Histopathology and Image Analysis of Brain Lesions in Ovine Scrapie in Japan

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The first case of scrapie in Japan was reported in Suffork sheep in Obihiro, Hokkaido in 1984 [5, 8, 9]. Pathologically natural scrapie in sheep is progressive encephalopathy characterized by the presence of vacuolated neurons and extensive astrogliosis [7, 9, 12], whereas in the experimentally transmitted disease the neuronal vacuolation proceeds to frank spongiform changes [3]. In sheep the presence of amyloid plaques is rare [3]. Since similar neuronal vacuolation proceeded to spongiform changes was observed in scrapie, Creutzfeldt-Jacob disease and kuru, these three disease were classified as subacute spongiform encephalopathies [2]. In the present investigation, spongiform lesions found in natural scrapie in a Corriedale sheep were studied histologically and with image analysis. Findings are discussed in comparison to the result previously reported in sheep.

One male Corriedale sheep histologically diagnosed as scrapie was used in this experiment. The sheep was transferred to the animal facility in Hokkaido Station of our institute from a farm in Hokkaido with past history of scrapie in Suffork sheep. The sheep exhibited rubbing of the buttocks and flanks against fixed objects from April, 1986. He was sacrificed by exsanguination on July 22, 1986. The animal was four years and three months old, when sacrificed. This was one of the three natural cases of scrapie observed in Corriedale sheep in Hokkaido during 1984–1986. Another four-year-old male Corriedale sheep, which was apparently healthy, was sacrificed for morphometric analysis.

The brains of the sheep were fixed in 10% formalin in phosphate-buffered saline for histological examination. Five μm paraffin sections were stained with hematoxylin and eosin (H-E), Luxol-fast-blue (LFB) and cresyl violet.

For the image analysis of sections, vacuolated and non-vacuolated areas of nerve cells were calculated by an analyzer (Japan PC Systems Co., Ltd., Osaka, Japan) and expressed as area

levels [4, 6]. One level was approximately $0.2 \mu\text{m}^2$.

A transverse section of the nucleus olivaris in the medulla oblongata, which was stained with H-E, was put into the image analyzer with sample image of 256×256 pixels with 16 levels (from 0 to 15) of lighting intensity. The images from the TV camera were transformed into a digital image, and the images of nerve cells and cytoplasmic vacuoles were then trimmed off. The lighting intensity of the control slide, that was the background intensity, was raised to the highest intensity (level 15) of this image analyzer. The threshold level of the lighting intensity of each pixel was determined in order to transform the sample image into a binary image. In order to quantitatively measure vacuolated area, the pixel with the highest intensity in the cytoplasm (level 15) was expressed in white. And the pixels with the other intensity in the cytoplasm of nerve cells (levels 0–12) were expressed in black to measure vacuolated and non-vacuolated areas [4, 6].

Histologically, characteristic bilaterally symmetrical lesions were observed in the brain. Affected nerve cells were observed throughout the brain stem and more frequently in the medulla oblongata than in the pons and mesencephalon. Acute swelling of nerve cells was found throughout the brain stem. Chromatolysis was frequently seen in nerve cells of the brain stem. Typical vacuolation was observed in the nucleus olivaris of the medulla oblongata (Figs. 1–3). No substance could be detected within the vacuoles. A few necrotic nerve cells were seen without vacuolation. Perineural glial infiltration was occasionally seen in the medulla oblongata. No focal glial infiltration was observed. Although no demyelinated tract was found in any affected area, a few vacuoles of white matter were observed in the medulla oblongata (Fig. 4). LFB staining did not show any demyelinated area.

Approximately 30% of nerve cells in the nucleus olivaris of the diseased sheep showed vacuolation. The mean values of vacuolated area

Fig. 1. Fig. 1. Localization of spongiform lesions in the medulla oblongata of diseased sheep. •, area of lesions. A, nucleus olivaris. B, nucleus ambiguus. C, nucleus nervi hypoglossi. D, nucleus dorsalis nervi vagi.

