182,183,188</sup> The H-type and L-type strains of BSE have been experimentally transmitted to bovinized mice (ie, mice carrying the bovine prion gene).<sup>183</sup> The L-type strain has been experimentally transmitted to cattle and nonhuman primates.<sup>189,190</sup> Results of recent experiments with humanized transgenic mice (ie, mice carrying the human prion gene) indicate that the L-type strain of BSE is capable of causing disease in humans.<sup>191</sup>

To date, atypical BSE has been identified only in cattle that appeared healthy at slaughter, and most of those animals were older than the animals typically affected with the classic strain of BSE.<sup>176,177</sup> It has been

hypothesized that these atypical cases could be the manifestation of rare prion isoforms that arise naturally in some older animals.<sup>192–194</sup> At least 1 case of H-type atypical BSE has been associated with a heritable mutation in the gene expressing the normal cellular prion protein.<sup>195–197</sup> The susceptibility to BSE disease strains may be related to genetic differences among cattle.<sup>194–196,198</sup>

Both of the BSE cases ascertained in the US native-born cattle were atypical cases (H-type), which contributed to the initial ambiguity of the diagnosis.<sup>174,185</sup> In Canada, there have been 2 atypical BSE cases in addition to the 14 cases of the classic UK strain of BSE<sup>2</sup>: one was of the H-type, and the other was of the L-type.<sup>198</sup>

There has been speculation that US outbreaks of transmissible mink encephalopathy, the last of which occurred in 1985,<sup>199</sup> might have resulted from ingestion of tissue from an animal infected with a hypothetical rare prion disease of cattle.<sup>199,200</sup> However, if this were so, this hypothetical disease was not classic BSE. When the BSE agent is experimentally transmitted to mink, the resulting disease is distinguishable from transmissible mink encephalopathy<sup>201</sup>; when the transmissible mink encephalopathy agent is experimentally transmitted to cattle, the resulting disease is distinguishable from BSE.<sup>76</sup> Experimental transmissions of BSE and transmissible mink encephalopathy to ovine transgenic mice (ie, mice that express the sheep native prion protein) suggest that L-type atypical BSE most closely resembles transmissible mink encephalopathy.<sup>202</sup> The L-type BSE has not been detected in US cattle.<sup>174,185</sup>

### **Age-Related Susceptibility of Cattle to BSE**

Young cattle appear to be more susceptible to infection with the BSE agent than older cattle. During the UK epizootic, innate age-based susceptibility appeared to play a greater role than age-related dietary patterns.<sup>89,203</sup> Cumulative field data fit best with an assumption that there was a true difference in age susceptibility among cattle—*independent of age-related dietary patterns*—and that the age susceptibility peaked very strongly at 1 year of age<sup>204</sup> or between 6 months and 1 year of age.<sup>205</sup> In cattle, the lower susceptibility to BSE observed in mature animals is not attributable to ruminal inactivation of prions.<sup>206,207</sup>

Although less susceptible than young cattle, it is evident from field data collected during the UK epizootic that older animals can indeed become infected with the BSE agent via consumption of contaminated feed. Animals born as early as 1977 developed clinical BSE during the BSE epizootic in the United Kingdom, albeit at an advanced age.<sup>95</sup> This suggests that most of the cattle from this earlier birth cohort were not exposed to the BSE agent until the epizootic was well underway in the mid-1980s. There have also been cases of BSE in cattle that were not fed meat-and-bone meal until after they were 2 years old.<sup>208</sup>

### **Genetic Susceptibility to BSE**

Bovine spongiform encephalopathy susceptibility to the classical strain of BSE among cattle does not ap-

pear related to underlying genetic variability in the prion coding gene.<sup>209–217</sup> There is some association of BSE with mutations of the promoter region of the normal cellular prion protein gene in cattle, but mutations are not sufficiently protective to enable a breeding program for resistant cattle.<sup>211,218–220</sup>

### **Incubation Period and Age of Onset of BSE**

In experiments in which cattle were exposed to infectious material via oral administration, the mean incubation period for BSE was inversely related to the dose of infectious material.<sup>100,101</sup> Among naturally occurring field cases, clinical BSE developed mostly among animals that were 4 and 5 years old.<sup>221</sup> Among cattle with BSE for which age at onset was known (124,000 clinical cases) in the United Kingdom, 7% were 3-year-olds, 31% were 4-year-olds, 33% were 5-year-olds, and 29% were 6-year-olds or older. Only 192 (0.02%) animals were clinically affected before they were 3 years old.<sup>95</sup>

