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Monoclonal antibody-based enzyme immuno-slide assay (EISA) in the rapid diagnosis of Peste des petits ruminants of goats

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Abstract

Monoclonal antibody (mAb)-based enzyme immune-slide assay (EISA) was used for the detection of Peste des petits ruminants (PPR) virus from field samples collected from a natural outbreak. The clinico-pathological study was undertaken to diagnose the case primarily of PPR. Antigen was detected from discharges and faeces of infected goats and swabs of postmortem lesions prepared on glass slide or glass plate using acetone fixation. Nasal discharge collected at the early stage of disease course or lung is an appropriate ante- or postmortem sample for this technique, respectively. Convalescent polyclonal sera collected from recovered animals which were diagnosed as PPR by EISA showed high antibody titer against PPR by C-ELISA, demonstrating the satisfactory specificity of the test. Therefore, EISA is a sensitive and specific assay to confirm PPR infection both in field and laboratory conditions and especially suitable for developing country.

Key words : Peste des petits ruminants (PPR), Goats, Enzyme immuno-slide assay

INTRODUCTION

Peste des petits ruminants (PPR) is a lethal contagion of goats and sheep (Shankar et al, 1998). The causative agent is PPR virus belonging to the *Paramixoviridae* family and genus Morbillivirus. Although PPR was first detected in West Africa in 1942 and Southern India in 1989 as a rinderpest-like disease, it was not confirmed as PPR by World Reference Laboratory until 1993 in Bangladesh (Debnath, 1995). It was later found that the causative virus has a close relation with Indian isolates (West Bengal) PPRV at a cluster with Asian group (Dhar et al, 2002). Clinically PPR is characterized by erosive stomatitis, enteritis with diarrhea and pneumonia. Prevalence is higher in indigenous Black Bengal goats than exotic breed like Jamunapari (Mondal et al, 1995). Islam et al (1996) reported even 100% morbidity and case fatality rates in native goats in Bangladesh.

The infected animals shed virus during the prodromal or erosive phases of the disease and act as a source of infection for susceptible animals (Plowright, 1968). The identification of virus in secretion and tissues of infected animals can be conducted by differential neutralization (Taylor, 1979), agar gel immunodiffusion (AGID) (Islam et al, 2001), electrophorectic profiling of the nucleocapsid (N) protein (Diallo et al, 1987) or antigenic profiling with monoclonal antibodies (Libeau and Lefevre, 1990). Recently, a differentiating cDNA probe has been used successfully to detect virus nucleic acid in postmortem tissue specimens (Diallo et al, 1989).

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PCR and DNA probe are highly specific and sensitive, but expensive and require well-equipped laboratory. Monoclonal antibody (mAb) based immunocapture ELISA (ICE) for the rapid and accurate diagnosis of PPR and rinderpest has also been developed (Libeau et al, 1994). The ICE is based on the attachment of PPR antigen tobroadly reactive pre-coated mAb and the detection of antigen: mAb complex using biotinylated mAb. However, this test needs antigen purification and protein quantification. Sil et al (2001) for the first time developed a monoclonal antibody based enzyme immuno slide assay (EISA) on glass slide for the rapid and accurate detection of PPR virus from excretion and homogenized tissue of infected animals. The sample can be used for the testwithout refrigeration for one week, which is especially suitable for laboratory with inadequate equipment. In this paper clinic-pathological study of a suspected PPR outbreak was conducted and PPR was confirmed by EISA conducted on glass slide or 12 well glass plate using smear from crude samples (excretions and tissues).

MATERIALS AND METHODS

Study population

Thirty one goats were observed in a natural field outbreak suspected of PPR in a village in Mymensingh district. The total population belongs to different age and sex groups.

Clinical study

The disease courses of the infected animal were observed and recorded. PPR was highly suspected based on clinical signs and symptoms. Among the 31 goats, 23 were found to be infected and later 17 goats died.

Test sample

At 4～6 days of the disease, nasal and oral swabs were collected using cotton swabs. Faecal swabs also were collected after diarrhoea onset. Necropsy of 4 dead goats was performed and lung, spleen, lymph nodes, and intestines were collected aseptically. Then, all the samples were kept at −80℃. Sera were collected from 6 recovered goats, transferred in ice box after centrifugation (at 3,000rpm for 10minutes), and kept at −20℃ until further use.

