DETECTION OF PESTE DES PETITS RUMINANTS VIRUS ANTIGEN IN IMPRESSION SMEARS BY AVIDIN-BIOTIN-PEROXIDASE STAINING

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ABSTRACT

An avidin-biotin-peroxidase immunoperoxidase staining method was adapted for detection of peste des petits ruminants virus antigen in impression smears of ocular, buccal, nasal and rectal swabs and tissue impressions from experimentatlly infected goats. Positive staining was demonstrated in 84% out of 100 swabs smears and 77% out of 100 tissue impressions. The test is recommended for use in any PPR surveillance programme.

INTRODUCTION

Peste des petits ruminants (PPR) is an acute, highly contagious and pathogenic morbillivirus disease of small ruminants and camels (Lefevre and Diallo, 1990). It is endemic in the tropical areas of Africa, Middle East and the Indian subcontinent. It is of great economic importance to these areas. Diagnosis of PPR is usually based on clinical signs, virus isolation and identification which is expensive, time-consuming and laborious (Durojaiye *et al*, 1983); immunofluorescence (Durojaiye, 1984) which requires specialized microscopes; precipitation methods (Abegunde *et al*, 1980; Obi and Patrick, 1984) the sentivities of which are relativeily low, and ELISASs (Libeau *et al*, 1994) which are also expensive.

Immunoperoxidase staining has been used for detection of antigens of morbilliviruses (Babu *et al*, 1984; Dandio Brown, *et al*, 1991; 1990). The avidin-biotin system has been employed by Dandio (1990) to detec PPR virus antigens in formalin - fixed, paraffin-embedded tissue sections of experimentally infected goats and has been found useful for retrospective studies. This paper reports the use of an avidin-biotin immunoperoxidase labelling technique for the detection of PPR virus antigen in smears from ocular, buccal, nasal and rectal swabs and tissue impressions from experimentally infected goats. The PPR virus antigen was detected in a sensitive and specific manner in infected tissues tested.

MATERIAL AND METHODS

Animals and samples

Seven five-month-old PPR virus antibody-free goats were infected subcutaneously with 10 $TCID_{50}$ of the Senegalese strain of the PPR virus. The virus was used after five passages in lamb kidney cell culture. Nasal, ocular, buccal and rectal swabs were collected from the goats daily from the third day post-inoculation (pi) through day 15 pi. Swabs were impressed onto clean microscope slides. Tissue impressions of the tongue, oesophagus, lungs, lymph nodes, liver, heart, kidney, spleen, abomasum, small intestines, ileum, caecum, colon, rectum and urinary bladder were also made on glass slides. Impression smears of cut surfaces of these tissues were air-dried, fixed with chilled acetone for one hour, dried at 37°c and kept for later use.

The Avidin-biotin-peroxidase Technique

An avidin-biotin peroxidase technique (Hsu *et al*, 1981) was developed. Several trials were carried out before the establishment of the final protocol.

After inactivation of the endogeneous peroxidase using 3% H_2O_2-methanol mixture, the slides were incubated for 45 minutes at 37°C in a 1:100 dilution of monoclonal antibody against PPR virus (McCullough *et al*, 1986). They were then washed three times in PBS for 10 minutes each, and incubated in 1:200 biotin-labelled antimouse IgG FOR 45 minutes at 36°C. After this, they were

washed in PBS three times and exposed to 1:200 HRP conjugated streptavidin for 45 minutes at 36°C. After washing as done previously, the peroxidase activity was developed by treating the impressions for 15 minutes in the dark with a solution of 0.02% diammobenzidine tetrahydrochloride (DAB) in Tris-buffered saline containing 0.01% freshly added H_2O_2. Slides were then rinsed in tap water, washwed in PBS and counterstained with fast green, mounted in buffered glycerol jelly, and examined under the light microscope. The monoclonal antibody was substituted by TBS in control slides.

RESULTS

The presence of PPR virus antigen was indicated by the positive appearance of brown granular staining of infected cells. This was found in both swab smears and tissue impressions. None was found in the negative controls. Positive staining was observed in 77 out of 100 (77%) tissue impressions and in 84 out 100 (84%) swab smears (Tables 1 and 2). Non-specific staining was highly reduced (Figs. 1 and 2).

There was no significant difference in the sen-

Table 1: Results of ABC-immunoperoxides test on tissue impression smears from goats experimentally invested with PPR virus.

Tissue	No of Impressions tested	No. Positive (%)
Abomasum	7	4 (57.1)
Caecum	7	6 (85.7)
Colon	6	6 (100)
Esophagus	6	4 (66.6)
Heart	6	4 (66.6)
Ileum	6	5 (83.3)
Jejunum	6	3 (50.0)
Kidney	6	4 (66.6)
Liver	7	6 (85.7)
Lungs	6	6 (100)
Lymph nodes	7	7 (100)
Rectum	6	5 (83.3)
Spleen	6	6 (100)
Trachea	6	4 (66.6)
Tongue	6	5 (83.3)
Urinary bladder	6	2 (33.3)
Total	**100**	**77(77.0)**

Table 2: Results of ABC immunoperoxides test on swab smears from goats experimentally infected with PPR virus.

