IMMUNOHISTOCHEMICAL DETECTION OF PNEUMONIC PASTEURELLOSIS CAUSED BY *PASTEURELLA MULTOCIDA* SEROTYPE A: 1 IN EXPERIMENTALLY INFECTED MICE

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Pasteurella multocida is an opportunistic pathogen in wild and domesticated animals, as well as in man (Adler, *et al.*, 1999). This bacterium was grouped into five serogroups as A, B, D, E and F based on capsular antigens. According to the Carter system, serotypes A and D cause severe bronchopneumonia in calves (Confer, *et al.*, 1996). Of the various diagnostic tests available, immuno histochemistry has become a valuable tool in both diagnosis and research of infectious and neoplastic disease in a variety of animals. Moreover immunoperoxidase technique can be used for the accurate diagnosis of pneumonic pasteurellosis. The present study provided an opportunity to know whether this method could be utilised for diagnosing pneumonic pasteurellosis caused by *P.multocida* serotype A.

In the present study one eighty Swiss albino mice of 4-5 weeks old weighing 18-20 gram body weight were obtained from laboratory animal research centre of the institute, were used in the present study. The animals were grouped into eighteen groups consisting of ten mice in each group. Of these nine groups were infected with 0.2ml of the inoculum containing $7.4x10^8$ cfu/ml of *P.multocida* serotype A: 1 obtained from the Division of Bacteriology and Mycology of the institute. The remaining nine groups were kept as control. Of the nine infected groups, each group was sacrificed at 6, 12, 24, 36, 48, 60, 72 and 96 hours of post inoculation (HPI) and all animals in ninth inoculated group were allowed to die due to infection. One control group of animals were also sacrificed along with each infected groups.

Immediately after euthanisation all the animals were necropsied and all the internal organs were thoroughly examined for gross lesions. Tissues from lungs were preserved in 10% neutral buffered formalin for immunohistochemical studies. The organism or its antigen detection in formalin fixed tissues sections was performed by indirect immuno peroxidase test using the method as described by Ramos-Vara (2005). The sections were counter stained by Mayer's Haematoxylin. The hyper-immune sera specific for *P.multocida* serotype A:1 used in this test was raised in rabbits.

The use of immunohistochemical staining in the present investigation provided an opportunity to know the distribution of the organism in lung tissue. Lung tissues of all the infected mice gave positive reaction whereas the lung tissues of control animals did not exhibit the positive reaction. The *P.multocida* antigen was detected by the brown deposit with 3, 3 diaminobenzidine (DAB) precipitate in tissue sections and was found to be insoluble in organic solvents (Fig 1). The reaction was seen in the alveolar linings as well as in the alveolar lumen

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(Fig 2). The intense immunostaining in the alveolar wall and alveolar lumen indicated that the presence of specific antibodies to the organism. In the present study, the results correlated well with the immunoperoxidase detection of bacterial antigen from pneumonic lungs caused by *P.multocida* as reported previously by earlier workers (Haritani, 1995; Haziroglu *et al.*, 2001 and Horadagoda, *et al.*, 1991). Hence we could conclude that this technique can be used for accurate diagnosis of pneumonic pasteurellosis.

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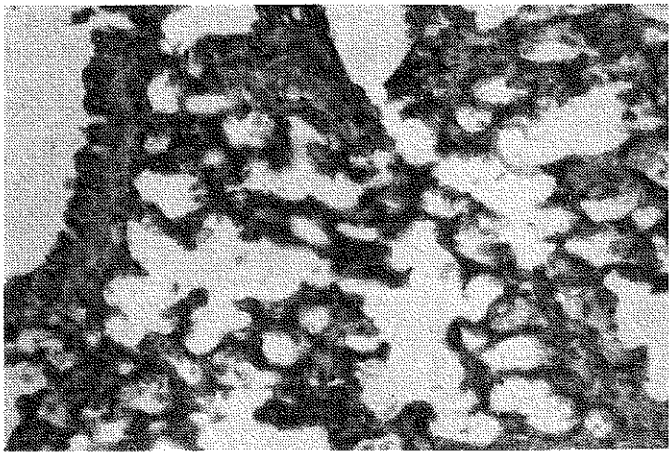

Fig. 1.

P. multocida positive reaction in the alveolar linings. Mayer's haematoxylin counterstain X 150

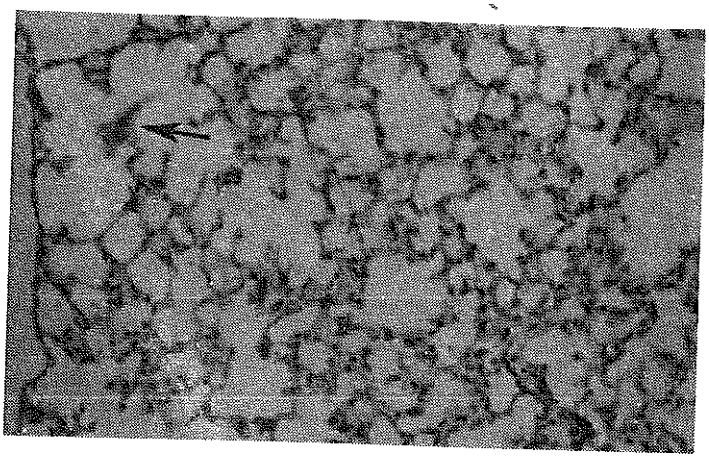

Fig. 2.
Positive immuno-reaction to *P. multocida* in the alveolar lumen (arrow) in immunoperoxidase test. Mayer's haematoxylin counterstain X 150