Viruses and antibodies

Reference PPR virus antigen and monoclonal antibodies used for the study were obtained from C-ELISA kit (IAEA AND BDSL, UK). Discharges, feces and tissue samples were used for the identification of antigen. Convalescent sera (collected from the recovered animal after a field outbreak) were used as polyclonal test sera.

Preparation of smear for EISA

Nasal, oral discharges and diarrhoeic faeces were smeared on glass slide or 12 well plate. Cotton swabbing of the infected tissue samples (lung, spleen, lymphnodes and intestines) were performed aseptically before smearing on slide or plate. The smear was air dried, fixed in ice cold acetone for 15 minutes, and used immediately or kept at 4℃ for further use.

EISA techniques

EISA test was conducted on samples coated on glass slides (acetone fixed) or 12 wells glass plates (Sil et al, 2001). Monoclonal antibodies against PPR virus (Virus specific, Anderson et al. 1991) were used as reference antibodies (1:100 in PBS) and added at 50μl/smear/well while positive (?)/negative control was kept using 50μl/well in blocking buffer solution. For each essay, the PPR reference antigen was included as a positive control. Slide/plates were incubated at 37℃ for an hour and wash with PBS (1 : 5) and finally air dried using table fan. After drying, 50μl of anti-mouse IgG conjugate (1: 1000 in buffer solution) was added to all wells. The slide was then incubated at 37℃ for 1 hour. Fifty μl of ortho-phenylendiamine (Sigma, UK) mixed with hydrogen peroxide (1 : 200) and added to each well and incubated for 15 minutes at room temperature .The reaction was stopped by adding 50μl of sulfuric acid (6.8%) and

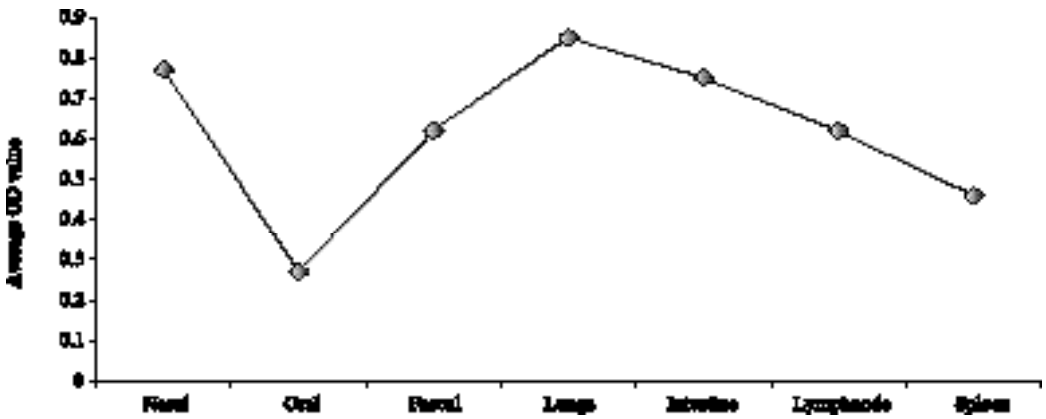

Fig. 1. The OD values of different discharges and tissue samples

examined by naked eyes (golden yellow color change in positive cases) or optical densities of the samples were measured at 492nm with a computerized ELISA reader.

Competitive ELISA

Sera collected from the recovered animals after field outbreaks from PPR infection (antigen detected by Mab based EISA) were tested for the presence of PPR specific antibody to confirm results obtained by EISA using Mab-based competitive ELISA against PPR antigen as per technique described by Anderson et al, 1991.

RESULTS

Clinical findings

Out of 31 goats from affected houses, clinical signs and symptoms were observed in 23 goats (morbidity 74.13%). The main clinical manifestations were fever (103°F～107°F), dry muzzle, anorexia followed by serous to mucopurulent nasal discharge, severe stomatitis with necrotic lesions in the buccal cavity, diarrhoea and respiratory distress. Dyspnoea with cough, frothy salivation, conjunctivitis, depression were also found. The disease was, therefore clinically suspected as PPR. All goats did not show all clinical signs and symptoms. Abrupt rise of body temperature which is the most striking feature of PPR infection and the temperature rising was occurred from the first day of infection. High fever was observed at the 2nd and 3rd day of illness and dropped down gradually. But in some cases high temperature was gained at the 5th day of illness also. A rise in body temperature was accompanied by watery ocular and nasal discharges which lasted for four to five days and later became thick and mucopurulent. Blocked nostrils caused painful respiratory discharges. Oral frothy discharge was not common.

