

ISOLATION AND IDENTIFICATION OF PESTE DES PETITS RUMINANTS VIRUS BY CELL CULTURE AND IMMUNOCAPTURE ENZYME LINKED IMMUNOSORBENT ASSAY

S. Bahadar, A. A. Anjum, M. D. Ahmad and A. Hanif

Department of Microbiology, University of Veterinary & Animal Sciences, Lahore.

ABSTRACT

Thirty tissue samples each of Necrotic debris in buccal mucosa, nasal discharges, ocular discharges and lymph nodes were collected from clinical cases and checked for Peste des Petits Ruminants (PPR) virus using Immunocapture Enzyme Linked Immunosorbent Assay (IC ELISA). The IC ELISA showed presence of PPR antigen 43.3% (13/30) in buccal mucosa samples, 36.6% (11/30) in nasal discharges, 40% (12/30) in ocular discharges and 80% (24/30) in lymph nodes. Overall percentage of PPR antigen in all different samples detected by IC ELISA was 50%. Samples found positive for Vero cell cultures were subjected to antigen detection by using IC ELISA. Out 30 buccal mucosa samples, 30 nasal discharges, 30 ocular discharges and 30 lymph nodes 11, 9, 9 and 22 samples were found positive respectively. Thus an overall 41% samples were found positive by cell culture isolation and IC ELISA. Lymph nodes were found to be the best organ for identification of PPR antigen by IC ELISA.

Keywords: PPR, IC ELISA, Cell Culture

INTRODUCTION

Pestes des Petits Ruminants (PPR) is an acute, contagious disease caused by a morbillivirus in the family Paramyxoviridae (Rashid *et al.*, 2008). On the basis of morphology, growth in cell culture, the nucleic acid composition, antigen and physio-chemical properties, PPR virus is classified as fourth member of the genus morbilli virus of the Paramyxoviridae family and grouped together with canine distemper, human measles and rinderpest viruses (Gibbs *et al.*, 1979).

The disease has been reported in Pakistan on the basis of clinical signs by Athar *et al.*, (1995) and Pervez *et al.*, (1993). One report by Ayaz *et al.*, (1997) described the signs, epidemiology and treatment of highly fatal form of pneumono-enteritis, which affected goats of all ages and breeds in Dera Ghazi Khan District of Punjab.

A tentative diagnosis of PPR is made on the basis of clinical signs but where rinderpest exists in cattle, laboratory identification is required. The disease must be differentiated from rinderpest, blue tongue, foot and mouth disease and other exanthemous conditions. The disease is usually more severe in goats and occasionally severe in sheep. The morbidity rate is 100 percent and in severe outbreaks with 100 percent mortality. In milder outbreaks, the morbidity may not exceed 50 percent (Losos, 1986). Serological tests are routinely used for the diagnosis of PPR. Various serological tests such as virus neutralization test, (Anonymous, 2000), Competitive ELISA (Lefevre *et al.*, 1991; Libeau *et al.*, 1995; Saliki *et al.*, 1993), counter immuno-electrophoresis (Forman *et al.*; 1983), indirect fluorescent antibody test (Hamdy *et al.*, 1975),

Precipitogen inhibition test and agar gel immunodiffusion test (Durojaiye, 1982) can be used for the diagnosis of PPR.

In present study identification of PPR virus was compared in different biological fluids and tissue samples by IC ELISA and virus isolation.

MATERIALS AND METHODS

The immuno capture ELISA was performed for the identification of PPR virus in different biological fluids and tissue samples. PPR virus was isolated by cultivating the suspected tissue materials on Vero cell line and then the virus was identified by the cytopathic effects (CPE) and IC ELISA.

