Demonstration of Aujeszky's Disease Virus Antigen in Naturally Infected Cattle by Immunoperoxidase Method

Toshikazu MATSUOKA, Tomio KURIHARA, Yasushi KONOSU, Yuji IJIMA, Minoru NARITA¹⁾, and Makoto HARITANI¹⁾

Omiya livestock Hygiene Service Center, 107-1 bessho, Omiya 331, and ¹⁾National Institute of Animal Health, 3-1-1 Kannondai, Yatabe, Tsukuba 305 Japan

(Received 12 February 1987/Accepted 18 April 1987)

Jpn. J. Vet. Sci. 49(3): 725-727, 1987

KEY WORDS: Aujeszky's disease, cattle, immunoperoxidase method.

Aujeszky's disease virus (ADV) is neurotropic in ruminant. Various techniques, such as a transmission electron microscopy, direct immunofluorescent antibody in frozen tissue and indirect immunofluorescent antibody in formalin-fixed tissue, have been used for the detection of the virus antigen in vivo [6, 7, 10]. Recently, several investigators have successfully applicated the immunoperoxidase technique in formalin-fixed tissues for the demonstration of herpes simplex virus, rabies virus, canine adenovirus and canine distemper virus antigens [3, 4, 8, 9].

The purpose of the present study was to demonstrate the ADV antigen in formalin-fixed and paraffin-embedded brain tissue sections from 3 naturally infected cattle in Japan. They were diagnosed by clinical and pathological examinations and virus isolation [5]. The tissues for histo- and immunopathological examinations were fixed in 10% buffered formalin, embedded in paraffin, stained with hematoxylin and eosin (H-E stain) and immunoperoxidase procedure as described below. After deparaffinization, endogenous peroxidase activity of each sections were blocked by treating with 0.3% H₂O₂ in absolute methanol. They were then incubated sequentially with normal goat serum, anti-ADV

Fig. 1. Immunoperoxidase staining of brain tissue from calf No. 3, showing positive reaction in the cytoplasm of pyramidal neurons from cerebral cortex. $\times 400$.

Fig. 2. Immunoperoxidase staining of brain tissue from calf No. 2, showing positive reaction in the cytoplasm of neuron and neuroglial cells. $\times 400$.

rabbit antibody, biotinylate anti-rabbit IgG goat antibody, avidin biotin peroxidase complex (ABC) reagent and 0.05% 3,3'-diaminobenzidine tetrahydrochloride and 0.1% H₂O₂ in Tris buffer pH 7.6. Then, they were counterstained with methyl green. The anti-ADV rabbit serum with a neutralization antibody titer of 1:32 was supplied

by Dr. Imada, National Institute of Animal Health, and used as primary antibody at a 1:2048 of dilution. The secondary biotinylated antibody and ABC reagent were obtained commercially (Vectastain, Vector Lab., Burlingame, U.S.A.). As specificity controls, normal rabbit serum was substituted for the first antiserum in staining

Table 1. Distribution of the lesion and ADV antigen in brain tissues from 3 naturally infected cattle

Region		Calf No.					
		1		2		3	
		Lesion ^{a)}	Antigen ^{b)}	Lesion	Antigen	Lesion	Antigen
Cerebrum	Lobus frontalis	—	—	—	—	++	++
	Lobus temporalis	—	—	—	—	+	+
	Lobus parietalis	—	—	—	—	—	—
	Lobus occipitalis	—	—	—	—	—	—
Pons		—	—	—	—	—	—
Medulla oblongata		+	+	++	++	+	+
Cerebellum		—	—	—	—	—	—
Spinal cord		—	—	+	+	NE ^{c)}	NE

a) Composed with neuronal degeneration and inclusion body; slight to marked (+ to ++).

b) ADV antigen immunoperoxidase staining; low to high (+ to ++).

c) Not examined.

Fig. 3. Intranuclear inclusion body in the neuron (a; H-E stain, $\times 1000$) and many virus particles in the nucleus and a few virus particles in the cytoplasm (arrow) of a neuron (b; $\times 26000$) from calf No. 3.

preparation as described above. Small parts of formalin-fixed brain tissue were fixed in 1% osmic acid and embedded in epon mixture. Ultrathin sections were made and stained with uranyl acetate and lead citrate, and were examined using JEOL 100CX electron microscope.