Diarrhoea was common and observed immediately after rising of body temperature. In most diarrhoeic cases, dark brown fluid faeces containing excess mucus, epithelial shreds and necrotic debris streaked with blood were observed. The profuse diarrhoea caused rapid dehydration and the affected goats wasted away while standing with lowering heads, shrunken eyes and arched back. Necrotic lesions were developed in the mouth of PPR infected goats and extended over the entire oral mucosa forming diptheric plaques. Seventeen goats showed watery diarrhoea and subnormal temperature before death.

Post mortem findings of PPR suspected goats

Amon the infected goats 17 goats died along with the disease course (mortality 54.83%). Postmortem examinations of 4 dead goats revealed severely dehydrated and emaciated carcasses. There were eroded and ulcerated mouths especially in the gums, inner lips, cheeks and hard palate, and dorsum of the free portion of

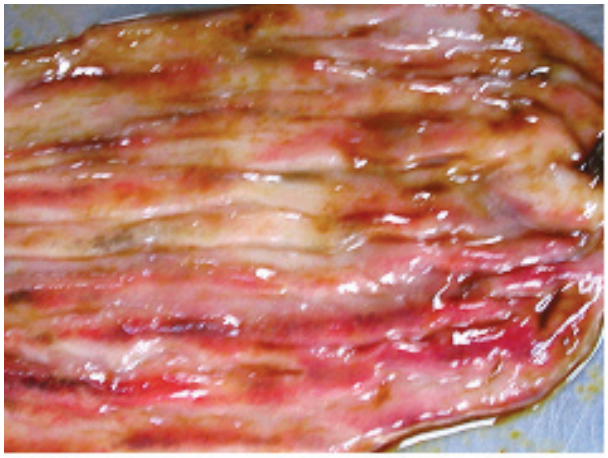

Fig. 2. Postmortem lesions in clinically PPR suspected goat showing Zebra striping in caeco-colic junction

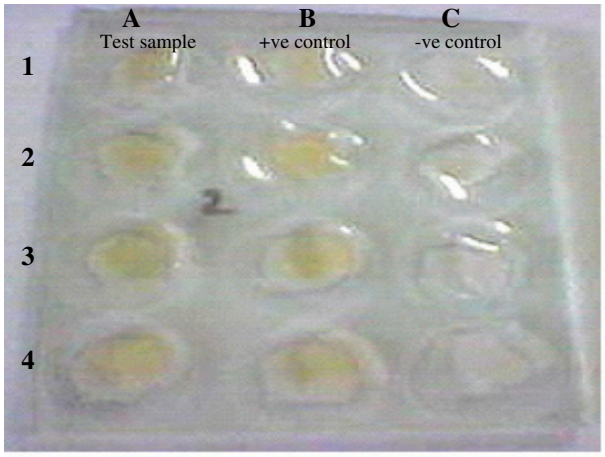

Fig. 3. 12 well glass slice showing golden yellow color change for PPR known and test sample in EISA.

tongue. All goats had the haemorrhages on the mucosa of the jejunum, ileum and colon. The pathognomonic lesion 'Zebra stripe' was observed in caeco-colic junction (Fig. 2). Spleen of goats was atrophied and lymphnodes were found enlarged. The tracheas were congested and in some cases frothy mass in the lumen of tracheas were observed. In all goats lungs showed congestion and consolidation. The congestion was more pronounced on the peripheral border. The uterus of one goat was found congested.

PPRV detection from discharges

The swab samples were collected from 23 clinically PPR suspected goats. Smears prepared with discharges (Nasal, oral and faeces) revealed color change (golden-yellow) (Fig. 3) when EISA was performed. Samples from PPRV infected goats reacted with PPR monoclonal antibody that again reacted with anti-species conjugate and this conjugate developed color after reaction with substrate/chromogen solution. The OD values of different discharges ranged from 0.211 to 0.945. Thus all the samples were found positive for PPRV. Positive control in EISA was found positive as the OD values ranged from 0.882 to 0.710 for PPR reference antigen. High level of PPR antigen was detected from nasal discharges ranging from 0.449 to 0.945. The average OD values of nasal, oral and faecal discharge were 0.77 ± 0.13, 0.27 ± 0.04 and 0.62 ± 0.11 respectively (Fig. 1). The optical density (OD) values less than or equal to 0.097 were considered as negative.

