Experimental infection of mink with bovine spongiform encephalopathy

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To determine whether the aetiological agent of bovine spongiform encephalopathy (BSE) is pathogenic for mink, standard dark mink were inoculated with coded homogenates of bovine brain from the U.K. Two homogenates were from cows affected with BSE. The third was from a cow that came from a farm with no history of having had BSE or having been fed ruminant-derived, rendered by-products, the proposed vehicle for introduction of the BSE agent. Each homogenate was inoculated intracerebrally into separate groups of mink and a pool of the three was fed to a fourth group. Signs of neurological disease appeared in mink an average of 12 months after intracerebral inoculation and 15 months after feeding. Decreased appetite, lethargy and mild to moderate pelvic limb ataxia were the predominant clinical signs, quite unlike the classic clinical picture of transmissible mink encephalopathy (TME). Microscopic changes in brain sections of most affected mink were those of a scrapie-like spongiform encephalopathy. Vacular change in grey matter neuropil was accompanied by prominent astrocytosis. Varying greatly in severity from one mink to another, the degenerative changes occurred in the cerebral cortex, dorsolateral gyri of the frontal lobe, corpus striatum, diencephalon and brainstem. Although resembling TME, the encephalopathy was distinguishable from it by less extensive changes in the cerebral cortex, by more severe changes in the caudal brainstem and by sparing of the hippocampus. The results of this study extend the experimental host range of the BSE agent and demonstrate for the first time the experimental oral infection of mink with a transmissible spongiform encephalopathy agent from a naturally infected ruminant species.

Introduction

Bovine spongiform encephalopathy (BSE) has affected more than 100000 adult, mainly dairy cattle in the U.K., since it emerged in 1985 (Anonymous, 1993a). Also, it has increased concerns about the possible transmission of BSE, sheep scrapie and related diseases from ruminants to other species (Southwood et al., 1989; Taylor, 1989) and fueled speculation that a transmissible encephalopathy exists undetected in the cattle population of the U.S.A. (Marsh et al., 1991). Contributing to this speculation are anecdotal associations between the use of ‘downer’ or non-ambulatory cattle by-products in mink feed and outbreaks of transmissible mink encephalopathy (TME) (Hartsough & Burger, 1965; Marsh et al., 1991).

TME arises from a food-borne infection of mink. The pathogen produces a disease that is similar to scrapie in sheep (Hartsough & Burger, 1965; Marsh et al., 1991). TME is not transmitted vertically and only rarely transmitted horizontally, in mink known to have cannibalized affected cage-mates. Historically, TME was thought to be caused by the use of scrapie-infected sheep in mink feed, but experimental efforts to reproduce the disease by oral transmission of scrapie-infected sheep brain have failed (Marsh & Hanson, 1979; Marsh et al., 1991).

The infrequent occurrence and sporadic nature of TME outbreaks has hindered systematic epidemiological investigations of its cause. To date, five outbreaks of the disease have been documented in the U.S.A., occurring in 1947, 1961, 1963 (two outbreaks) and 1985 (Hadlow et al., 1987a; Hartsough & Burger, 1965; Marsh et al., 1991), and involving 11 mink ranches. Three outbreaks occurred in relatively large mink production facilities that had histories of on-site preparation of feed involving the use of non-ambulatory cattle (Hartsough & Burger, 1965; Marsh et al., 1991). A non-ambulatory animal is defined in this context as one that is unable to rise or remain standing without support. This condition may be
attributed to a number of causes including trauma, metabolic disorders, or neurological dysfunction (Anonymous, 1993b).

Because non-ambulatory cattle have been identified as a feed component in three outbreaks of TME and could not be excluded as possible feed components in the other two (Hadlow et al., 1987a; Hartsough & Burger, 1965; Marsh et al., 1991), we felt it was necessary to determine whether mink are susceptible to infection by a known agent of transmissible encephalopathy of bovine origin. As the only known transmissible encephalopathy agent in cattle, the BSE agent from the U.K. represented the logical choice for testing. Here we report the results of intracerebral inoculations and oral dosing of mink with brain homogenates from BSE-affected cattle.

Methods

Animals. Male and female standard dark mink were purchased from the Oregon State University Furbearing Animal Facility (Corvallis, Ore., U.S.A.). All of the mink were serologically negative for Aleutian disease. Each was inoculated with distemper and enteritis vaccine, and Clostridium botulinum and Pseudomonas aeruginosa bacterin-toxoid. They were housed individually in standard cages in a covered outdoor facility until moved to a biohazard level 3 (BL-3) containment facility for experimental purposes. A wet-mix formula that contained no bovine or ovine components was fed to the mink twice daily and water was available ad libitum.

