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DEVELOPMENT OF A CELL-ELISA USING ANTI-NUCLEOCAPSID PROTEIN MONOCLONAL ANTIBODY FOR THE TITRATION OF PESTE DES PETITS RUMINANTS (PPR) VACCINE VIRUS

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ABSTRACT

Peste des petits ruminants (PPR) is an acute and highly contagious viral disease of sheep and goats. Indian veterinary research Institute has developed a very efficacious vaccine using lineage IV virus of PPR being used under field condition. Infectivity assay and identity test of the vaccine virus are two important tests to be carried out routinely for quality assurance of PPR vaccine. We developed a monoclonal antibody based cell-ELISA for simultaneous titration and identity test of vaccine virus using 4G6 anti-nucleocapsid monoclonal antibody. Cell-ELISA developed correlated well (r=0.96) with the conventional titration technique using cytopathic effect of virus as indicator. Cell ELISA has a relative sensitivity and specificity of more than 97% compared to infectivity titration. Findings indicated that, cell-ELISA can be successfully used for infectivity and identity test of virus in vaccine samples.

INTRODUCTION

Peste des petits ruminants (PPR) is an acute and highly contagious viral disease of sheep and goats, characterized by pyrexia, necrotizing and erosive stomatitis, enteritis and pneumonia followed by death and recovery. The causative virus has been classified under the genus *Morbillivirus* within the family *Paramyxoviridae*. In India, PPR was first reported from Tamilnadu in 1987 and now this disease is endemic throughout the country (Singh *et al.*, 2004c). Control of the disease is possible through mass vaccination campaigns using homologous PPR vaccines. A live attenuated PPR vaccine has been developed (Sreenivasa *et al.*, 2000; Sarkar *et al*., 2003) and used at large scale for vaccination in the country.

The conventional method for virus quantitation in vaccine sample is by observation of CPE (cytopathic effect) microscopically in microtiter plates or in tubes in case of tube titration (Rossitter *et al*., 1982). This technique requires an expert eye and at times suffers from ambiguity. Moreover, screening of large number of virus samples becomes difficult through microscopic observation of CPE in titration wells or tubes. Present investigation deals with the development of a cell-ELISA as an alternate technique to microtitration method, using PPR specific monoclonal antibody (clone no 4G6) to nucleocapsid protein (Singh *et al.*, 2004a). The test has been compared with a gold standard conventional microtitration technique using identical vaccine virus samples. The test will also help as identity test of vaccine virus for vaccine manufacturers involved in PPR vaccine production.

MATERIALS AND METHODS

Vero cells (African Green Monkey cells) between 130-150th passage levels were used for the titration of PPR vaccine samples. The cells were propagated in Eagle's Minimum Essential Medium (EMEM) supplemented with 10% bovine calf serum. These cells were maintained in the same medium with 2% bovine calf serum.

PPR virus, Sungri/96 at passage 59 in Vero cells, was used throughout the study. The samples used in this study were derived from thermostability trials using a combination of different chemical stabilizers (Sarkar *et al.*, 2003). Virus titration was carried out in 96 well plates using Vero cells. Briefly, co-cultivation of 10-fold dilution of each virus sample was carried out with 10^5 cells /well of micro-titer plate using four replicates per dilution. After final reading (Reed and Muench, 1938) on 6th day, these samples were processed for cell-ELISA.

Cell-ELISA was carried out as per the technique described earlier (Samuel *et al.*, 2000) with certain modifications. Cytopathic effect of the virus was observed at a regular interval. After visual observation of PPR spe-

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cific CPE on 6th day of titration, the supernatant of each well was discarded gently. Cells were fixed with chilled acetone/PBS (100ml /well) in the ratio of 80:20 for 10 minutes (Samuel *et al.*, 2000). After fixation, acetone/PBS was aspirated completely and wells were air-dried. The micro-titer plates were blocked using 200µl of blocking buffer (PBS with 1% gelatin, 0.5% fetal calf serum and 0.1% tween 20) for one hour at 37°C. Then 100µl of detection antibody (clone no. 4G6) diluted 1:20 in blocking buffer was added in each well. The detection antibody used in this test is an anti-nucleocapsid (N) protein monoclonal antibody raised against an Indian isolate of PPR virus. Plates were incubated for 1 hour at 37°C. The wells were gently washed three times with PBS (0.0025M, pH7.4±0.2). Rabbit anti-mouse antibodies conjugated to HRPO (Dako patts, Denmark) in blocking buffer (1:1500) was then added to each well of the ELISA plate. Plates were further incubated for one hour at 37°C. The wells were washed three times and substrate (H_2O_2) chromogen (OPD) mixture (100µl) was added. Colour reaction was observed for 4-5 minutes at room temperature and reaction was stopped using equal volume of 1M H_2SO_4. Optical density was measured at 492 nm (A_{492}) in an ELISA reader. A cut-off value with two times the absorbance (A_{492}) compared to mock-infected cells was set for declaring a well as positive.