PPRV detection from postmortem samples

Lungs, spleen, lymphnodes, intestine from goats were used to detect PPRV in EISA. Postmortem samples smeared on the glass slides revealed color change (Golden yellow). Optical density values were measured and varied from 0.307 to 0.892 for different tissue samples. There was found that PPRV concentration was higher in lung (0.85 ± 0.06) compared to intestine (0.75 ± 0.04), lymphnodes (0.62 ± 0.08) and spleen (0.46 ± 0.15) (Fig. 1).

Sensitivity and specificity of EISA

The sensitivity of EISA in this study was estimated by determining the positive OD values of reference antigen (antigen from C-ELISA Kit, BDSL, Uk) at 1 : 100 dilution, 2µl/smear/well. Convalescence sera of the recovered animal after a field outbreak diagnosed by EISA were tested by C-ELISA against PPR antigen and found high level of PPR specific antibody (average percent of inhibition was 81.99 ± 3.82).

DISCUSSION

The study was carried out for the detection of Peste

des petits ruminants (PPR) from PPR virus-infected goats. Investigation of clinical signs and postmortem features was considered for primary clinical diagnosis of PPR. An immunocapture ELISA (ICE) has already been developed for the specific identification of PPR virus antigen (Libeau et al, 1994). The test was based on the use of virus specific mouse monoclonal antibody (mAb) and detection of antigen by another specific biotinylated mAb. ICE is able to detect viral antigen from discharges and tissue sample. Sil et al (2001) developed a field adaptive technique; monoclonal anti-body based enzyme immuno slide assay (EISA) on glass slide for the rapid and accurate detection of PPR virus from excretion and pathological homogenized tissue sample of infected animals following fixation of smears in acetone. In this study smear was prepared by swab-bing of crude tissue sample, faeces and discharge. Result obtained from the suspected field outbreak showed that EISA was able to detect PPR antigen from nasal and oral discharge, faecal sample and postmortem tissues. These results have later been confirmed by C-ELISA using convalescent sera against PPR antigen. This test is rapid, economic and can be performed under field condition.

Infected goats with PPR showed depression, inappetence, abrupt rise in body temperature (103°F to 107°F), oculonasal discharges, erosive stomatitis, respira-tory distress, later diarrhoea with decreasing body temperature, dehydration and death. There were variations in clinical signs in different goats. The clinical signs and symptoms observed in the present outbreak were similar to those reports in case of PPR of goats from different geographical areas (Mondal et al, 1995; Shanker et al, 1998; Kumar et al, 2001; Debasis et al, 2002 and Pawaiya et al, 2004). Necropsy findings con-sisted of necrotic and ulcerative lesions on lips, oral mucosa, pharynx and nasal mucosa. Similar lesions were also described by Toplu (2004). Hemorrhagic ulceritis on the mucosa of the colon, jejunum, ileum and 'Zebra stripe' found in the study was also reported by Mondal et al (1995); Debasis et al (2002). Consolidation and congestion of the lung was consistent feature also observed by Islam et al (1996) and Kumar et al (2001).

In the early stage of disease course, nasal discharge showed higher level of viral antigen than oral discharge and faecal sample was considered as a suitable source of virus antigen for diagnosis after onset of diarrhoea. Similar result obtained by Libeau et al (1994) using ICE and Sil et al (2001) using EISA. Nasal discharge sampling seemed to be less traumatic and remain as a good source of antigen until mucopurulent. Smear prepared from lung samples revealed more deep golden brown color change in EISA test. Less virus antigen was detected in spleen than intestine and lymphnodes, confirming the previous result obtained by Wamwayi et al (1991).

The sensitivity of EISA in this study was established by finding the positive OD values of reference antigen (antigen from C-ELISA Kit, BDSL, Uk) at 1 : 100 dilution. This might be due to the ability of mAb to react with haemagglutinin protein epitope against envelope virus particle. Convalescence sera of the recovered animals after a suspected field outbreak diagnosed by EISA were tested by C-ELISA against PPR antigen to confirm PPR was involved in the outbreak. The high level of PPR specific antibody supports the specificity of EISA as one of the major diagnostic tools for epidemiological investigation of PPR infection

This method can detect PPR virus from the sample kept at room temperature for one week with not reduction of OD values lees than 40%; not studied in this study. The EISA was considered as the most suitable technique to operate both in field and laboratory condition as compared to ICE, PCR and cDNA probe which require costly reagent, plastic materials, and well-equipped laboratories.

