Demonstration of Infectious Bovine Rhinotracheitis Virus Antigen by Immunoperoxidase Method in Tissues of Aborted Bovine Fetuses Preserved for 25 Years in Paraffin Blocks

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ABSTRACT. Infectious bovine rhinotracheitis (IBR) virus antigen was demonstrated by the immunoperoxidase method in tissues of two aborted bovine fetuses, which had been stored for 25 years after fixing in formalin and embedding in paraffin. Necrotic foci were detected in the liver, kidney, adrenal gland, and thymus of the fetuses. Coincidently with the distribution of the necrotic foci, IBR virus antigen was demonstrated by immunostaining. The present study proved the immunoperoxidase method in one of useful techniques to demonstrate IBR virus antigen in tissue sections from preserved paraffin blocks. — KEY WORDS: aborted bovine fetus, IBR virus, immunohistochemistry.


Infectious bovine rhinotracheitis (IBR) virus, both field and vaccine strains, has been suspected to cause abortion in cattle. The diagnosis of IBR is made by virus isolation and the detection of characteristic necrotic foci in various organs [1, 2, 6]. Recently, the immunoperoxidase technique has been applied to the diagnosis of several infectious diseases [3–5]. IBR virus antigen was demonstrated by the immunofluorescent microscopy on frozen sections [7]. However, there has been no report on the stability of viral antigens in paraffin-embedded tissues which are detected by the immunoperoxidase method. The purpose of the present study was to demonstrate the IBR virus antigen in paraffin-embedded tissues of the aborted fetuses, collected in 1970.

The history of the disease and the results of virus isolation from two aborted bovine fetuses have been described previously [8]. The specimens were fixed in 10% buffered formalin and embedded in paraffin wax. The paraffin blocks were kept in the room temperature from 1970 to 1995, when the present study was performed. The sections were cut and stained with hematoxylin and eosin (HE).

IBR virus antigen was demonstrated by the avidin-biotin-complex (ABC) immunoperoxidase method using a Vectastain ABC kit (Vector Lab., Burlingame, CA, U.S.A.). The rabbit anti-IBR virus serum was kindly provided by Dr. S. Taniguchi, Kyoritsu Shoji Co., Ltd., Central Research Laboratory, Ibaraki, Japan, and used as primary antibody at a dilution of 1:4092. After deparaffinization, endogenous peroxidase activity was blocked by treatment with 0.3% H2O2 in absolute methanol for 30 min. Then, the sections were treated with 0.1% actinase E (KaKen Pharmaceutical Co., Ltd., Tokyo, Japan) in phosphate-buffered saline for 5 min at 37°C. They were then incubated with normal goat serum, primary antibody, biotinylated goat anti-rabbit IgG, ABC reagent and 0.05% 3,3-diaminobenzidine tetrahydrochloride and 0.1% H2O2 in Tris buffer, pH 7.6 (DAB-H2O2). The sections were counterstained with methyl green. Serum from a non-immunized rabbit was used for the control study.

The microscopic findings were characterized by multifocal necrosis with hemorrhage. The lesions were in the liver, kidney, adrenal gland and thymus. Necrotic foci in the liver were distributed throughout the lobules and were more obvious in the central and para-central areas. Small necrotic foci in the kidney were observed in the cortex near the medullary-cortical junction and appeared to involve the collecting tubules. Lesions similar to those in the kidney were also frequently located in the cortex of the adrenal gland and medulla of the thymus. There was no inflammatory response associated with necrosis in the liver, kidney, adrenal gland, and thymus. The cells adjacent to necrotic foci contained an intranuclear inclusion body which stained purple or red in color in HE-stained sections. However, intranuclear inclusions were hardly detected within necrotic lesions.

Immunohistochemically, IBR virus antigen was detected as brown granules in the cytoplasm and nuclei of the degenerating and necrotizing parenchymal cells (Figs. 1 and 2). The distribution of IBR virus antigen was correlated with necrotic foci.

Distribution of necrotic foci in the present cases was similar to that reported in fetuses infected experimentally or naturally with IBR virus [1, 2, 6, 7]. It has been well known that various infectious agents, such as bacteria, fungi, protozoa [5] and virus [3, 4], affect the uterus and placentia of pregnant animals or fetuses leading to abortion and stillbirth. The fetuses died in the uterus and then underwent autolytic changes. It is rather difficult to detect the typical lesions or infectious agents under such autolytic conditions. In the present study, we could demonstrate IBR virus antigen by the immunohistochemical procedure in tissues of the aborted fetuses which had been preserved for 25 years after fixing in formalin and embedding in paraffin. Hence it appears that the immunoperoxidase method is one of the useful

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Fig. 1. IBR virus antigens are seen in necrotic area of the liver. ABC method, counterstained with methyl green. × 400.

Fig. 2. a: IBR virus antigens are shown in necrotic area of the adrenal gland. b: Part of the necrotic area is shown at higher magnification. ABC method, counterstained with methyl green. × 100 (a) and × 400 (b).

techniques to demonstrate IBR virus antigen in tissues kept a long time in paraffin blocks. Further studies are obviously needed to determine the stability of antigens of other infectious agents in a paraffin-embedded state.

REFERENCES