Sensitivity and specificity of cell-ELISA was determined using two-sided contingency table, wherein the number of wells positive in cell-ELISA out of the wells positive by PPR virus specific CPE was taken as the sensitivity. Specificity of the test was determined as the number of the wells negative in cell-ELISA out of the total wells negative for PPR virus specific CPE.

RESULTS AND DISCUSSION

Based on the screening of 247 identical virus samples, a good correlation (r=0.96) between cell-ELISA and

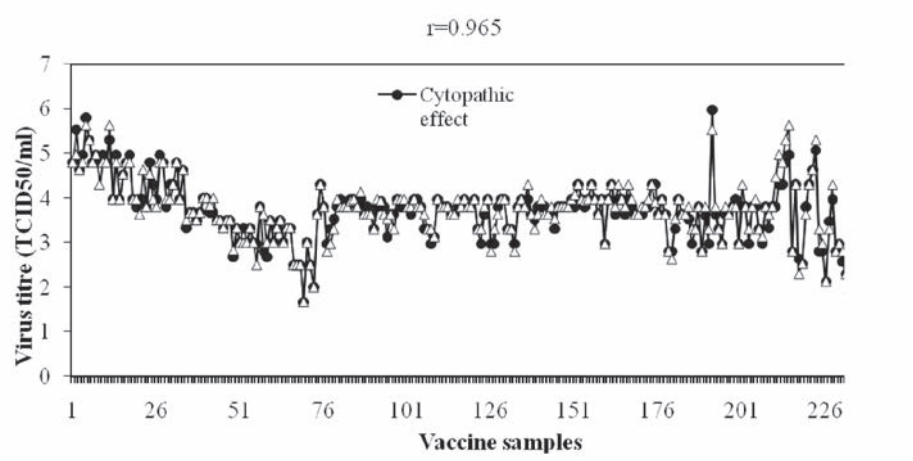

Figure 1. Infectivity titration on 247 identical PPR vaccine virus samples using cytopathic effect and cell-ELISA.

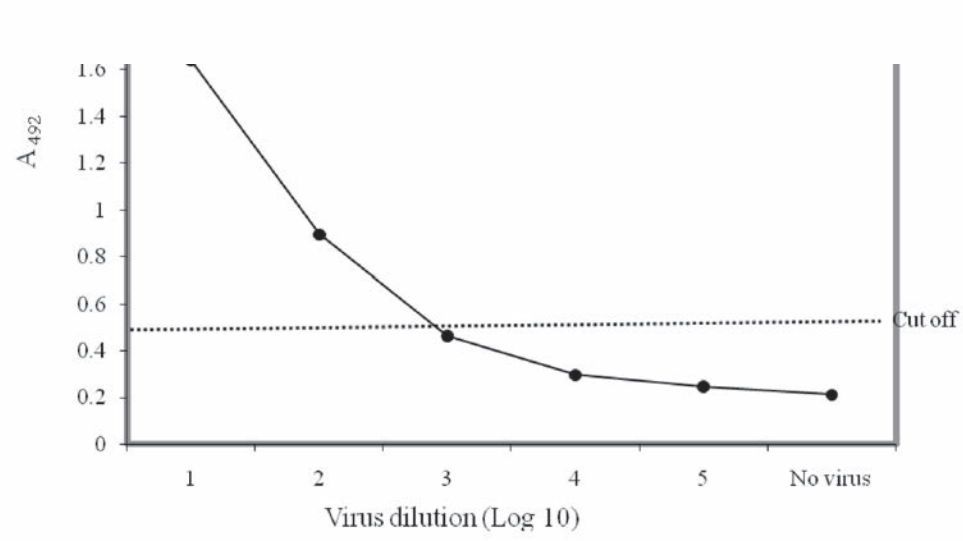

Figure 2. Detection of PPR vaccine virus in vero cells using 4G6 MAb based cell-ELISA. The cells were fixed 6th day post infection.