Collection of samples: 120 tissue samples (30 Necrotic debris in buccal mucosa, 30 each nasal and ocular discharges and 30 lymph nodes) were collected from clinical cases labeled and transported to laboratory under refrigerated conditions

Immunocapture ELISA (IC ELISA): The Immunocapture ELISA test kit was purchased from CIRAD / EMVT, Montpellier (France). For the detection of PPR Nucleoproteins antigens (Libeau *et al.*, 1995). Briefly, ELISA plates (Nunc Maxisorp) were coated with polyclonal anti-PPR antibodies as a capture antibody with dilution (1/500) in coating buffer (PBS 1X pH 7.2–7.6). Plates were incubated at 37 °C for 1 h and washed. Samples were added (50 µl/ well); then specific PPR biotinylated monoclonal antibodies diluted 1/500 in blocking buffer were added along with the enzyme conjugate, consisting of streptavidin-peroxidase. Plates

were incubated at 37 °C for 1 h and washed. Chromogen, orthophenyldiamine and hydrogen peroxide substrate were added and incubated for 10 minutes. The color developed by sample and reference positive and negative controls was read on an ELISA reader (Multiskan EX Thermo USA) at an absorbance of 492 nm. Any samples giving an OD value greater than twice the mean OD value of the Blank controls (PPRV) were considered positive.

Sample Preparation and virus cultivation on Vero cells: The tissue samples were ground and made in 30% (w/v) suspension in F-12 medium without serum (Sigma chemicals). The suspension was centrifuged at 1000g for 15 minutes. The supernatant was collected and antibiotics

were added (penicillin 10,000 units, streptomycin 10 mg/ml, and amphotericin B 25 mg/ml). Supernatants were inoculated in Vero cell cultures, maintained in Dehydrated Modified Essential Medium (DMEM) with 5% foetal bovine serum. The cultures were incubated rotating at 37 °C and daily examined for the appearance of cytopathic effects. Positive culcers were retested with IC ELISA KIT.

Source of reference virus: The reference Peste des Petitis Ruminants virus (local strain) was obtained from National Agriculture Research Council (NARC), Islamabad and Veterinary Research Institute (VRI), Lahore. The data thus obtained were statistics analysed through caley t. test (Steel *et al.*, 1997)

Table-1 Identification of PPR antigen using IC ELISA and Vero cell culture isolation + IC ELISA in various samples

Sample	Total number of tissue Samples	Positive Samples by IC ELISA	Positive Samples by cell culture isolation and IC ELISA	Percentage of Positive Samples by IC ELISA	Percentage of Positive Samples by cell culture isolation and IC ELISA
Mouth swabs	30	13* ^a	11* ^a	43.3	36.6
Nasal swab	30	11* ^a	9* ^a	36.6	30
Ocular swab	30	12* ^a	9* ^a	40.0	30
Lymph nodes	30	24** ^a	22** ^a	80	73
Total	120	60	51	50	41

** indicating the significant difference for detection limits among samples

RESULTS AND DISCUSSION

An overall prevalence of PPR antigen was detected in 50% (60/120) of various samples. Highest presence of PPR was recorded in 80 percent (24/30) of the lymph nodes samples followed by mouth lesion samples where it was found in 43.3 percent (13/30), where as almost same has found in 40 percent (12/30) of ocular samples, however a least percentage of presence of PPR antigen was recorded at 36.6 percent (11/30) of nasal discharges. Present findings are not in agreement with the results of Diop *et al.*, (2005), who reported the highest values 84.6 percent (22/26) from ocular, nasal and mouth lesions during outbreak in goat flocks in Senegal (Table-1). The higher values may correspond to the difference in the nature of the infectious agent or sensitivity of IC ELISA kit. Abraham and Berhan (2001) also reported that Antigen capture ELISA is rapid, sensitive and virus specific for the identification of PPR virus.

The samples obtained from different sources were also subjected to isolation of virus using Vero cell cultures. An overall prevalence of PPR antigen was detected in 41% (51/120) of samples by virus isolation and confirmation by IC ELISA. Highest presence of PPR antigen 73% (22/30) was found in lymph nodes where as

36.6% (11/30) was detected in buccal mucosa samples and a least percentage 30% (9/30) was recorded in nasal and ocular discharges by virus isolation. The present study is closely related to the previous findings by Housawi *et al.*, (2004); Libeau *et al.*, (1995); Forsyth and Barret, (1995) regarding isolation of PPR virus using Vero cell line. Although the number of samples detected by Vero cell cultured and IC ELISA was less than the direct tissue IC ELISA but statistically non significant difference was found in both methods.

It is observed that the ICE ELISA is sensitive and useful tool for rapid diagnosis and implementation of quick and efficient control measures.

