Veterinary Research Communications, 31 (2007) 355–364
DOI: 10.1007/s11259-006-3442-x

Development of an Indirect ELISA for the Detection of Antibodies against Peste-des-petits-ruminants Virus in Small Ruminants

V. Balamurugan[1,*], R.P. Singh[2], P. Saravanan[1], A. Sen[1], J. Sarkar[1], B. Sahay[1], T.J. Rasool[1] and R.K. Singh[1]
[1]*Division of Virology, Indian Veterinary Research Institute, Mukteswar, Uttaranchal;*
[2]*Division of Biological Products, Indian Veterinary Research Institute, Izatnagar, Bareilly, India*
[*]*Correspondence: E-mail: balavirol@rediffmail.com; balavirol@email2me.net; balavirol@gmail.com*

ABSTRACT

Peste des petits ruminants (PPR) is an acute, febrile, highly contagious and economically important viral disease of small ruminants. A polyclonal antibody based indirect ELISA was developed for detection of antibodies to PPR virus in the serum samples of goats and sheep using purified PPR viral antigen propogated in Vero cell culture. A threshold (cut-off) value was set as twice the mean of the negative population based on the distribution of known negative serum samples in respect of PPR virus antibodies in the test. A total of 1544 serum samples from goats and sheep were screened by indirect ELISA and competitive ELISA. The indirect ELISA compared very well with competitive ELISA, with a high degree of specificity (95.09%) and sensitivity (90.81%). When compared with virus neutralization test, the present assay had 100% specificity and 80% sensitivity. With serum samples, the assay could clearly differentiate animals from the infected population from uninfected ones. These results suggest that the indirect ELISA may be a good alternative tool to competitive ELISA for seroepidemiological surveys.

Keywords: indirect-ELISA, peste des petits ruminants, antibody detection, small ruminants

Abbreviations: c-ELISA, competitive enzyme-linked immunosorbent assay; CPE, cytopathic effect; HRPO, horseradish peroxidase; OD, optical density; OPD, orthophenylenediamine; PEG, polyethylene glycol; PPR, peste des petits ruminants; VNT, virus neutralization test

INTRODUCTION

Peste des petits ruminants (PPR) is an acute, highly contagious and economically important viral disease of small ruminants, especially goats and sheep, with morbidity and mortality rates as high as 100% and 90%, respectively (Abu-Elzein *et al*., 1990). Clinically the disease resembles rinderpest and is characterized by severe pyrexia, oculonasal discharges, necrotizing and erosive stomatitis, enteritis and pneumonia (Diallo *et al.*, 1989; Jones *et al*., 1993; Ismail *et al*., 1995). The causative agent is PPR (PPRV)virus, a member of the genus *Morbillivirus* of the family *Paramyxoviridae* (Van Regenmortel *et al.*, 2000). PPR was first described in the Ivory Coast, West Africa (Gargadennec and Lalanne, 1942) and later spread widely across sub-Saharan Africa, the Arabian Peninsula and south-west Asia (Taylor, 1984). PPR was first reported in 1987 from Arasur village, Villupuram district Tamil Nadu (Shaila *et al*., 1989) and was subsequently reported in other parts of the country (Mondal

et al., 1995; Nanda *et al*., 1996), with a unique report in Indian buffalo (Govindarajan, *et al.*, 1997). Of the four known lineages of PPR virus, one (lineage 4) is restricted to Asia but the other lineages are prevalent in Africa (Shaila *et al*., 1996; Dhar *et al*., 2002).

PPR virus is antigenically closely related to other morbilliviruses, the closest being the rinderpest virus (Gibbs *et al*., 1979). Various diagnostic techniques have been applied for diagnosis of PPR in goats and sheep. These include agar gel immunodiffusion test (Durojaiye, 1982; Obi and Patrick, 1984), counterimmunoelectrophoresis, indirect fluorescent antibody test (Durojaiye, 1984; Durojaiye and Taylor, 1984), indirect ELISA (Obi *et al*., 1990), virus neutralization test (VNT) (Rossiter *et al.*, 1985) and monoclonal antibody-based competitive-ELISA(c-ELISA) (Anderson *et al*., 1991; Saliki *et al*., 1993; Libeau *et al*., 1995; Singh *et al*., 2004). Among these tests, VNT and ELISA are commonly employed for testing serum samples. VNT is laborious and is unsuitable for large numbers of serum samples because it requires tedious cell culture procedures and skilled personnel. Therefore, a diagnostic method that is simple, rapid, specific and sensitive is preferred over VNT for intensive surveillance. ELISA is a rapid, inexpensive and sensitive serological test that has been applied for the diagnosis of many diseases.