Table 1. Comparative efficacy of cell-ELISA with conventional infectivity titration in Vero cells

		Infectivity Titration		
		Positive	**Negative**	**Total**
Cell-ELISA	Positive	1848	89	1937
	Negative	52	3118	3170
	Total	1900	3207	5107

Relative Sensitivity of cell-ELISA Vs Infectivity Titration= 1848/1900=97.26%; Relative Specificity of Cell-ELISA Vs Infectivity Titration=3118/3207=97.22%

CPE was observed (Fig. 1). Relative sensitivity and specificity of the cell-ELISA compared to virus infectivity titration by CPE method was 97.26% and 97.22% respectively in a two-sided contingency table (Table 1).

PPR vaccine developed by IVRI is under commercial manufacture/production by Industries and Government Institutions. The presently developed cell-ELISA test serves as an alternate method to infectivity titration and at the same time proves identity of the PPR vaccine virus in sample. Identity test using PPR specific 4G6 monoclonal antibody based cell-ELISA in combination with conventional virus titration may some time avoid the routine potency testing of PPR vaccine batches, which involves animal inoculations. With the increasing pressure on quality of vaccines being manufactured in the developing countries, several of the manufacturers claim to follow Good Manufacturing Practices (GMP). This may not always be true, as there are several instances of vaccine safety failures in the field, majority of which are not publicized due to unknown reasons. The cell-ELISA test developed is likely to help in improving the quality of PPR vaccine by identity test and also quantitative infectivity titration. High degree of sensitivity and specificity (more than 97.0%) of newly developed cell-ELISA compared to conventional infectivity titration in microtitre plates indicates that, measurement of virus titration by microscopic observation can be replaced by cell-ELISA technique. In other way the cell-ELISA can be done after visual measurement in order to rectify mistakes, which may happen during microscopic observation. The anti-nucleocapsid protein monoclonal antibody selected for the purpose recognizes the most abundant viral protein of PPR virus in infected cells (Singh *et al.,* 2004a). This monoclonal antibody is being extensively used in the investgation of PPR specific outbreaks in India as a part of sandwich-ELISA kit (Singh *et al.,* 2004b). Subsequently sandwich-ELISA kit has been used for quick titration of PPR vaccine samples (Saravanan *et al*., 2008).

Further, cell-ELISA test helps in keeping a permanent record through a micro plate reader. Optical densities (A_{492}) in the cell-ELISA are indicative of the extent of replication of PPR virus in the specified well. Therefore the presently developed technique can lead the way for development of techniques to monitor the extent of growth of intracellular viruses in vitro, as in the case of viral interference and investigations relating to antiviral drugs. This approach can also be extended to monitor replication or adaptation of certain viruses like Classical swine fever virus and Bovine viral diarrhea virus which produce poor or no cytopathic effects in cell culture.

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REFERENCES

Reed, L. J. and Muench, H. (1938). *Amer. J. Hygiene*, **27:** 493.

Rossitter, P.B. and Jessett, D.M. (1982). *Res. Vet. Sci.,* **32:** 253-256.

Samuel, D., Megson, B., Strang, M. and Appleton, H. (2000). *J. Virol. Methods*, **90:** 125-133

Sarkar, J., Sreenivasa, B. P., Singh, R. P., Dhar, P. and Bandyopadhyay, S. K. (2003). *Vaccine*, 21, 4728-35

Saravanan, P., Sen, A., Balamurugan, V., Bandyopadhyay, S.K., Singh, R.K. (2008). *Biologicals*, **36:** 1-6.

Singh, R. P., Bandyopadhyay, S. K., Sreenivasa, B. P., Dhar, P. (2004a). *Vet. Res. Comm.*, **28:** 623-639

Singh, R.P., Sreenivasa, B.P., Dhar, P. and Bandyopadhyay, S.K. (2004b). *Arch. Virol.*, **149** (11): 2155-2170.

Singh, R.P., Saravanan , P., Sreenivasa , B.P., Singh, R.K. and Bandyopadhyay, S.K. (2004c). *Rev. sci. tech. Off. int. Epiz*., **23** (3): 807-819.

Sreenivasa, B. P., Dhar, P., Singh, R. P. and Bandyopadhyay, S. K. (2000). Abstract in proceedings of "XX annual conference of Indian association of veterinary microbiologists, immunologists and specialists in infectious diseases" October, pp84. Pantnagar.