Inocula. Three coded samples (PG29/90, PG30/90 and PG45/90) of bovine brain homogenate were obtained from the Central Veterinary Laboratory (CVL, Weybridge, U.K.). Two homogenates were from cows with clinical BSE, which had been confirmed microscopically. The third was from a cow that had lived on a farm with no history of having had BSE or having been fed ruminant-derived, rendered protein supplements. The source of each homogenate was unknown to those who carried out the inoculations.

Each bovine brain homogenate was prepared at the CVL by mincing and mixing the basal nuclei, thalamus, midbrain, pons and medulla from one animal in a sterile disposable plastic tray with sterile disposable scalpel blades. The homogenates were transported from the U.K. under permit (USDA Animal and Plant Health Inspection Service Import Permit #24170) to Pullman, Wash., U.S.A. in double-sealed containers packed in dry ice. Each was thawed in a room temperature water bath and either used undiluted for the feeding study or brought to 10% (w/v) in sterile Tris-buffered saline (TBS; 50 mM-Tris-HCl, 100 mM-NaCl, 1 mM-dithiothreitol, pH adjusted to 7.8 with glacial acetic acid) in a separate, new, sterile glass tissue homogenizer immediately before use in intracerebral inoculations.

Inoculations. Ten-month-old standard dark mink were anaesthetized by intramuscular injection of a xylazine-ketamine solution (4 mg/kg xylazine, 20 mg/kg ketamine). Sterile instruments were used and aseptic technique was observed for the inoculation procedure. Hair was shaved from the scalp. With a specially designed jig, the skin, underlying muscle and calvarium in the left parietal region were perforated with a number 12 diameter wire bit attached to a hand drill. Each mink received 0.1 ml intracerebrally (i.c.) of one of the 10% brain homogenates or TBS diluent control through a 25-gauge, 16 mm needle.

Each of the 10% homogenates of bovine brain was used in a group of 10 mink. Eight received the homogenate i.c., one received the TBS diluent i.c. and one was left uninoculated as a physical sentinel separating adjacent inoculation groups.

Ten standard dark mink were also used for the feeding study. A pool of the three homogenates was prepared by mixing equal parts of the homogenates into a uniform paste. For each mink, 1 g of the pool was mixed with 4 g of normal mink food. The mink were not fed for 24 h prior to being given the mixture. Each mink was fed its 5 g mixture and observed until the mixture had been consumed.

Housing and observation. The mink were housed in separate cages in a climate-controlled BL-3 facility. They were fed the same wet ration used for mink in other parts of our facility. The ration did not contain tissues of bovine or ovine origin. The mink were observed daily for clinical signs of encephalopathy. Once a mink became incapacitated and was unable to reach food or water, it was euthanized by intracardiac injection of sodium pentobarbitol. The brain was removed and fixed in 10% formalin. Paraffin sections were stained with haematoxylin and eosin for microscopic examination. Mink found dead were necropsied, but their brains were not examined microscopically because of postmortem autolysis. All experimental mink still healthy at 20 months post-inoculation, as well as two mink chosen at random as external controls from uninoculated groups kept outside the BL-3 facility, were euthanized and necropsied. Representative brain samples from each of these groups were prepared for microscopic examination as described.

Results

All mink inoculated with two of the brain homogenates (PG30/90 and PG45/90) and all fed the pool of three homogenates developed clinical signs of encephalopathy and were euthanized (23 in total) or found dead (three in total) within 17 months of experimental inoculation (Table 1). Because the clinical signs in affected mink were often subtle and the clinical period was relatively short, the results are reported in terms of time-to-death (t.t.d.) for comparative purposes.

The average t.t.d. for mink inoculated i.c. was 12 months. The average for those fed the pooled homogenates was 15 months (Table 1). All mink inoculated i.c. with PG29/90, two of the three inoculated i.c. with the TBS diluent control and the three kept as uninoculated sentinels remained healthy for the 20 month observation period. One of the TBS-inoculated controls died at 18 months owing to asphyxiation from tracheal obstruction.

The clinical signs of mink inoculated i.c. were subtle compared to the usual signs of TME. The initial signs generally included reduced aggressive behaviour and inappetence. These were followed by lethargy and a mild to moderate hindlimb ataxia. The clinical period lasted 10 days or less for 12 of the 16 affected mink. Each mink would reach a state where they would no longer attempt to drink water or eat feed even if stimulated to do so. At this stage of morbidity, death was likely within 1 or 2 days and so the mink was euthanized.