Swab	No. of Smears tested	No. Positive (%)
Buccal	25	18 (72.0)
Conjunctival	25	23 (92.0)
Nasal	25	19 (76.0)
Rectal	25	24 (96.0)
Total	**100**	**84 (84.0)**

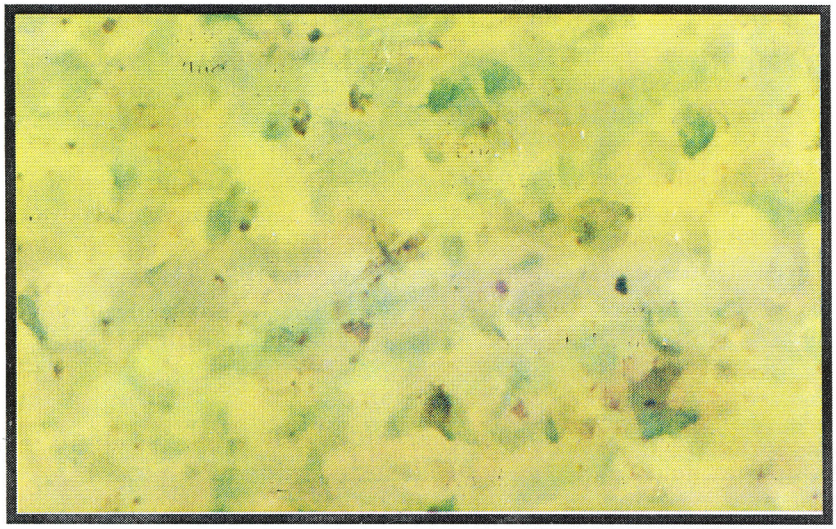

Plate 1: Buccal Swab; Day 5 Pi
Counter stained with Fastgreen

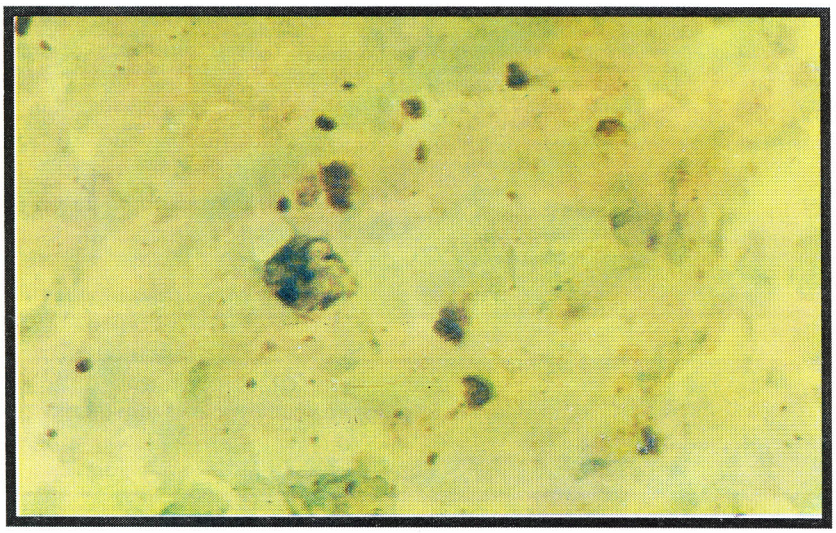

Plate 2: Color Impression; Day 11 Pi
Counter stained with Fastgreen

There was no significant difference in the sensitivity of the test when applied to either swab smears or tissue impressions.

Discussion

Lesions of PPR virus have been reportedly found in various tissues of the body of infected animals (Obi *et al.* 1983; Isitor *et al.* 1984) and the presence of the virus in body secretions has also been demonstrated (Abegunde and Adu. 1977). The results obtained in this study suggest that the avidin-biotin complex (ABC) immunoperoxidase test could be useful for the sensitive and specific detection of PPR virus antigen in infected tissues. the immunoperoxidase test therefore offers a useful tool for the demonstration of virus antigens in infected tissues and secretions. Although the technique has limited advantage over conventional methods. the visualisation gives a very good indication of the presence of virus antigens. The test could be useful in surveillance programmes necessary for the control of the disease since the demonstration of the virus antigens provides a reliable means osf establishing the PPR infection status of herds or flocks.

However. the ABC-IPT has technical but not diagnostic limitations. which could include expenses and long time spent on the bench. Nevertheless. background reaction could be reduced to a minimum by the use of specific monoclonal antibodies.

It is envisaged that the ABC-IPT will provide a very useful diagnostic tool for PPR virus infection in future disease surveillance programme.

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