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*Brief Communication*

# Demonstration of Bovine Viral Diarrhoea Virus Antigens in Cell Cultures and in Paraffin-embedded Tissue Sections by the Peroxidase-antiperoxidase (PAP) Technique Using Monoclonal Antibodies

The diagnosis of both bovine viral diarrhoea (BVD) and mucosal disease (MD) is usually made on the basis of characteristic clinical and pathological findings. The definitive etiological diagnosis by virus isolation is time consuming, expensive and elusive. Isolation of the virus in cell cultures is rather difficult since it has no characteristic cytopathic effect (CPE). Furthermore, many strains have no CPE at all. Due to these uncertainties, virus isolation trials are generally supported by additional tests (*Radostits & Littlejohns* 1988).

The most common additional methods used to detect BVDV antigens in cell cultures are: direct immunofluorescense (*Carbrey et al.* 1972), direct immunoperoxidase (*Ward & Kaeberle* 1984), indirect immunoperoxidase (*Meyling* 1984, *Katz et al.* 1987), and the avidin-biotin-peroxidase complex (ABC) methods (*Smith et al.* 1988).

In cryostat tissue sections the BVDV antigens have been detected by direct (*Meyling* 1970) and indirect immunofluorescence (*Bielefeldt Ohmann & Dalsgaard* 1980), and by the indirect immunoperoxidase method (*Bielefeldt Ohmann et al.* 1981, *Bielefeldt Ohmann* 1987).

In this report we describe the use of monoclonal antibodies for the demonstration of BVDV antigens in both cell cultures and paraffin embedded tissues by the peroxidase-antiperoxidase (PAP) technique.

Monolayers of bovine turbinate (BT) cells were inoculated with $10^4$ $TCID_{50}$ of the cytopathic BVDV strain Borgen or of a non-cytopathic local isolate. Non-infected cell cultures were used as negative controls. The cultures were incubated at 37°C for 5 days and then rinsed in PBS and fixed in cold acetone for 10 min.

Tissue samples from 2 calves showing typical symptoms of BVD were fixed in 96 % ethanol for 24 h and embedded in paraffin according to routine histological processes. Sections from small intestines, abomasum and spleen were tested. BVDV infection in the calves was comfirmed by virus isolation from the same organs. Tissue samples from a non-infected calf were used as negative controls. Three different BVDV monoclonal antibodies (MoAbs): CA3, CA34 and CT6 were kindly provided by Dr. V. Moennig, Hannover, F.R.G.

Sections were deparaffinized and rinsed in 0.05 mol/l Tris-HCl buffer ph 7.6, containing 0.15 mol/l NaCl. This Tris buffered saline (TBS) buffer was used through the whole procedure. Endogenous peroxidase was inactivated by rinsing the sections in 0.3 % (v/v) $H_2O_2$ in TBS buffer for 20 min.

Both cell cultures and tissue sections were briefly rinsed in TBS buffer and incubated with a 1:5 dilution of pooled supernatants of 3 hybridoma cell lines. The MoAbs raised against different epitopes on the 56 kD poly-

peptide of the BVDV were used as a pool in order to increase the sensitivity and safety of our diagnostic procedure. All the 3 epitopes appear to be broadly distributed among various strains of BVDV (*Bolin et al.* 1988, *V. Moennig* personal communication).

After a thorough rinse in TBS buffer the specimens were incubated with rabbit anti-mouse IgG and mouse PAP-complex (DAKOPATTS, Glostrup, Denmark) diluted 1:20 and 1:100, respectively. Peroxidase activity was visualized by rinsing the sections in 0.06 % (w/v) diaminobenzidine (DAB; SIGMA, St. Louis, U.S.A.) and 0.34 % (v/v) $H_2O_2$ in TBS buffer for 8 min. After a rinse in tap water the specimens were counterstained in Mayer's haematoxylin and finally mounted with Eukitt (KINDLER, Freiburg, F.R.G.). All dilutions were made in TBS buffer containing 1 % BSA.

The non-infected cell cultures and the tissue samples of the non-infected control calf did not show positive immunostaining. The results were also negative when TBS buffer containing 1 % BSA was used as primary reagent on sections of the infected calves.