Recently, we developed the monoclonal antibody (anti-H protein of PPR virus) based competitive ELISA for serosurveillance and seromonitoring of PPR throughout the country (Singh *et al.*, 2004). However, the accidental loss of the monoclonal antibody-producing hybridoma clone owing to a laboratory accident or improper storage conditions (McCullough *et al.*, 1986) constitutes a limitation of the competitive assay. To accomodate such a situation in future, we have developed a polyclonal antibody-based indirect ELISA for the detection of PPR virus antibodies for serological survey of PPR using purified PPR viral antigen propogated in Vero cell culture (Singh *et al.*, 2004) from an attenuated PPR virus (Sreenivasa *et al*., 2000).

MATERIALS AND METHODS

Viruses

The Sungri 96 isolate attenuated (60th passage) in Vero cells–PPR vaccine virus (Sreenivasa *et al*., 2000) was used for the preparation of ELISA antigen. Data for VNT were generated while studying the experimental PPR vaccine (Sreenivasa *et al*., 2000).

Serum samples

Serum samples with VNT titre of $\leq$1:2 (n = 382: goat = 251; sheep = 131) and $\geq$1:8 (n = 309: goat = 252; sheep = 57) were considered negative and positive, respectively, based on VNT status with respect to PPR virus. The majority of positive serum samples (goat = 187) having VNT titre between 1:8 and 1:256 were obtained from experimental animals 21 days after vaccination with live-attenuated PPR vaccine. Serum samples (n = 122: goat = 65; sheep = 57) from non-vaccinated but naturally PPR virus-infected animals with a VNT titre of $\geq$1:8 were also included in the study. Post-challenge serum from a

PPR-vaccinated animal was used as strong positive control. Serum samples of unknown antibody status ($n = 853$: goat = 588; sheep = 265) from different geographical locations of the country obtained through various organizations including organized goat/sheep farms, the field, state animal husbandry laboratory and samples from small-herd owners submitted to the laboratory for PPR diagnosis were also included. Serum samples collected periodically (over a period of three years) from three PPR-vaccinated goats were also included in this study for seromonitoring. Samples came from different goat and sheep breeds originating from diverse geographic regions of India.

Antigen preparation

PPR virus antigen was prepared according to the methods used for the preparation of rinderpest and PPR antigens (Singh *et al*., 2000, 2004). Briefly, Vero cell cultures infected with PPR virus showing >80% CPE were harvested and frozen–thawed thrice. The cell debris was clarified by centrifugation at 1000 *g* for 15 min. The supernatant was subjected to precipitation using 8% (w/v) PEG 6000 in the presence of 2.3% (w/v) sodium chloride. The mixture was centrifuged at 8500 *g* for 30 min following overnight incubation at 4°C. The pellet was dissolved in TNE buffer (10 mmol/L Tris, 150 mmol/L NaCl, 1 mmol/L EDTA, pH 7.4) in one-tenth of the original volume. This partially purified antigen was lyophilized for long-term storage at −20°C and used in indirect ELISA after reconstitution with PBS (0.01 mol/L, pH 7.2).

VNT and c-ELISA

For the detection of neutralizing antibodies to PPR virus, a microassay was carried out as described by Bandyopadhyay and colleagues (1999) in Vero or B95a cells. VNT results were available in laboratory records (Sreenivasa *et al.*, 2000). The serum samples ($n = 770$) used in this experiment had already been tested by competitive ELISA (Singh *et al*., 2004). The remaining serum samples ($n = 774$) were tested according to the protocol described by Singh and colleagues (2004). Briefly, ELISA plates (NUNC Maxisorp, Hamburg, Germany) were coated with the PPR virus antigen (50 µl/well). After incubation, at 37°C for 1 h, the wells were washed three times with 0.002 mol/L phosphate-buffered saline(PBS). Next, all the wells of the plates received 40 µl of blocking buffer (PBS with 0.2% PPR-negative goat serum and 0.1% Tween 20). The test serum samples (20 µl) were added to duplicate sets of well followed by addition of 40 µl of monoclonal antibody in each well (except conjugate control wells) at a final dilution of 1:500. Anti-mouse–HRPO conjugate (Dako, Glostorp, Denmark) diluted 1:1000 in blocking buffer was added to each well (50 µl/well) after incubation. Finally, substrate solution (orthophenylene diamine, OPD) was added in each well and colour reaction was developed for 10 min before stopping the reaction with 1 mol/L H_2SO_4 and OD was measured at a wavelength of 492 nm.

