Epidemiological and experimental studies on a new incident of transmissible mink encephalopathy

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Epidemiological investigation of a new incident of transmissible mink encephalopathy (TME) in Stetsonville, Wisconsin, U.S.A. in 1985 revealed that the mink rancher had never fed sheep products to his mink but did feed them large amounts of products from fallen or sick dairy cattle. To investigate the possibility that this occurrence of TME may have resulted from exposure to infected cattle, two Holstein bull calves were injected intracerebrally with mink brain from the Stetsonville ranch. Each bull developed a fatal spongiform encephalopathy 18 and 19 months after inoculation, respectively, and both bovine brains passaged back into mink were highly pathogenic by either intracerebral or oral inoculation. These results suggest the presence of a previously unrecognized scrapie-like infection in cattle in the United States.

Introduction

Transmissible mink encephalopathy (TME) is a rare disease of ranch-raised mink with clinicopathological features similar to scrapie (Marsh, 1976). In their initial description of TME, Hartsough & Burger (1965) reported that the disease was associated with common or shared feeding practices and was experimentally transmissible to mink after long incubation periods. Subsequent studies on the physicochemical properties of the TME agent showed that it was indistinguishable from the scrapie agent (Marsh & Hanson, 1969) and that the disease caused was transmissible to hamsters (Marsh et al., 1969), subhuman primates (Marsh et al., 1969; Eckroade et al., 1970), striped skunks, ferrets and raccoons (Eckroade et al., 1973) and sheep and goats (Hadlow et al., 1986).

To test the hypothesis that TME resulted from feeding mink scrapie-infected sheep products, mink were inoculated intracerebrally (i.c.) or orally with several different sources of the scrapie agent from both the United States and Great Britain (Hanson et al., 1971; Marsh & Hanson, 1979). Some mink inoculated i.c. with American sources of scrapie developed TME-like disease after between 11 and 24 months. Inocula from Great Britain produced a fatal spongiform encephalopathy in only one of 65 inoculated mink after an incubation period of 22 months. No scrapie inoculum was found to be pathogenic for mink exposed orally after observation periods as long as 4 years (unpublished results). Since epidemiological investigations of natural cases of TME have indicated that the disease is caused by some unknown feed ingredient and can have an incubation period as short as 7 to 12 months (Hartsough & Burger, 1965), the experimental testing of mink susceptibility to scrapie did not confirm that TME was caused by feeding mink scrapie-infected sheep products. It was concluded that if TME did result from exposure to a subpopulation of the scrapie agent in sheep, additional sources of scrapie would need to be tested to identify such a pathogen (Marsh & Hanson, 1979).

This report describes a new incident of TME in Stetsonville, Wisconsin, U.S.A., the first to occur in the United States since 1963. Epidemiological and experimental studies indicate that cattle may have been the source of infection.

Methods

Tissue collection and preparation. On 13 April 1985, a female Pastel mink showing advanced signs of the debilitating neurological illness affecting other animals on the ranch was killed and its brain collected for histopathological and microbiological examination. One half of the brain, sectioned longitudinally, was frozen at −80 °C for 72 h and then thawed and homogenized to 5% (w/v) using a buffer containing 10 mM-Tris, 100 mM-NaCl, 1 mM-EDTA adjusted to pH 7.8 by the addition of acetic acid. Sterile disposable plastic syringes and tissue culture flasks were used to homogenize the tissue which was then briefly clarified by centrifugation at 500 g for 1 min. This inoculum was used for all primary animal passages; all brain tissues used for serial passages were prepared in an identical manner with the exception that some tissues were frozen at −80 °C for longer than 72 h before homogenization.

Animal inoculation and observation. Neonatal (6 to 8 days old) and adult (1 to 2 years old) mink and ferrets were obtained from the
University of Wisconsin Mink Unit. Weanling, outbred male Syrian hamsters were purchased from Harlan Sprague Dawley. Random-bred female white mice, Charmany strain, were obtained from the Department of Veterinary Science, Charmany Farms, as were the two adult squirrel monkeys and two 6-week-old Holstein bull calves. The mink were in captivity to parents purchased from the Tarpon Springs Zoo, Fla., U.S.A.; the bull calves were castrated and dehorned before inoculation.

I.c. injections were into the right cerebral hemisphere and contained either 30 µl (mice), 50 µl (hamsters, and neonatal mink and ferrets), 100 µl (adult mink and ferrets), or 200 µl (calves and squirrel monkeys) of inoculum. Oral exposure of mink was by direct consumption of 1 g of mink or bovine brain stem tissue.