The incubation period for naturally occurring BSE in cattle has been estimated from data in thousands of case records from the UK and French epizootics.<sup>89,102,203</sup> These calculations, accounting for incomplete information about animals that may have been incubating BSE at the time of slaughter, estimated the mean incubation period as 4.5 to 5.5 years.<sup>204,205,222</sup> However, compared with this mean value, there were many cattle with shorter incubation periods and relatively fewer cattle with longer incubation periods. In other words, the few animals with extremely long incubation periods effectively inflated the population-wide mean incubation period to an estimate (4.5 to 5.5 years) that is larger than the incubation period in most of the cattle in the epizootic. Hence, the estimates for population-wide mean age of greatest susceptibility (0.5 to 1.5 years) and population-wide mean incubation period (4.5 to 5.5 years) do not add to reflect the most common age of onset for clinical cases during the epizootic (4 to 5 years of age).

### **Pathogenesis of BSE in Cattle**

Following oral ingestion of infective material, the route by which prions propagate through the body of cattle is not completely understood. Unlike the pattern of pathogenesis for several other TSEs, there is very little prion replication in lymphoid tissue in cattle with BSE.<sup>105,223</sup> Results of recent studies<sup>224,225</sup> suggest that, in cattle, prions usually infect the CNS via the autonomic tracts that innervate the gastrointestinal tract.

### **Infective Tissues in Cattle with BSE**

Because of the risk of zoonotic transmission via ingestion of infective tissue, the distribution of the BSE agent in tissues of infected cattle and the time course for development of tissue infectivity have been carefully investigated.

**CNS tissue**—In cattle with clinical signs of BSE, the CNS contains the largest concentration of BSE agent.<sup>226</sup> European scientists estimate that approximately 90% of the infectious prions are found in the brain and spinal cord in clinically affected cattle.<sup>226</sup> On the basis of

sequential examination of tissue from experimentally (orally) infected calves, CNS tissue infectivity in cattle becomes apparent only toward the end of the incubation period; at that time, the BSE agent is present in the brain, spinal cord, dorsal root ganglia, and trigeminal ganglia.<sup>223,227</sup>

**Autonomic and peripheral nerve tracts**—In cattle that have been administered infective material orally, disease-associated prions are present in the myenteric nerve plexus in the distal portion of the ileum, which indicates that the agent travels to the CNS through autonomic nerve tracts that supply the gastrointestinal system.<sup>224,228</sup> There is also the possibility of more rapid transit to the brain via cranial nerves or the vagus nerve. In cattle infected with the classic UK strain of BSE prion, the solitary tract nucleus and the spinal tract nucleus of the trigeminal nerve appear to be primary and early sites of neural pathogenesis; the dorsal motor nucleus of the vagus nerve is also commonly affected.<sup>229</sup>

In the L-type atypical strain of BSE identified in some Italian cattle, infectious prions were not detected in the dorsal motor nucleus of the vagus nerve, and the brainstem was not as heavily infected as the supratentorial brain regions, both of which suggest that invasion was not via the alimentary tract.<sup>177</sup> In these atypical cases of BSE, the olfactory bulb stained for prion more heavily than the olfactory bulb in typical cases of BSE, indicating possible infection via the olfactory nerve to the brain.<sup>177</sup>

Infectivity is rarely found in peripheral nerves. Compared with development of CNS tissue infectivity, peripheral nerve infectivity becomes apparent in the later stages of the incubation period. Also, when the BSE agent is detected in peripheral nerves, the concentration is much lower than that detected in CNS tissues. These facts suggest retrograde travel of the BSE agent following CNS infection.<sup>226</sup> In a study<sup>223</sup> of cattle that had been administered infective material orally, samples of the sciatic nerve collected from several animals before the development of clinical signs were infective, but at an extremely low level. In a clinically advanced field case, samples from the optic, facial, and sciatic nerves were infective but, again, at a very low level; a sample from the radial nerve (located more distant from the CNS) was not infective.<sup>105</sup> Bovine spongiform encephalopathy-associated prions have also been detected via western blot analysis in several peripheral nerves from cattle that had been administered infective material orally and from infected cattle (field cases) prior to and after development of clinical signs.<sup>224,230,231</sup>

**Lymphoreticular system**—Although in many other TSEs, there is widespread infection throughout the lymphoreticular system, this does not occur in cattle with BSE.<sup>33,232–235</sup> The small degree of lymphoreticular tissue involvement may not be a crucial step in the pathogenesis of BSE.<sup>33,105,223</sup>

In cattle, Peyer's patches become infected with BSE prions as early as 6 months after exposure.<sup>105,223,236</sup> The tonsils from BSE-infected cattle are also presumed to be infective, but the concentration of BSE agent is low in tonsils of animals with experimentally induced BSE.<sup>223,226</sup> Samples of the tonsils of a cow with late-stage BSE (field case) were not infective.<sup>105</sup> Infectivity of re-

gional or mesenteric lymph nodes or spleens collected from cattle challenged with the BSE agent and infectivity of the thymuses of calves with experimentally induced BSE have not been detected.<sup>105,223,226</sup>