In the cell cultures inoculated with the Borgen strain of BVDV the areas of CPE showed positive staining. Monolayers infected with the non-cytopathic virus showed focally distributed groups of stained cells. Thus, the pool of the 3 monoclonal antibodies reacted with both the cytopathic and with the non-cytopathic biotypes of BVDV.

Regarding tissue sections, BVDV antigens were detected in all the tested organs. In the ileum an abundant number of epithelial crypt-cells were positive (Fig. 1). Immunoperoxidase specific staining was also seen in some mononuclear cells in the lamina propria of the mucosa and throughout within the Peyer's-patches. Some neurons of the myoenteric or Auerbach plexus were also positive. In abomasum BVDV antigens were detected especially in parietal cells located at the deeper half of the mucosal glands. Very few cells were positive in the spleen sections.

For a wide array of viral antigens the PAP technique proved to be more sensitive than the peroxidase-conjugated antibody methods (*Stenberger* 1986). Thus, the PAP method has an excellent applicability in diagnostic virology.

Our results indicate that the PAP technique using monoclonal antibodies is a suitable tool for routine diagnosis of BVDV infections both in cell cultures and in ethanol fixed organ sections.

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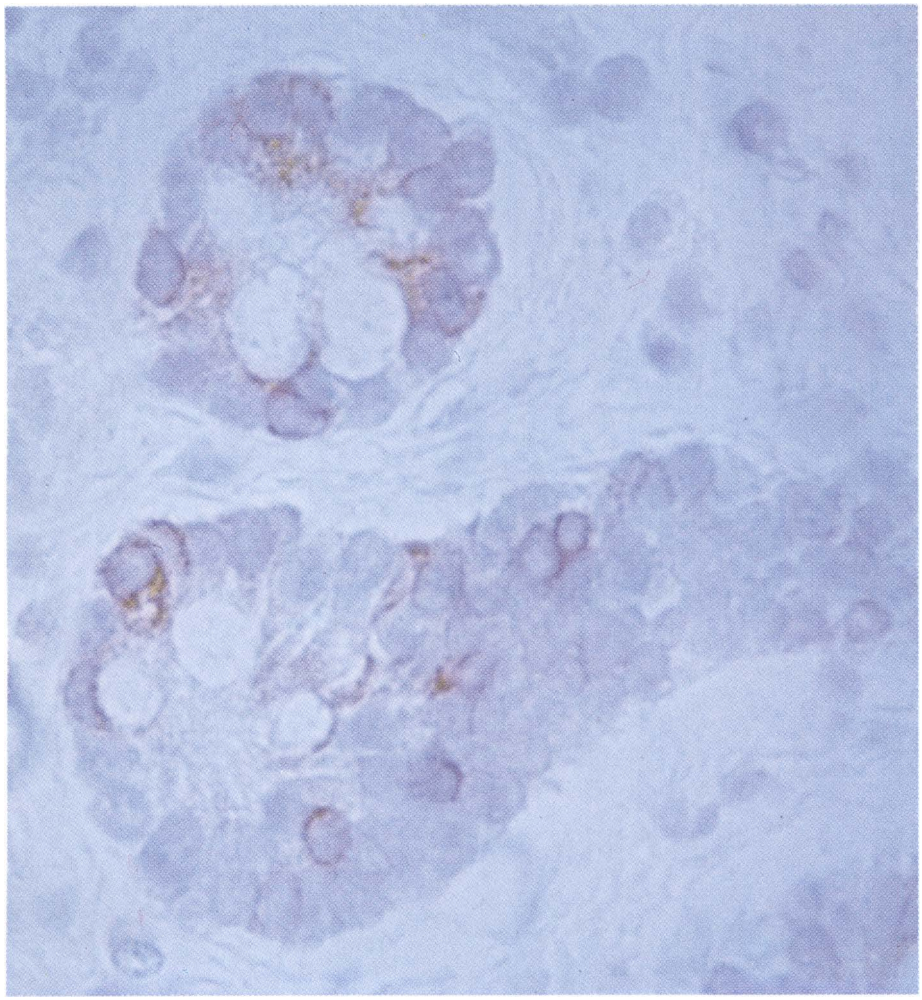

Figure 1. Monoclonal antibody immunolabelling for BVDV antigens: an abundant number of epithelial crypt-cells are stained. 252 ×.

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