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Short Communication

Exacerbated Spongiform Lesions in the Cerebral Cortex in Japanese Sheep, in an Outbreak of Scrapie during 1984-1987

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SUMMARY: The present study dealt with the pathology of natural scrapie in Japanese Suffolk sheep in a certain selected area. Vacuolations in the cytoplasm of neurons were conspicuous. They were particularly evident in many areas of the medulla and pons, extending into and through pedunculus cerebri and thalamus to the septal area and olfactory tubercle. Proliferation of astrocytes was also easily observed with glial fibrillary acidic protein staining. Neural vacuolations in the cerebral cortex were observed in 73% of the cases. Abnormal prion protein deposits were seen in all cases observed by hydrolytic autoclaving, and subsequent peroxidase and anti-peroxidase immunostaining. Abnormal prion protein staining was the most conspicuous in the polymorphic layers of the hippocampus.

Worldwide attention has been focused on scrapie because of experimental results revealing the oral transmission of bovine spongiform encephalopathy (BSE) to sheep (1) and plausible transmission to beef-consuming humans (2). If contaminated meat and bone meals fed to sheep affect humans, then it is possible that an ovine form of BSE, clinically identical to scrapie, may exist. The Japanese Ministry of Agriculture, Forestry and Fisheries has declared scrapie a notifiable disease on April 26, 1996, and decided to organize a national surveillance scheme against this disease.

It has long been established that there is much variation in the pathology of natural scrapie. The pattern and distribution of lesions vary with the breed of sheep; the nature of lesions also differs within breeds (3). The present study considers the pathology of natural scrapie in Japanese Suffolk sheep in a certain selected area. This paper describes the results of a study designed to determine whether any temporal changes in the pathology of naturally occurring scrapie in sheep occurred concurrently with the epidemic of BSE in England since 1986.

Suffolk or Corriedale sheep were bred in a restricted area in Hokkaido, Japan. Sheep were fed with grasses and silage without the addition of processed food (4). In England, the BSE epidemic originated by the use of processed food (4). Although in Japan, such tainted feed is not given to sheep, yearly surveillance is necessary to exclude accidental use of contaminated products. For this study, we used 15 sires that displayed clinical signs of the disease. The average age of sheep at the onset of scrapie symptoms is 3.5 years. Most animals between the ages of 2.5 and 4.5 years were affected. Clinical aspects including age, duration of disease, and symptoms from 15 cases were recorded before the animals were sacrificed. A flock of about 100 sheep was confined to a 20-hectar field where lambing has been conducted for the past 20 years. Scrapie-affected cases were removed from the restricted field and penned separately when symptoms became obvious. The sheep were sacrificed at an advanced stage of the disease, and scrapie was then confirmed by pathological examination of the brain and spinal sections (3). Sheep that were killed or died of other causes were similarly examined. The histopathological tissues studied included the spinal cord, medulla oblongatae (at least two), cerebellum, pons, mesencephalon, diencephalon, septal area, basal ganglia, and frontal cortex.

Table 1 shows that the dominant sign in the fully developed disease was pruritus. Ataxia was a common finding, which presented as gait incoordination, hypermetria, trotting, or bunny hopping. Some sheep were hypersensitive to sound, movement, or touching (stimulation). Not all sheep demonstrated all signs, which are progressive and normally last several weeks or months.

In all cases, there was moderate to severe neuropil vacuolation in many areas of the medulla and pons, extending into and through pedunculus cerebri and thalamus to the septal area and olfactory tubercle. Lesions were visible in the neocortex of 73% of the cases (Table 2), centered around the superior frontal gyrus. Vacuolation of the neuronal perikarya, and vacuolation of the surrounding neuropil were observed as occurring with variable severity. On the other hand, the frequency of lesions in the neocortex was relatively rare (37%) in the case of British sheep (5). The severity of histological lesions in the brain exacerbated progressively, resulting in a shorter lifespan of the animals. A major difference between this study and most previous reports was the finding of vacuolar change in the cerebral cortex (Table 2). The severity of histopathological lesions in brain cortex exacerbated progressively in succeeding generations within 4 years. White

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Table 1. Relationship between year of incidence, age, and clinical sign

Case	Year	Breed	Age (year)	Sex	P[1]	I	D	S
S1	1984	S[2]	NR[3]	F[4]	+	–	–	–
S2	1984	S	NR	F	+	–	–	–
S3	1984	S	4	F	+	–	–	–
S4	1984	S	4	F	+	–	–	–
S6	1984	C	5	F	+	–	–	–
S7	1984	S	6	F	+	–	–	+
S8	1984	S	6	F	+	–	–	+
S10	1986	S	3	F	+	+	–	+
S11	1987	S	2	F	+	–	+	–
S12	1987	S	3	F	+	–	+	–
S13	1987	S	NR	F	+	–	+	–
S14	1987	S	NR	F	+	–	–	+
S15	1987	S	NR	F	+	–	–	+
S16	1987	S	NR	F	+	–	–	–
S17	1987	S	NR	F	+	–	–	–

[1]P: pruritus, I: incoordination, D: depression, S:stimulation. [2]S:Suffolk, C: Corriedale. [3]NR: not recorded. [4]F: female.