Fig. 2. Multiple vacuoles in large nerve cells in the nucleus olivaris. H-E staining ($\times 600$).

and non-vacuolated area of nerve cells computed with the image analyzer are shown in Table 1. The vacuolated areas occupied 46.2% of the total area of nerve cells in the nucleus olivaris of the diseased sheep. An age-matched control sheep did not show any vacuoles in nerve cells of the nucleus olivaris. Non-vacuolated area of nerve cells increased by 72.4% in the nucleus olivaris of

Fig. 3. Single vacuoles and degeneration in medium-sized nerve cells in surrounding area of the nucleus olivaris. H-E staining ($\times 375$).

Fig. 4. Small spongiform lesions in myelinated area of the medulla oblongata. H-E staining ($\times 375$).

Table 1. Image analysis of vacuolated and non-vacuolated nerve cells in the nucleus olivaris of the medulla oblongata from a scrapie sheep

Sheep	nerve cells		
	vacuolated area	non-vacuolated area	total area
scrapie	267.3±119.3 ^{a)} (53 ^{b)})	311.7±48.5(61 ^{c)})	579.0
control	0	180.8±97.8(309 ^{c)})	180.8

The sizes of vacuolated and non-vacuolated areas were computed with an image analyzer and expressed as area level.

a) Area levels. Mean±standard deviation.

b) Number of vacuoles counted.

c) Number of nerve cells counted.

the diseased sheep as compared to the control sheep.

This is the first natural case of scrapie in Corriedale sheep in Japan, although approximately 70 natural cases of scrapie were known in Suffolk sheep in Japan since 1984 (unpublished observations). Since the diseased Corriedale sheep was transferred from a farm with past history of scrapie in Suffolk sheep, this scrapie could be horizontally transmitted from Suffolk sheep to Corriedale sheep. Major pathological alterations in scrapie have been reported to be status spongiosus and astrogliosis in the brain and spinal cord [10, 12]. The largest concentration of vacuolated nerve cells was found in the medulla oblongata just behind the posterior olive [12]. Vacuolation of nerve cells in the medulla oblongata, microglial proliferation in the brain stem and perivascular cuffings were found in sheep with experimentally induced scrapie. This proved that the experimental disease was similar to natural case [11]. However, the other report showed that vacuolated nerve cells were seen in non-scrapie animals [1], while another report indicated that the number of vacuoles in sheep affected naturally with scrapie was much higher than in apparently healthy sheep [12]. Our results showed that vacuolations were seen in the brain stem of scrapie sheep, but not in the brain stem of an age-matched healthy sheep. In our case vacuolation was usually severe in large-and medium-sized nerve cells, while a previous report indicated that vacuolation was mostly seen in medium-sized nerve cells [12].

Besides, swollen nerve cells were frequently seen in the nucleus olivaris in the present scrapie sheep. Swollen cells could further be vacuolated, although a few severely degenerated necrotic

cells were seen without vacuolation. Although very few reports indicated the swelling of the nerve cells in the scrapie sheep [12], our image analysis showed the numerical difference in the degree of swelling and vacuolation between the scrapie sheep and apparently healthy sheep.

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要 約

スクレイピー羊脳病変の画像解析および病理組織学的研究(短報): 小野寺 節・百溪英一¹⁾・清水真也・吉原一浩・吉野知男(家畜衛生試験場, ¹⁾家畜衛生試験場北海道支場)——北海道においてコリデール羊に初めて亜急性海綿状脳症が観察された。4才3ヶ月の雄羊で組織学的に左右対称の両側性変性病変が延髄に見られた。延髄全体に神経細胞の腫大が見られたが、オリブ核では大型および中程度の大きさの神経細胞において、空胞化および変性が見られた。また少数の空胞化を伴わない神経細胞の変性、壊死も見られた。スクレイピー羊と対照健康羊のオリブ核組織切片を画像解析したところ、スクレイピー羊では空胞化のみならず神経細胞の面積の増加(腫大)が数量化された。