**Muscle**—Muscle tissues from cattle with BSE do not appear to be infective. In cattle that were experimentally infected with the BSE agent via oral administration of infective materials as calves, samples of the diaphragm, tongue, semitendinosus, so-called longissimus dorsi (likely the longissimus thoracis and longissimus lumborum muscles although not specified in the reports), sternocephalicus, triceps brachii, masseter, and cardiac muscles were all non-infective.<sup>223,237</sup>

Samples of cardiac, semitendinosus, and so-called longissimus dorsi muscles from a naturally infected cow from Germany were assessed by use of a highly sensitive transgenic mouse bioassay.<sup>105</sup> Inoculation of bovinized transgenic mice with cardiac and longissimus dorsi muscle samples did not transmit disease, but inoculation of 1 of the 10 samples of semitendinosus muscle was associated with evidence of transmitted BSE.<sup>105</sup> The investigators considered that a technical error could account for this result. The semitendinosus muscle is innervated by the sciatic nerve; samples of this nerve from the same cow were also infective (9/13 samples yielded positive results). The investigators concluded that “the potential level of infectivity in the examined bovine muscle is lower than that in the brain by at least 6 log steps, as can be deduced from the results of our titration experiment.”<sup>105</sup>

**Blood and bone marrow**—Although blood of persons with vCJD (the human manifestation of BSE) is infective,<sup>123–127,238,239</sup> blood samples from BSE prion-infected cattle have been tested and are not infective.<sup>105,223,240</sup>

The BSE agent was detected in a pooled sample of bone marrow obtained from 3 calves at 38 months after oral challenge with infective material.<sup>241</sup> Bone marrow was not infective in the 2 calves killed at 40 months after exposure.<sup>241</sup> The detection of infectivity in the pooled bone marrow sample may have been the result of tissue cross-contamination because this was the last sample collected at necropsy. If truly infective, it may be surmised that prion infection can reach bone marrow through its autonomic innervation (ie, innervation of the walls of the marrow-associated vasculature). A hematogenous route seems unlikely because samples of spleen, lymph nodes, and blood from cattle with BSE have never been found to be infective.<sup>105,223</sup>

## **BSE Diagnostic Evaluations**

Currently, there is no test for BSE in living animals. The concentration of infectious prions increases throughout the incubation period, but during most of that period, an infected animal has no clinical signs. A definitive diagnosis of BSE in a suspect bovid requires the detection of characteristic BSE-associated prions in a sample of brain tissue.<sup>221</sup> Current diagnostic tests can determine infection in cattle only during the latter part of the incubation period, after detectable concentrations of BSE-associated prions have appeared in the CNS.

Rapid-acting high-throughput immunoassays have become the first line of testing in BSE surveillance programs in cattle populations. These postmortem assays are conducted on fresh tissue samples from the obex region of the midbrain<sup>221</sup> and can detect disease-associated prions in brain tissue at least several months before the onset of diagnostic histopathologic change.<sup>223,227</sup> Approved rapid tests use several different methods to detect infective prions; typically, assessments are preceded by removal of the normal cellular form of prions to eliminate interference with detection of the disease-associated form.<sup>242</sup> Detection of infective prions is then performed via western blot analysis, ELISA, or lateral flow immunoassay (a modified dipstick version of the ELISA).<sup>243</sup> The European Union has approved 12 rapid tests for BSE screening of cattle, and some are licensed for use in the US testing program.<sup>244</sup> All of those tests are considered to have equivalent sensitivity for detection of the BSE agent.<sup>245–248</sup>

If the results of a rapid screening test are positive, BSE is confirmed by positive results of immunohistochemical testing<sup>35,249–251</sup> of formalin-fixed brain tissue or via detection of BSE prions in brain extracts by use of a western blot immunoassay.<sup>221,252–254</sup> Immunohistochemical testing of fixed brain tissue reveals both the characteristic vacuolization and the distinctive patterns of prion deposits; western blot analysis confirms a BSE diagnosis by the presence of the characteristic migration pattern of BSE-associated prions.<sup>243</sup> Characterization of the molecular signature via western blot analysis enables identification of the strain of BSE prion; the atypical L-type and H-type BSE prions can be distinguished from the classic form of BSE prion.<sup>176,177</sup> A recently developed ELISA is able to distinguish the 3 strains of BSE prion.<sup>255</sup>