Table 2. Histopathology of the frontal cortex (superior frontal gyrus)

	Spongiform of		Increase in astrocytes	Intra-cytoplasmic vacuolation	
	White Matter	Gray Matter		central	periph.
S1	+	–	–	–	–
S2	+	–	–	–	–
S3	+	–	–	–	–
S4	+	–	–	–	–
S6	–	–	+	–	–
S7	–	–	+	–	–
S8	–	–	+	–	–
S10	–	+	+	–	–
S11	++	+	++	+	++
S12	++	+	++	–	–
S13	–	+	++	–	+
S14	+	+	+++	–	–
S15	+	+	++	–	++
S16	–	–	+	–	–
S17	–	+	++	–	+

\+ Limited, often confined to a single brain nucleus.
++ Moderate, present in more than one nucleus.
+++ Severe, spongiform in many nuclei and neuropils.
periph.: peripheral

matter vacuolation is not normally associated with natural scrapie, as is addressed in a previous report (3). However, the white matter vacuolation found in the present study, especially the vacuolation found in polymorphic layers (stratum multiforme) of the hippocampus, was associated with abnormal prion protein by immunoperoxidase staining (Fig. 1a) (6).

For immunostaining of abnormal prion protein, peroxidase-anti-peroxidase (PAP) method was used. Sections were deparaffinized and dehydrated, washed in running tap water and rinsed with distilled water before pretreatment with hydrolytic autoclaving. For the hydrolytic autoclaving pretreatment, sections were completely immersed in 2.5 mM HCl and autoclaved at 121°C. Endogenous peroxidase was blocked by immersing sections in freshly prepared 3% hydrogen peroxide (100 volumes) in methanol for 10 min. Sections were washed in running tap water, rinsed with

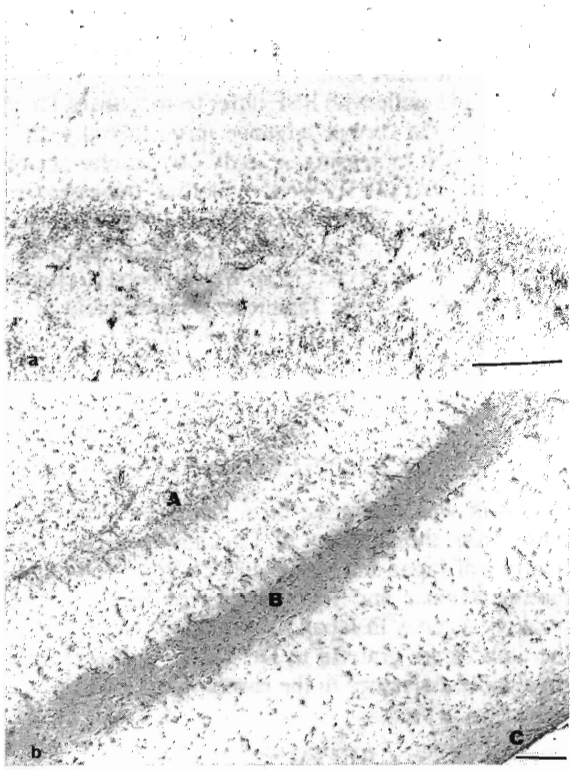

Fig. 1. a) Immunodetection of abnormal prion protein in the hippocampal area observed by in situ immunohistochemistry using anti-SAF (scrapie-associated fibril) rabbit antibody (6). Abnormal prion protein was seen as dark spots in the stratum multiforme. Bar shows 100 μm. b) Immunohistochemistry directed against GFAP (glial fibrillary acidic protein, a specific marker of astrocyte cytoplasm) using anti-human GFAP rabbit antibody (8), in the hippocampal area. A: stratum multiforme, B: stratum reticulare, C: alveus. Bar shows 200 μm.

Tris-buffered saline (TBS, pH 7.6, 1.6% sodium chloride), and then incubated with a mixture of 10% (v/v) normal goat serum and 3% (w/v) bovine serum albumin in TBS for 15 min. After draining, sections were incubated overnight at 4°C with a rabbit antiserum to the prion protein (7) at a dilution of 1:200. This antiserum has been characterized by Western blotting and has been shown to identify ovine prion protein (7). After washing with TBS, all sections were incubated at room temperature with goat anti-rabbit immunoglobulins (Dako Japan, Kyoto, 1:50), and then with rabbit PAP complex (1:320), each for 30 min, with a TBS wash after the incubations. Diaminobenzidine tetrahydrochloride (DAB) was used to visualize immunoreactivity, and the sections were lightly counterstained with Mayers haematoxylin.

Glial fibrillary acidic protein (GFAP) staining revealed active astrocyte proliferation in the hippocampus (Fig. 1b)(8). In the same area, positive staining of abnormal prion proteins was observed, even in the absence of vacuolated neuronal cells.

In Japanese natural cases, a majority showed spongiform lesions in the cerebral cortex (Table 2). Wood et al. (5) recently completed a report on the neuropathology of natural sheep scrapie in the United Kingdom (submitted to the Central Veterinary Laboratory, Weybridge, U.K., for more than a decade). According to their study, in some cases, lesions in the cerebral cortex afforded certain discriminating patterns of the disease between different breeds. Japanese scrapie cases in 1984 matched well with Wood's type III, and cases in 1987 corresponded well to Wood's type IV. In cases 6-8, no

spongiform lesions were observed in the frontal cortex. However, GFAP staining showed an increased number of astrocytes in the same area.

The lesions in cattle with BSE differ from those of Japanese scrapie in that the former advance more rostrally and are concomitant with increasing severity of vacuolar change at the level of the obex (3). By Western blotting, our cases (frozen brain samples) were shown to correspond well to Collinge's glycosylation type IV: new variant Creutzfeldt-Jakob disease (CJD), and to the BSE type (7, 9). We still need to clarify the relationship between the Japanese scrapie agent and the British BSE agent; this can be accomplished by comparing pathological lesions in the brain using several different strains of mice, as has been studied previously (10).

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