All animals were housed at Charmany Farms and handled and cared for according to NIH and University of Wisconsin Research Animal Resources Center guidelines. Animals were observed daily for signs of neurological disease. However, it was not possible to observe the locomotor coordination of the injected cattle because they and the mink were placed in a tight containment facility in which the 8' x 12' rooms limited observation of movement.

Examination for prion protein. Brain tissue was extracted by the method of Hilmert & Diringer (1984) to enrich for the scrapie-specific, protease-resistant prion protein (PrP<i>res</i>), a 27K to 30K sialoglycoprotein. This purified preparation was separated by gel electrophoresis and silver stained or immunoblotted, as described previously (Aiken et al., 1989), using a PrP antipeptide serum provided by B. Caughey.

Results

Clinical observations

In April 1985, the owner of a mink ranch in Stetsonville, Wis., U.S.A. called the Ranch Service to report that many of his animals were behaving abnormally and some had died. Upon visiting the ranch (G.R.H. and R.F.M.), it was apparent that approximately 400 animals were showing various clinical stages of TME (Hartsough & Burger, 1965). The earliest signs were behavioural changes, in which the mink appeared to be hyperexcitable and no longer deposited faeces in a single area of the pen but distributed faecal material randomly throughout the cage. Arching of the tail over the back in a 'squirrel-like' manner was observed in many animals. Mink at more advanced stages showed locomotor incoordination, as evidenced by the inability to climb into their nestboxes or to maintain their hindquarters in a straight sagittal plane when at rest. Some mink appeared completely somnolent with their noses in the corner of the cage as if in a trance. The length of clinical illness varied from 2 weeks to 6 weeks in others.

The disease persisted on the ranch for 5 months. Of the total breeding herd of 7300 adult animals, approximately 60% developed clinical signs and all of these died. The morbidity rate was slightly higher among females than males. The incidence of disease in the three colour phases on the farm (Violets, Pastels and Blue Iris) appeared to be equal, as was the incidence in first year breeders, born in May 1984, and older animals. A group of 600 Blue Iris mink received from another mink ranch on 17 July 1984 remained unaffected. Kits born to affected mothers did not develop the disease, as has been observed previously in other reports of natural TME (Hartsough & Burger, 1965).

Epidemiological investigation

Because studies of previous occurrences of TME had indicated that some unknown contaminated feed ingredient was the source of infection (Hartsough & Burger, 1965), the ranch owner was carefully questioned about the mink ration formulation; he used commercial sources of fish, poultry and cereal. Most of the fresh meat portion of the ration came from fallen and sick dairy cattle which were picked up within a 50 mile radius of the mink ranch and returned for processing (butchering, grinding and freezing); a few horses had been used. Sheep products were never fed to the mink and there were no feed supplements of meat and bone meal.

Diagnosis

The diagnosis of TME was confirmed by histopathological examination of brain tissue, experimental transmission to neonatal and adult mink (Table 1) and demonstration of PrP<i>res</i>. Brain lesions were similar to those seen previously with natural (Burger & Hartsough, 1965) and experimental (Eckroade et al., 1979) TME; diffuse microvacuolation (spongiform degeneration) of the grey

| Table 1. Experimental inoculation of the Stetsonville source of transmissible mink encephalopathy into various species |
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| Species* | Inoculated/affected† | Passage (incubation period in months) |
| | (incubation period in months) | |
| Mink | 14/14 | I.c. in neonatal mink (4) |
| | (4) | Per os in adult mink (7) |
| Ferret | 8/7 | 15/15 |
| | (28 to 38) | (8 to 9) |
| Squirrel monkey | 2/2 | I.c. in mink (4-5) |
| Cattle | 12/10 | I.c. in neonatal mink (4) |
| | (15 to 16) | Per os in adult mink (7) |
| Hamsters | 90/0 | I.c. in hamsters (7, 4 and 2) |

* Inoculated i.c. with a 5% brain suspension prepared from a female Pastel mink killed in extremis on the Stetsonville ranch on 13 April 1985.

† Number inoculated/number affected with neurological disease and having spongiform degeneration at necropsy.

‡ Mice were inoculated i.c. with either mink brain (45 animals) or cattle brain (45 animals).
matter was present throughout the corpus striatum, midbrain, brain stem and cerebral cortex (Fig. 1). No inflammatory cell infiltrates were observed. The spongiform degeneration was accompanied by an intense reactive astrocytosis.