Autolysis of a tissue sample will obscure the pattern of disease-associated prion deposition. However, because of their extreme physical and chemical stability, the infectious prions remain intact and can be detected in samples that have undergone autolysis to the point of liquefaction.<sup>221,256,257</sup> In these highly decomposed samples, BSE infection can still be confirmed by use of a western blot immunoassay.<sup>221</sup>

As the capability to detect extremely low concentrations of infectious prions continues to improve, the hope is that, in the future, there will be a test to detect prions (or some other potential biomarker for BSE) in an easily accessible body compartment prior to the onset of clinical signs.<sup>243,258–266</sup>

### **Prevalence of BSE in a Cattle Population**

Because of the nature of cattle production systems, any disease in cattle that has a long incubation period is difficult to detect. When few animals mature to the age at which an infection becomes clinically evident, the disease can circulate silently; in a country without vigilant surveillance systems or stringent interdiction of ruminant tissue from bovine feed, the spread of BSE can remain undetected for years.

The 1988 meat-and-bone meal feed ban in the United Kingdom did not prevent the exportation of this product to other countries and with it the spread of BSE. Cattle producers in the United States, who are

largely reliant on soybean or other vegetable-based dietary supplements, had little need for meat-and-bone meal transported from the United Kingdom. Between 1981 and 1985, the United States imported only a small amount—24 metric tons—of rendered material from the United Kingdom<sup>267</sup>; US agricultural officials forbade its import after 1985. However, other countries continued to import meat-and-bone meal from the United Kingdom after the advent of BSE.<sup>267</sup>

Throughout Europe, BSE detection at first depended on a passive surveillance system; veterinarians and farmers were required to report animals with CNS signs compatible with BSE, but there was no organized effort to search for them. In 1996, the startling confirmation that BSE was a zoonosis gave new impetus to devising a means of conducting active surveillance of cattle. By 2001, several rapid screening tests for use on brain samples had been approved. These new surveillance tools revealed that BSE has already penetrated continental Europe to a much greater degree than previously suspected.<sup>268,269</sup>

The principles of BSE surveillance rest on the assumption that there is a background level of CNS disease in any population of adult cattle. To assure a high probability that any BSE case will be captured in a surveillance system, it is recommended that field investigations of adult cattle that have BSE-compatible CNS signs should be conducted at the rate of at least 100 investigations annually for every 1 million cattle > 30 months old; fewer investigations would result in some BSE cases remaining unidentified and unreported.<sup>205</sup>

Bovine products are used extensively in medical applications, including injectable collagen, gelatin for the production of capsules, and bovine serum albumin for use in vaccine production. These products are not made from neurologic tissue, but it is important to assure that no contamination from bovine neurologic tissue occurs during manufacture and that manufacturing processes include sufficient steps to eliminate any potential prion contamination.<sup>270,271</sup> To protect human consumers, it is recommended that these products originate from countries that have no evidence of BSE and have a strong BSE surveillance program in place, as certified by the World Organization for Animal Health (Office International des Epizooties).<sup>272</sup>

### **Decontamination of Infectious Prions**

Prions are extremely stable, and it is difficult to completely inactivate them in tissues, in liquid waste, or on contaminated surfaces.<sup>273</sup> The susceptibility to inactivation also depends somewhat upon the prion strain.<sup>273,274</sup> In laboratory or surgical settings, when humans or other animals are known or suspected to be infected with TSEs, use of disposable instruments is preferred; these items should be subsequently incinerated.<sup>275</sup>

Prion-contaminated materials should never be allowed to dry onto surfaces because drying renders the prions more difficult to remove.<sup>276</sup> Formaldehyde or aldehyde disinfectants (eg, glutaraldehyde-based disinfectants) are contraindicated because they can further stabilize the prion molecule by cross-linking

or fixing the protein chains<sup>277</sup>; BSE infectivity remains almost unaltered in tissue exposed to 10% formol-saline solution for 2 years.<sup>187</sup> Steam cleaning is not recommended because the high temperature renders the prions more difficult to remove from contaminated surfaces.<sup>278</sup>

Protein denaturants, such as guanidine salts, are effective inactivants.<sup>279</sup> Incineration at 1,000°C (1,832°F)<sup>280</sup> or extended autoclave runs (4.5 hours) at high temperatures (134°C [273.2°F]) or in combination with sodium hydroxide will inactivate prions.<sup>281</sup> A phenolic disinfectant has been shown to greatly diminish prion infectivity,<sup>282</sup> and under appropriate conditions, cleaning solutions that are as mild as acidic 5% SDS may markedly reduce infectivity.<sup>274,283</sup> For TSE prion decontamination of medical and veterinary necropsy and surgery facilities, there are several best practices guidelines that are generally based on the guidelines published by the World Health Organization.<sup>284,285</sup>

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