The orally inoculated group exhibited similar signs, though the incubation period and clinical duration were longer. In addition, postorialateral hair loss, due to hindlimb incoordination, was noticeable and hindlimb
Table 1. BSE infection of mink

<table>
<thead>
<tr>
<th>Inoculum</th>
<th>Route</th>
<th>Number affected/inoculated</th>
<th>T.t.d.</th>
<th>Evidence of spongiform encephalopathy</th>
</tr>
</thead>
<tbody>
<tr>
<td>PG29/90*</td>
<td>I.c.</td>
<td>0/8</td>
<td>NA†</td>
<td>0/42</td>
</tr>
<tr>
<td>PG30/90</td>
<td>I.c.</td>
<td>8/8</td>
<td>290-447</td>
<td>364 (22)</td>
</tr>
<tr>
<td>PG45/90</td>
<td>I.c.</td>
<td>8/8</td>
<td>300-429</td>
<td>375 (14)</td>
</tr>
<tr>
<td>Pool</td>
<td>Oral</td>
<td>10/10</td>
<td>422-505</td>
<td>462 (11)</td>
</tr>
<tr>
<td>TBS diluent</td>
<td>I.c.</td>
<td>0/2§</td>
<td>NA</td>
<td>0/1</td>
</tr>
<tr>
<td>None (sentinel)</td>
<td>~</td>
<td>0/3</td>
<td>NA</td>
<td>0/1</td>
</tr>
<tr>
<td>None (external control)</td>
<td>~</td>
<td>0/2</td>
<td>NA</td>
<td>0/2</td>
</tr>
</tbody>
</table>

* Negative control brain.
† NA, Not applicable; healthy when euthanized at 20 months post-inoculation.
§ Number of positive brains/number of brains examined.

Fig. 1. Severe spongiform change in the caudate nucleus of mink injected i.c. with BSE-infected brain homogenate. Stained with haematoxylin and eosin. The bar represents 100 μm.

paralysis supervened towards the end of the clinical period.

None of the clinically affected mink had the classic signs of TME, which include tail curling, scattering of faeces, hyperexcitability, and hyperaggressiveness (Hart-sough & Burger, 1965; Hadlow et al., 1987b; Marsh et al., 1991; Marsh & Hadlow, 1992). However, some occasionally exhibited a tenacious biting of the cage wires similar to that seen in the later stages of TME (Hadlow et al., 1987b; Marsh & Hadlow, 1992).

Two of the mink from the PG45/90 i.c. inoculated group and one from the group fed the pooled homogenates exhibited clinical signs of encephalopathy, but were found dead despite daily observation. Necropsies were performed on each of these mink to rule out causes of death other than encephalopathy and none were found. However, the brains were not examined microscopically because of the high probability of postmortem autolysis. Brains from euthanized mink, including all of the other clinically affected mink from the PG30/90, PG45/90 and the pooled homogenate groups, four of the eight unaffected mink from group PG29/90, one of the sentinels, one of the TBS diluent-inoculated controls and two uninoculated age-matched mink from outside the facility (external controls), were examined microscopically for comparison of normal structure with pathological changes.

Microscopic changes were found in the brains of animals from all groups. In the uninoculated sentinel, the TBS-inoculated control and those inoculated with PG29/90, bilaterally symmetrical vacuolar change, comprising a few scattered neuropil spaces, was distributed in, or adjacent to, nuclei of the dorsal medulla, the vestibular nucleus and the cerebellar nuclei. The change was also evident in the brains of the two uninoculated age-matched external control mink. Vacuoles were generally larger and often irregular compared to those of mink with transmissible spongiform encephalopathies, and tended to occur in white matter bundles rather than grey matter. This vacuolation was not accompanied by glial responses and was not considered indicative of a transmissible encephalopathy.

Unequivocal evidence of spongiform encephalopathy was present in mink inoculated with PG30/90 (six out of eight), PG45/90 (five out of six) and in mink fed the pooled brain homogenates (nine out of nine) (Table 1). Lesions were similar among affected animals, but varied widely in severity between animals and between inoculation groups. Spongiform change predominated, but vacuolation of neuronal perikarya was common in certain locations. The remaining brains from animals injected with PG30/90 (two) or PG45/90 (one) had minimal vacuolar changes, comparable with those in controls and insufficient to confirm a diagnosis of spongiform encephalopathy. Because of the marked variation in severity of vacuolation, possible differences in the distribution of changes between i.c. inoculated mink and those fed the pooled homogenates were difficult
to vacuolar changes, a prominent astrocytic reaction occurred in most of the brains (Fig. 3).