REFERENCES

- Abraham, G. and A. Berhan (2001). The use of antigen-capture enzyme-linked immunosorbent assay (ELISA) for the diagnosis of rinderpest and peste des petits ruminants in ethopia. *Trop. Anim. Health Prod.*, 33(5): 423-430.
- Anonymous (2000). OIE (Office International des Epizooties / World organization for Animal Health), Manual of Standards for diagnostic tests and vaccines. Ed. 4. Ch., 2. 1. 5.
- Athar, M., G. Muhammad, F. Azim, A. Shakoar, A. Maqbool and N. I. Chaudhry (1995). An

- Outbreak of Peste des Petits Ruminants like disease among goats in Punjab, Pakistan. *Vet. J.*, 15: 140-143.
- Ayaz, M. M., G. Muhammad and S. M. Rehman (1997). Pneumo-enteritis syndrome amongst goats in D.G. Khan. *Pakistan. Vet. J.*, 17(2): 97-99.
- Diop, M., J. Sarr and G. Libeau (2005). Evaluation of novel diagnostic tools for peste des petits ruminants virus in naturally infected goats. *Epidemiol. Infect.*, 133: 711-717.
- Durojaiye, O. A. (1982). Precipitating antibody in sera of goats naturally affected with Peste des Petits Ruminants. *Trop. Anim. Health Prod.*, 14: 98-100.
- Forman, A. J., L. W. Rowe and W. P. Taylor (1983). Detection of rinderpest antigen by agar gel diffusion and counter immunoelectrophoresis. *Trop. Anim. Health Prod.*, 15: 83-85.
- Forsyth, A. and T. Barret (1995). Isolation and identification of Peste des Petits Ruminants virus. *J. Vet. Med. Series B*, 42: 61-69.
- Gibbs, E. P., W. P. Taylor, M. P. J. Lawman and J. Bryant (1979). Classification of peste des petits ruminants virus as the fourth member of the Genus Morbillivirus, *Inter Virology*, 11: 268-274.
- Hamdy, F. M., A. H. Dadiri and S. S. Breese (1975). Characterization of Peste des Petits Ruminants virus and its immunological relationship to rinderpest virus. *Abst. Annual Meeting. Amer. Societ. Microb.*, 75:265.
- Housawi F.M.T., E. M. E. Abu Elzein, G. E. Mohamed, A. A. Gameel, A. I. Al-Afaleq, A. Hegazi and B. Al-Bishr (2004). Emergence of Peste des Petits Ruminants in sheep and goats in eastern Saudi Arabia. *Revue Elev. Med. Vet. Pays. Trop.*, 57 (1-2): 31-34.
- Lefevre, P. C., A. Diallo, F. Schenkel, S. Hussein and G. Staak (1991). Serological evidence of peste des petits ruminants in Jordan. *Vet. Record*, 128: 110-112.
- Libeau, G., C. Prehaud, R. Lancelot, F. Colas, L. Guerre, D. H. L. Bishop and A. Diallo (1995). Development of a competitive ELISA for detecting antibodies to the Peste des petits ruminants virus using a recombinant nucleoprotein. *Res. Vet. Sci.*, 58: 50-55.
- Losos, J. G. (1986). *Infectious tropical diseases of domestic animals*. Longman Scientific and Technical, Canada. : 549-58.
- Pervez, K, M. Ashraf, M. S. Khan, M. A. Khan, M. M. Hussain and F. Azim (1993). Rinderpest like disease in goats in Punjab, Pakistan *J. Livestock Res.*, 1: 1-4.
- Rashid, A., M. Asim and A. Hussain (2008). An outbreak of peste des petits ruminants in goats at district Lahore. *The J. Anim. and Plant Sci.* 18(2-3): 72-75.
- Saliki, J. T., G. Libeau, J. A. House, C. A. Mebuus and E. J. Dubovi (1993). Monoclonal antibody based blocking ELISA for specific detection and titration of PPR virus antibody in caprine and ovine sera. *J. Clin. Microb.*, 31 (5): 1075-1082.
- Steel, R. G. D., J. H. Torrie and D. A. Dickey (1997). *Principal and procedure of statistics. A biochemical approach*. 3rd edition McGraw Hill International book company USA, 64: 362-364.