Application of the immunoperoxidase procedure to the paraffin sections yielded a dark brown fine granular reaction in the positive cells. The cytoplasm of the neurons and neuroglial cells were stained equally all over, but the nucleus was not stained (Figs. 1 and 2). After counterstaining, neuronal degeneration, ranging from mild chromatolysis to complete necrosis, was prominent. From the findings of H-E stain, the nuclei in some affected neurons had become rounded and homogeneous basophilic masses. Some neurons contained basophilic or eosinophilic intranuclear inclusion bodies. A slight neuroglial cell infiltration and perivascular cuffing by lymphocytes were observed around the affected areas. Neurophagia was not a common finding. The distribution of the ADV antigen by immunoperoxidase staining was much coincident with the result of histopathological lesion (Table 1). Intranuclear and intracytoplasmic herpetic virus particles were found in the neuronal cells under the electron microscope (Fig. 3). Although, the particles in the nucleus consisted of nucleocapside, the particles in the cytoplasm consisted of nucleocapsid and envelope. The cause that the nucleus was not stained by immunoperoxidase staining is considered as the properties of the anti-ADV rabbit serum used as primary antibody.

Non-suppurative encephalitis in naturally ADV infected cattle were similar to those of experimentally infected cattle [1, 2]. Generally, the detection of the intranuclear inclusion bodies

in the ADV infected cells provided sufficient evidence for the diagnosis, although it was sometimes difficult.

The present study has shown that demonstration of ADV antigen in formalin-fixed and paraffin-embedded tissue sections was undertaken with satisfactory accuracy by using the immunoperoxidase staining technique. Therefore, immunoperoxidase staining using together with a routine histologic procedure is effective for the pathologic diagnosis of Aujeszky's disease.

ACKNOWLEDGEMENTS. The authors wish to thank Dr. H. Yamamoto, National Institute of Animal Health, for his valuable advice.

REFERENCES

1. Dow, C., and McFerran, J. B. 1962. *J. Comp. Pathol.* 72: 337-347.
2. Dow, C., and McFerran, J. B. 1966. *J. Comp. Pathol.* 76: 379-385.
3. Ducatelle, R., Coussement, W., and Hoorens, J. 1980. *Am. J. Vet. Res.* 41: 1860-1862.
4. Lascano, E. F., and Berria, M. I. 1890. *Arch Virol.* 64: 67-79.
5. Matsuoka, T., Iijima, Y., Sakurai, K., Kurihara, T., Konosu, Y., Tamiya, K., Oki, M., Haritani, M., and Imada, T. 1987. *Jpn. J. Vet. Sci.* 49: 507-510.
6. McCracken, R. M., McFerran, J. B., and Dow, C. 1973. *J. Gen. Virol.* 20: 17-28.
7. Narita, M., Inui, S., Kobayashi, M., and Fukusho, A. 1983. *Natl. Inst. Anim. Health Q. (Jpn)*. 23: 105-107.
8. Palmer, D. G., Ossent, P., Suter, M. M., and Ferrari, E. 1985. *Am. J. Vet. Res.* 46: 283-286.
9. Rakich, P. M., Prasse, K. W., Lukert, P. D., and Cornelius, L. M. 1986. *Vet. Pathol.* 23: 478-484.
10. Sabo, A., Rajcani, J., and Blaskovic, D. 1968. *Acta Virol.* 12: 214-221.

要 約

免疫酵素抗体法によるオーエスキー病ウイルス感染牛におけるウイルス抗原の証明：松岡俊和・栗原富男・鴻巣泰・飯島雄二・成田 實¹⁾・播谷 亮¹⁾（埼玉県大宮家畜保健衛生所，¹⁾農林水産省家畜衛生試験場）——3例のオーエスキー病ウイルス感染牛のホルマリン固定，パラフィン包埋切片の脳標本について，免疫酵素抗体法を用いてウイルス抗原の証明を行った。ウイルス抗原は神経細胞とグリア細胞の細胞質に認められその分布は病理組織学的病変と一致していた。