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*Brief Communication*

# Monoclonal Antibodies Applied in an Immunoperoxidase Method for Detection of Parvovirus in Specimens of Small Intestine from Dog and Mink

Virus isolation and the haemagglutination test are traditionally used for detection of parvovirus in faeces and intestinal contents (*Weber* 1983). In 1980 *Osterhaus et al.* demonstrated parvovirus-like particles in faeces of dogs by means of negative contrast electron microscopy. Lately, ELISA tests have been developed for the detection of canine parvovirus (*Mildbrand* 1984, *Fiscus* 1984, *Juntti et al.*, in manuscript). Further, the presence of parvovirus in histological specimens, fixed either in formalin-sublimate (*Macartney & Macartney* 1986) or in 10 % buffered formalin (*Ducatelle et al.* 1981), has been demonstrated using polyclonal antibodies and the immunoperoxidase technique.

As immunohistology enables a correlation between the pattern of immunolabelling and the histopathological lesions in the same tissue section this technique is applicable in routine diagnosis. In this report we describe how the use of a pool of 3 monoclonal antibodies (*Rimmelzwaan et al.* 1987), directed to 3 epitopes on the parvoviral haemagglutinin, was applied for the immunohistological detection of parvovirus in formalin-fixed, paraffin-embedded tissues from dog and mink.

Deparafinished tissue sections were rinsed in 0.05 mol/l TRIS-buffer, pH 7,6, containing 0.9 % (w/v) NaCl (this buffer was used throughout the experimental procedure). Endogenous peroxidase was inactivated by rinsing sections in 0.3 % (v/v) $H_2O_2$ in buffer for 20 min. After a brief rinse in buffer the sections were incubated with 2 % BSA in buffer for 10 min. Sections were then gently drained and incubated with the pool of monoclonal antibodies to the haemagglutinin of parvovirus (10 µg/ml) for 1 h. The same pool of MAbs has previously been used in an ELISA-system for detection of parvovirus in samples obtained from dog, cat and mink (*Juntti et al.*, in manuscript). After a thorough rinse in buffer the sections were consequtively incubated with swine anti-rabbit IgG and rabbit PAP-complex (DACOPATTS) diluted 1/20 and 1/100, respectively. The tissue sections were rinsed in buffer between and after these steps which both lasted for 30 min. Peroxidase activity was visualised by rinsing sections in 0.06 % (w/v) diaminobenzidine (DAB; SIGMA) and 0.34 % (v/v) $H_2O_2$ in buffer for 8 min. After a rinse in tap water the sections were counterstained in Mayer's haematoxylin and finally mounted in Eukitt. All dilutions were made in buffer containing 1 % BSA.

With this immunostaining technique it was possible to detect parvovirus in specimens from dog and mink. Attempts to demonstrate parvovirus in tissue from cat were also made but so far no one has been positive.

Fig. 1 shows a typical positive staining reaction in an infected small intestine from a dog. The labelling of the ileum was confined to infected epithelial cells in areas subjected to crypt degeneration. The staining product was thus located in areas showing lesions

typical for parvovirus infection. However, the mononuclear cells in the Peyer's patches did not exhibit any labelling. Non-infected tissues or tissues incubated with only TRIS-buffer as primary reagent were devoid of staining.

It could be of advantage to use a polyclonal antiserum in routine immunohistology as the formalin fixative might reduce tissue antigenicity (*Brozman* 1987), rendering antigenic sites unrecognizable for a monoclonal antibody. This problem was overcome by using a pool of three monoclonal antibodies directed to different sites on the parvoviral haemagglutinin. Thus, background staining, a common problem with polyclonal antisera, was minimised but still the specificity of the primary reagent was "broad" enough to detect the formalin-fixed antigen.

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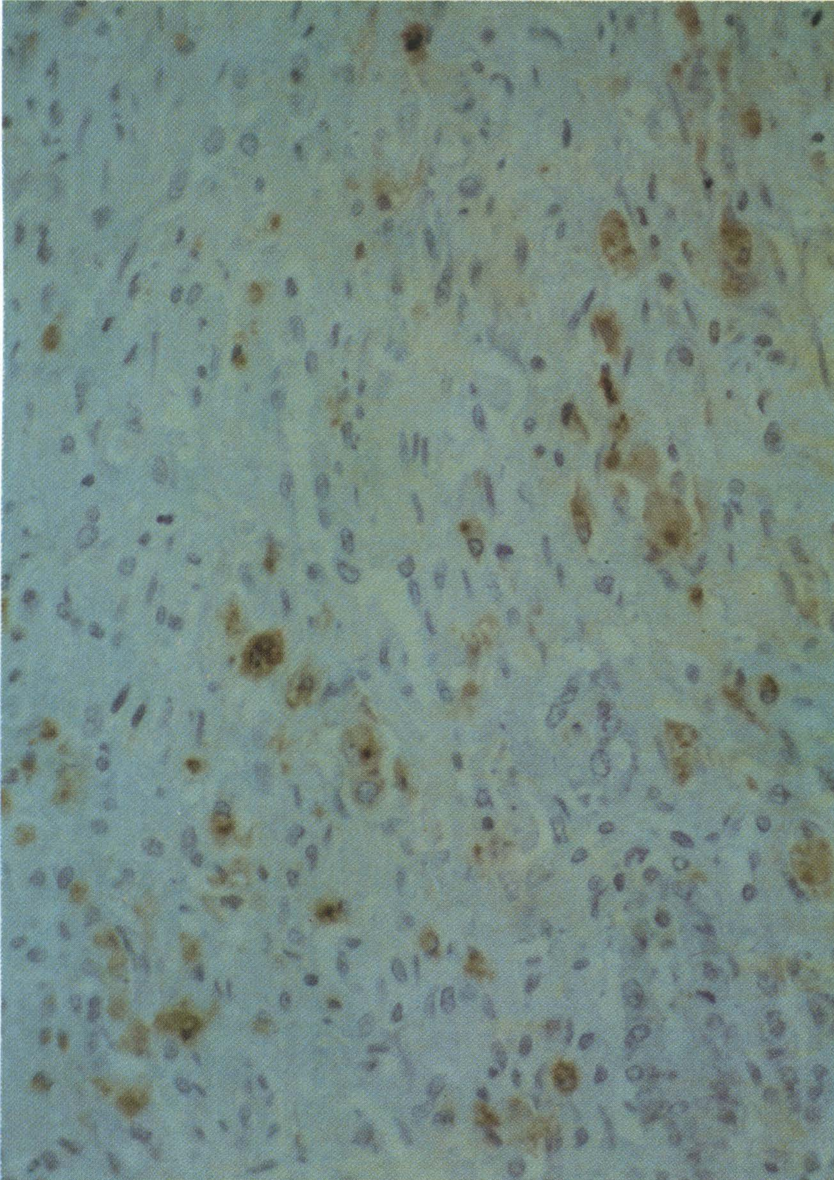

Figure 1. Monoclonal antibody immunolabelling for canine parvovirus: epithelial cells in degenerated crypts of Lieberkühn are prominently stained. 400 ×.