REFERENCES

Anderson J. 1991. The use of monoclonal antibodies in competitive ELISA for the detection of antibodies to rinderpest and peste des petits ruminants viruses: the sero-monitoring of rinderpest throughout Africa. Phase one. Proceedings of a final research coordination meeting of the FAO-IAEA-SIDA-OAU-IBAR-PARC Co-ordinated Research Programme. Held in Bingerbille, Cote d'Ivoire, 19-23 Nov 1990. *IAEATECH-DOC* 623: 45-53.

Dhar P, Sreenivasa BP, Barrett T, Corteyn M, Singh RP, Bandyopadhyay SK. 2002. Recent epidemiology of peste des petits ruminants virus (PPRV). *Vet Microbiol* 88(2): 153-159.

Debasis J, Mousumi G, Jana D, Ghosh M. 2002. Incidence of an explosive outbreak of peste des petits ruminants (PPR) in Black Bengal goats in Bankura district of West Bengal. *Indian Vet J* 79(7): 739-740.

Debnath NC. 1995. Peste des petits ruminants (PPR); An overview. Proceedings of the BSVER symposium on eradication of rinderpest and related disease. 2 December, 1995, Dhaka: 9-13.

Diallo A, Barrett T, Barborn M, Subbarao SM, Taylor WP. 1989. Differentiation of rinderpest and peste des petits ruminants viruses using specific cDNA clones. *J Virol Method* 23(2): 127-136.

Diallo A, Barrett T, Lefevre PC, Taylor WP. 1987. Comparison of proteins induced in cells infected with rinderpest and peste des petits ruminants viruses. *J Gen Virol* 68(Pt 7): 2033-2038.

Islam MR, Shamsuddin M, Rahman MA, Das PM, Dewan ML. 2001. An out break of Peste des petits ruminants in Black Bengal goats in Mymensingh, Bangladesh. *Bangladesh Vet* 18(1): 14-19.

Kumar A, Singh SV, Rana R, Vaid RK, Misri J, Vihan VS. 2001. PPR outbreaks in goats: epidemiological and therapeutic studies. *Indian J Anim Sci* 71(9): 815-818.

Libeau G, Lefevre PC. 1990. Comparison of rinderpest and PPR viruses using antinucleoprotein monoclonal antibodies. *Vet Microbiol* 25(1): 1-16.

Libeau G, Diallo A, Colas F, Gueree L. 1994. Rapid differential diagnosis of Rinderpest and PPR using an immunocapture ELISA. *Vet Rec* 134(12): 300-304.

Mondal AK, Chottopadhuay AP, Sarkar SD, Saha GR, Bhowmik MK. 1995. Report on epizootological and clinico-pathological observationon peste des petits ruminants (PPR) in West Bengal. *Indian J Animal Health Bull* 64: 261.

Pawaiya RVS, Misri N, Bhagwan PSK, Dubay SC. 2004. Pathology and distribution of antigen in goats naturally infected with peste des petits ruminants virus. *Indian J Anim Sci* 74: 35-40.

Plowright W. 1968. Rinderpest Virus. *Virology monograph*. Eds S Gard, C. Halluauer, K. F. Meyer, Vienna, Springer Verlag: 25.

Shankar H, Gupta VK, Singh N. 1998. Occurrence of peste des petits ruminats like disease in small ruminants in Uttar Pradesh. *Indian J Animal Sci* 68: 38-40.

Sil BK, Taimur MJFA, Hossain KM, Giasuddin M, Haque ME, Rahman MM, Roy A, Alam J, Islam MR, Rasid B, Akter N, Pramanic BK, Anower AKMM. 2001. Development of Mab-based Enzyme immuno slide Assay (EISA) for the rapid detection and accurate field diagnosis of peste des petits ruminants virus. *Bangladesh J Lives Res* 7: 48-63.

Taylor WP. 1979. Serological studies with the virus of PPR in Nigeria. *Res Vet Sci* 26: 236.

Toplu N. 2004. Characteristic and non-characteristic pathological findings in peste des petits ruminants (PPR) of sheep in the Ege District of Turkey. *J Com Pathol* 131: 135-141.

Wamwayi HM, Rossiter PB, Wafala JS. 1991. Confirmation of riderpest in experimentally and naturally infected cattle using microtitre techniques. *Trop Animal Health Product* 23(1): 17-21.