Species susceptibility

(i) Ferrets
Intracerebral inoculation of mink brain into adult European ferrets (*Mustela putorius furo*) resulted in the development of progressive neurological disease in seven of eight animals after incubation periods of between 28 and 38 months (Table 1). On a second passage into 15 neonatal ferrets, the incubation period was reduced to between 8 and 9 months. Microscopic lesions in brain tissue were similar to those observed in mink but were less intense with a more focal distribution of spongiform degeneration in the cerebral cortex.

(ii) Hamsters
Of twelve weanling, outbred male Syrian hamsters, 10 developed progressive neurological disease characterized by cerebellar ataxia 15 to 16 months after intracerebral inoculation with mink brain (Table 1). On second passage, the incubation period was reduced to 7 months and the clinical syndrome was again characterized by signs of hyperexcitability and cerebellar ataxia. On the third serial passage, two clinical forms emerged. The first had an incubation period of 4 months and featured hyperexcitability and cerebellar ataxia as observed in the previous two passages. The other clinical syndrome had an incubation period of between 5 and 7 months and was characterized by complete lethargy with no hyperexcitability and no cerebellar ataxia. Passage of the first, or 'hyper' syndrome resulted in similar clinical signs after only 2 months. Passage of the second, or 'sleepy' syndrome using a 5% brain homogenate resulted in some animals showing the 'hyper' syndrome after a 2 month incubation period and some having the 'sleepy' syndrome 3 to 4 months after inoculation.

The brain lesions in hamsters infected with the mink brain inoculum, second hamster passage, and third passage showing the 'hyper' syndrome were characterized by mild spongiform degeneration in the midbrain and brain stem. Hamsters showing the 'sleepy' syndrome had more intense vacuolation in these same areas with some animals also having mild microvacuolation in the cerebral cortex.

(iii) Squirrel monkeys
Squirrel monkeys have previously been shown to be susceptible to the Hayward source of TME (*Eckroade et al.*, 1970) so it was thought important to test their susceptibility to this new Stetsonville source. Both inoculated animals developed progressive debilitating neurological diseases after incubation periods of 9 and 13 months (Table 1). Profound lesions of spongiform degeneration of the grey matter were present in the cerebral cortex, corpus striatum, midbrain and pons of both brains. Each brain also reinfected mink, causing disease symptoms after an incubation period of 4-5 months (Table 1).

(iv) Cattle
Owing to the extensive use of fallen or sick dairy cattle in the mink diet, two 6-week-old Holstein bull calves were inoculated i.c. with mink brain. Eighteen months after inoculation, one animal suddenly collapsed in its holding room and could not be returned to a standing position. This animal had shown no previous signs of behavioural change or loss of body condition. After 24 h, the animal was killed and brain tissue was collected for histopathological and microbiological examination. The second animal was normal until 19 months after inoculation when it too suddenly collapsed. Observations over the next 4 days revealed a rapid nystagmus,
opisthotonos, and hyperexcitability when handled. The animal was killed and brain tissue and spinal cord were collected.

Histopathological examination of both bovine brains revealed spongiform degeneration of the grey matter in the midbrain and brain stem (Fig. 2); no lesions were seen in the cerebral cortex. Homogenates from each brain produced disease in mink 4 months after intracerebral inoculation and feeding whole brain tissue to mink produced disease after 7 months (Table 1). PrP\textsuperscript{res} was present in both brains. Extraction of 17.5 g of cerebral cortex from bull 1, which had an 18 month incubation period, resulted in a yield of 14.4 µg in the final pellet enriched for PrP\textsuperscript{res} as compared to extraction of 4 g of hamster or mink brain which yielded pellets of 96 µg and 26.4 µg, respectively.

(v) Mice

Forty-five random bred Charmany strain female white mice were inoculated i.c. with either the mink brain inoculum or a 5% homogenate from the second bull brain. These animals were observed for 700 days for signs of neurological disease. Over the course of this period, especially during the last 100 days, 12 mice developed debilitating diseases characterized by weight loss and incoordination; some animals developed tumours. Histopathological examination of the brains from these animals revealed no obvious lesions characteristic of spongiform degeneration and PrP\textsuperscript{res} could not be demonstrated by Western blot analysis.

**Discussion**

These studies on a new occurrence of TME in the United States confirm previous observations that the disease can have a natural incubation period of less than 12 months. They suggest a very short exposure period between approximately 1 June 1984, when the mink kits first began eating the food ration, until 17 July when the 600 unaffected Blue Iris mink were introduced to the ranch. They further suggest a possible new source of infection.