In summary, clinical and morphologic evidence of spongiform encephalopathy was obtained in mink after intracerebral inoculation with either PG30/90 or PG45/90 and after oral inoculation with the pool of brain homogenates. Further observations of the distribution of lesions is required before statements regarding possible patterns or clinico-pathological relationships can be made. However, the general distribution of lesions throughout the affected groups tentatively indicated a greater degree of brainstem involvement than is seen with TME in mink.

Discussion

BSE has been transmitted experimentally to mice, cattle, pigs, sheep, goats and marmosets (Baker et al., 1993; Barlow & Middleton, 1990; Dawson et al., 1990a, b; Fraser et al., 1988). The present study extends the experimental host range to mink. Although successful intracerebral transmission to mink was largely expected, successful oral transmission was not because of previous failures to transmit sheep scrapie to mink by oral dosing (Marsh & Hanson, 1979). We estimate that each mink in the orally inoculated group received 0.67 g of BSE-infected bovine brain, because equal amounts of the three homogenates were used to make the pooled inoculum and each mink was fed 1 g from the pool. Although the minimum oral dose of BSE-infected brain homogenate necessary to cause disease was not determined, it is interesting that an oral dose of 0.67 g was sufficient to cause disease in an adult mink. One implication of this finding is that the central nervous system tissue in a 500 kg cow could contain enough infectious agent to cause disease in more than 1000 mink.

One reason for investigating the susceptibility of mink to the BSE agent was to test the hypothesis that TME is caused by the consumption of cattle affected with a transmissible scrapie-like encephalopathy. Since no such disease has been identified in the cattle population of the U.S.A., BSE-infected bovine brain from the U.K. was used. The fact that BSE has proven transmissible to mink does not prove that a transmissible encephalopathy exists in the U.S. cattle population. However, it does show that mink could become infected following oral exposure to a bovine scrapie-like agent if one existed in the U.S.A.

The clinical signs observed in BSE-infected mink bore only minimal resemblance to those of natural or experimental TME. Absent were tail curling and scattering of faeces, clinical hallmarks of documented TME outbreaks and experimental studies (Hartsough & Burger, 1965; Hadlow et al., 1987b; Marsh et al., 1991;
Marsh & Hadlow, 1992). The BSE-infected mink appeared to become docile instead of hyperaggressive and excitable. Furthermore, the clinical period was significantly shorter than that seen in TME outbreaks (Hartsough & Burger, 1965; Marsh et al., 1991; Marsh & Hadlow, 1992). Lastly, the incubation period for oral transmission of BSE averaged 15 months, whereas the estimated incubation period for at least two of the documented outbreaks of TME was less than 1 year (Hartsough & Burger, 1965; Marsh et al., 1991). However, it is possible that the level of exposure of ranch mink to the TME agent was greater than that simulated here, and the effective oral dose may be inversely related to the incubation period as it is with intracerebral inoculation in mink and other species.

The pattern of lesions seen in BSE-infected mink brain was similar to, but not the same as that seen with TME. While cerebrocortical and midbrain changes were seen in natural TME (Hartsough & Burger, 1965; Marsh & Hadlow, 1992), in experimental TME (Eckroade et al., 1979) and in mink in the present study, the pattern in BSE-infected mink involved brainstem structures to a greater extent than has been seen previously with TME.

It is interesting to note that TME has been linked historically to the use of scrapie-infected sheep in mink feed, even though the epidemiological information available did not identify sheep carcasses or offal as a feed component prior to any U.S. outbreak (Hartsough & Burger, 1965; Hadlow et al., 1987a; Marsh et al., 1991). In addition, experimental evidence has shown that (i) mink are slightly susceptible to U.K. scrapie isolates following i.c. inoculation and (ii) they are not susceptible to U.S. scrapie isolates inoculated orally (Marsh & Hanson, 1979). It is clear from the present study that a spongiform encephalopathy can be transmitted experimentally from BSE-infected brain homogenates to mink with relative ease by both intracerebral and oral routes. The fact that the clinical signs and the pattern of histopathological changes observed in BSE-infected mink were different from those in mink with TME suggests possible differences between the BSE and TME agents. A better understanding of the significance of these findings may be possible after the results of current transmission studies of scrapie in cattle and mink (Cutlip et al., 1994; M. M. Robinson et al., unpublished results) become available.

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References


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