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Brief Communication

Demonstration of *Pasteurella multocida* Type 6:B (B:2) in Formalin-Fixed Paraffin-Embedded Tissues of Buffaloes by the Peroxidase Anti-Peroxidase (PAP) Technique

Pasteurella multocida type 6:B is the specific serotype of the genus *Pasteurella* responsible for the cause of haemorrhagic septicaemia (HS), a highly fatal, septicaemic disease in cattle and buffaloes in Asia. The disease is endemic in almost all South East Asian countries and is ranked as the major cause of mortality in buffaloes, a species of vital importance in the rural economy of the region. Several authors (*Carter & De Alwis* 1989, *Losos* 1986, *De Alwis* 1984) have extensively reviewed the literature on HS. Reference has been made to a carrier status in HS and the dearth of information on the pathology of this infection.

Recent studies by *De Alwis et al.* (1990) have demonstrated the persistence of the organism in the tonsil of HS carriers for prolonged periods, in one instance extending upto 229 days. Studies on the pathology of HS in the buffaloes have been conducted and preliminary findings have been reported (*Horadagoda et al.* 1989). In order to pursue the findings of the afore mentioned studies on the carrier status and the pathological investigations, it was decided to examine the possibility of using an immunolabelling technique to determine the distribution of the organism in tissues.

In this report we describe the use of a rabbit polyclonal antibody for immunohistological detection of *P. multocida* type 6:B antigens in formalin-fixed, paraffin-embedded tissues from the buffalo.

Tonsils from experimentally induced HS carriers (*Horadagoda et al.* in prep.) and lungs, kidney, intestines and liver from animals that died following experimental infection were tested. The presence of *P. multocida* in these tissues was confirmed by isolating the organism according to the method described by *De Alwis* (1989). Tissue samples from noninfected buffalo calves and tonsils collected from cattle calves at the Uppsala slaughter house were used as negative controls. All tissues were fixed in 10 % neutral buffered formalin and embedded in paraffin according to routine histological procedures. Sections were deparaffinised and rinsed in 0.5 mol/l Tris-HCl buffer, pH 7.6 containing 0.15 mol/l NaCl (TBS). Endogenous peroxidase was inactivated by incubating sections for 20 min in TBS containing 0.3 % (v/v) hydrogen peroxide. The sections were then rinsed thoroughly in TBS and incubated for a further 10 min with 2 % BSA in TBS buffer. A hyperimmune antiserum to *Pasteurella multocida* type 6:B raised in rabbits, essentially according to the procedure described by *Carter* (1984) was used as the primary antibody. The sections were incubated for 45 min with a 1:5000 dilution of the *Pasteurella* antiserum followed by 30 min incubation with swine anti-rabbit IgG and rabbit PAP-complex (DAKOPATTS, Glostrup, Denmark) diluted 1:20 and 1:100, respectively. All dilutions were made in TBS containing 1 % BSA; the

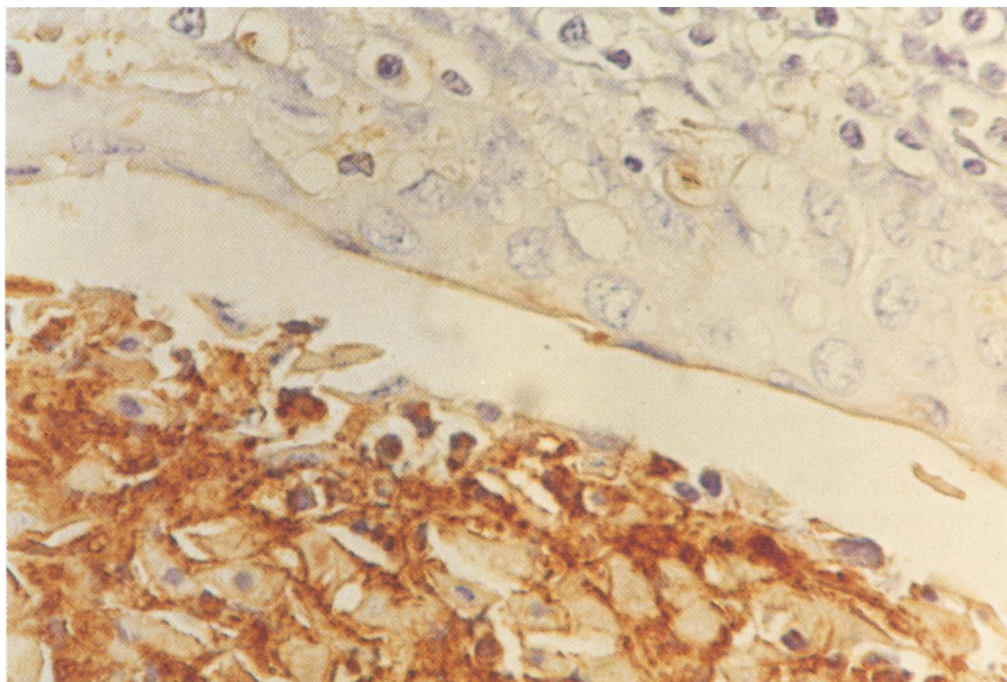

Figure 1. Immunostaining for *P. multocida* revealing bacterial antigen in the lumen of the tonsillar crypt. ×250.

sections were thoroughly rinsed in TBS between each incubation. Peroxidase activity in tissues was visualised by incubation sections for 8 min in TBS containing 0.06 % (w/v) diaminobenzidine (DAB, Sigma, St. Louis, USA) and 0.034 % (v/v) hydrogen peroxide. Finally, the sections were rinsed in tap water, counterstained in Mayer's haematoxylin and mounted with Entellan (Merck, Darmstadt, F.R.G.).

Fig. 1 shows a typical positive staining reaction of the tonsil in a HS carrier. Antigens to *P. multocida* were confined to the lumen of the tonsillar crypt while the lining epithelium and lymphoid tissues were completely devoid of any immunoreaction.

In the lungs of animals that succumbed to the infection diffuse immunostaining was present in alveoli with concentration within alveolar macrophages. Bacterial antigen was also present in lymphatics of the interlobular septae and pleura. In the kidneys, intestines and the liver staining was present exclusively in the blood vascular system indicating the terminal septaemia associated with HS. Immunostaining was neither observed in tissues from non-infected buffaloes nor in cattle tonsils collected at the Uppsala abattoir.

The results were negative when the non-immune rabbit serum was used as primary antibody on sections from infected animals. Immunoreactions were not present in infected tissues when the primary antibody was preabsorbed with the organism (1.6×10^{10}) prior to it been used in the staining proce-

dure. Crossreactivity of the rabbit antiserum with other species of *Pasteurellae* was tested by incubating bovine and porcine lung from which *P. haemolytica* and *P. multocida* respectively, has been isolated. Murine tissues positive for *P. pneumotropica* were also tested. Staining was not observed in the bovine or murine sections but the porcine lung did show immunostaining. Crossreactivity of the primary antiserum may be expected since we used a polyclonal antibody raised against the entire organism.

This crossreaction may also underline the close similarities of the antigens present in the two types of *Pasteurella*. As far as the present study is concerned, the tissues tested were from experimentally infected animals with HS caused by *P. multocida*. Moreover, *P. multocida* species affecting pigs do not produce HS in buffaloes (*Carter* 1967).

The results of this study indicate that the PAP technique is a suitable immunochemical procedure for locating *P. multocida* in tissue sections. This method also offers the opportunity to simultaneously appreciate the histopathological changes associated with the infection.

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