The origin of TME remains unknown. Experiments to test mink susceptibility to various sources of sheep scrapie have failed to demonstrate a pathogen with biological properties similar to TME and scrapie has never been shown to be transmissible to mink by oral exposure. The results of previous epidemiological investigations on TME, which tried to identify the contaminated feed ingredient, have been inconclusive because of the long interval between exposure and the onset of disease and the fact that most mink ranchers feed by-product mixtures to mink, the exact composition of which is not known. There has been anecdotal evidence that sheep products may have been fed to mink in a few instances of TME but, before this occurrence, no rancher has been able to state with a high degree of certainty that they did not feed sheep products to their mink.

If TME does not arise from the sheep scrapie pool, then what is its origin? We believed that the large amount of products from fallen or sick dairy cattle fed to mink on the Stetsonville ranch indicated a possible source of infection and we inoculated two calves to test this hypothesis. The production of a fatal spongiform encephalopathy in this species was not particularly remarkable since these diseases are transmissible to a wide variety of animals. What is meaningful was the biological behaviour of the bovine agent when back-passaged into mink. There was no evidence for any deadaptation of the bovine agent for mink compared to the i.c. and oral pathogenicity of non-bovine-passaged mink brain (Table 1). This suggests that there are no species barrier effects between mink and cattle in relation to the Stetsonville source of TME and may indicate that the subpopulation of TME from the mink brain which produced disease in cattle may also represent the major pathogen for mink.

The experiments on species susceptibility aimed to
characterize the biological properties of the Stetsonville source of TME and compare them with those previously reported for the Hayward TME source. The results were remarkably similar. The Stetsonville source is pathogenic for European ferrets but only after a much longer incubation than in mink. This same observation has been made with the Hayward source of TME (Marsh et al., 1969; Eckroade et al., 1973). Hamsters and squirrel monkeys are susceptible to both the Stetsonville and Hayward sources of TME (Marsh et al., 1969; Eckroade et al., 1970), both producing similar clinicopathological syndromes in these species. It is especially interesting to note that the initial passage of the Hayward TME mink brain also produced a 'hyper' syndrome in hamsters (Marsh et al., 1969) which changed to a 'sleepy' syndrome on subsequent passage (Marsh & Kimberlin, 1975). We are presently attempting to clone the agents responsible for these two syndromes by endpoint animal inoculation. Purified 'strains' will be characterized in hamsters and possible differences in mink pathogenicity will be examined.

If mink on the Stetsonville ranch were exposed to TME by feeding them infected cattle, there must be an unrecognized scrapie-like disease of cattle in the United States. If this is true, the disease is rare. The low incidence rate of TME and the fact that the Stetsonville mink rancher had fed products from fallen or sick cattle to his animals for the past 35 years suggests a very low prevalence of this disease. If this were the total scope of the problem, remedies such as recommending that mink ranchers do not feed products from fallen cattle to their animals (Marsh & Hartsough, 1988) and increasing our surveillance for a possible scrapie-like disease of cattle at veterinary diagnostic laboratories (Marsh & Hartsough, 1985) might suffice. However, there is a greater concern. Bovine spongiform encephalopathy (BSE) was first recognized in Great Britain in April 1985 (Wells et al., 1987). The disease has had a large impact, closing foreign markets to British beef and raising public awareness as to their possible susceptibility to 'mad cow disease.' Studies on the epidemiology of BSE have indicated that exposure was via a feed ingredient and that it began in 1982, with a 3 to 8 year incubation period (Wilesmith et al., 1988). The studies showed further that rendered animal protein, probably from scrapie-infected sheep, in the cattle feed was the most likely source of infection (Wilesmith et al., 1988). These findings on BSE are relevant to the spread of a possible scrapie-like disease of cattle in the United States. There has been a recent trend in the American cattle industry to use more meat and bone meal in their rations and these unusual neuropathological agents are likely to survive any rendering process (Brown et al., 1990). The implication is that these new feeding practices could cause a rare, insignificant disease to become one causing considerable economic hardship.

Finally, what is the relationship between a possibly unrecognized scrapie-like disease of cattle in the United States and BSE? If the two are similar, BSE brain should be pathogenic for mink and, after mink passage, have biological properties like those reported here for the Stetsonville source of TME. There is, however, some preliminary information indicating that the two diseases may be different. BSE has not yet been shown to be transmissible to hamsters but does produce disease in mice (Fraser et al., 1988). Our results showed that the Stetsonville source of TME, either before or after cattle passage, was not transmissible to random-bred mice. However, these findings must be assessed with regard to the likely possibility that mink passage changed the biological properties of the original agent which naturally infected the animals. TME has never been shown to be transmissible to mice (Marsh et al., 1969; Taylor et al., 1986), not even after passage in sheep or goats (Hadlow et al., 1986), and scrapie is not transmissible to mice after mink passage (unpublished results).

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References


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