Indirect ELISA

The indirect ELISA was carried out using partially purified attenuated PPR virus as coating antigen (in 50 µl volume) at 1:100 dilution (∼0.7 µg of antigen/well) in PBS in 96-well,

flat-bottomed, polystyrene microtitre plates (Maxisorp). The plates were incubated at 37°C for 1 h under constant orbital shaking for each step. After incubation, the wells were washed three times with washing buffer PBS-T (0.002 mol/L PBS containing 0.05% Tween 20) to remove unbound antigen and then the remaining sites in each well were blocked with 100 µl of blocking buffer (PBS-T containing 3% lactalbumin hydrolysate and 5% skim-milk powder). After incubation and washing of the plate, serum diluted in blocking buffer (1:200) was added in 50 µl volume and incubated. The anti-goat–HRPO conjugate (Dako) diluted in blocking buffer (1:1000) was added (50 µl/well) and incubated for 1 h at 37°C. Substrate solution (OPD 1 mg/ ml containing 4 µl 3% H_2O_2) was added in each well and the colour reaction was developed for 15 min before stopping the reaction with 1 mol/L H_2SO_4. The absorbance values were measured at a wavelength of 492 nm using EDI software developed by FAO(IAEA (Jeggo and Anderson, 1992).

Optimization of ELISA

A checkerboard titration was performed for optimization of working dilution of antigen and antibodies in indirect ELISA. The specific dilution of the PPR virus antigen and standard positive serum that induced approximately 75% absorbance (A_{492}) of the plateau was arbitrarily selected as described elsewhere (Saliki *et al*., 1993; Singh *et al*., 2000, 2004). For this purpose; the antigen, the reference serum samples from animals of various immune status (negative serum [VNT titre $\leq$1:2] from healthy or unvaccinated animals, and positive serum [VNT titre $\geq$1:128] from PPR vaccinated animals) were tested in twofold dilutions starting from 1:16 and 1:25, respectively. The antigen and serum dilutions that gave maximum difference in absorbance at 492 nm between positive and negative (P/N) were selected for testing the serum samples on larger scales. Test sera also included standard controls such as strong and weak positive and negative samples. These controls were based on the percentage inhibition (PI) values in c-ELISA. Optimized indirect ELISA was validated by detection of antibody in known positive (post-vaccinated $n = 187$) and negative (pre-vaccinated, $n = 382$) serum samples (on the basis of vaccination as well as VNT status) and serum samples from naturally infected animals ($n = 122$), before testing the field samples of unknown status.

Comparative efficacy of indirect ELISA with VNT and c-ELISA

The performance of the indirect ELISA was compared with VNT, the most reliable test for detection of morbillivirus antibodies (Rossiter *et al*., 1985) and c-ELISA, which is the currently employed test for serosurveillance of PPR throughout the country (Singh *et al*., 2004). The data from the laboratory records on VNT status of the serum samples ($n = 658$) and immune status of donor animals were used (Sreenivasa *et al*., 2000). Diagnostic sensitivity and specificity of indirect ELISA were calculated from various serum samples of known positive (vaccinated and naturally infected) and negative (healthy or pre-vaccinated) populations using a two-sided contingency table (Jacobson, 1998) in correlation with VNT and c-ELISA according to the methods described by Singh and colleagues (2004). The proportions of positive and negative samples detected, out of the known actual positive and negative samples, were taken as the sensitivity and specificity of the assay, respectively.

RESULTS AND DISCUSSION

PPR disease has a great potential to cause heavy economic losses to the livestock industry in Africa and Asia. In India, PPR is one of the main constraints affecting the productivity of small ruminants. Eradication of the disease depends on rapid and accurate diagnosis of infection and the implementation of prompt control measures. Because of the immense economic importance of the disease, there is a need for a battery of affordable and alternate tools for rapid diagnosis of PPR infection. Strict standards are necessary to ensure that the diagnostic tests used by field laboratories meet a minimum standard of diagnostic performance (Wright, 1998). Most field laboratories cannot afford the cell culture facilities that are indispensable for performing VNT. Epidemiological surveys of large number of serum sample using VNT are laborious and time-consuming. A rapid serological test suitable for open bench work would have obvious advantages. The indirect ELISA reported here could be a good alternative assay to c-ELISA for the detection of antibodies to PPRV and can successfully be applied for PPR seroepidemiological surveys.

To select a single dilution of test serum for use in the assay, it was necessary to titrate the serum over a twofold dilution series. This was done to determine whether there was an inhibitory effect at high serum concentration and also to determine the levels of non-specific activity present in negative serum. The optimal antigen dilution was established by titrating an antigen dilution series against a varying dilution of a positive serum (VNT titre $\geq$ 128). The results are shown in Figure 1, where maximum OD (75% plateau) reading was obtained between 1:64 and 1:128 antigen dilution with 1:200 dilution of antibody, after which OD decreased with the change in antigen concentration. The titration curve for known positive and negative sera obtained by measuring OD is shown in Figure 2. In this study, we established the highest dilution of positive serum that gave maximum OD

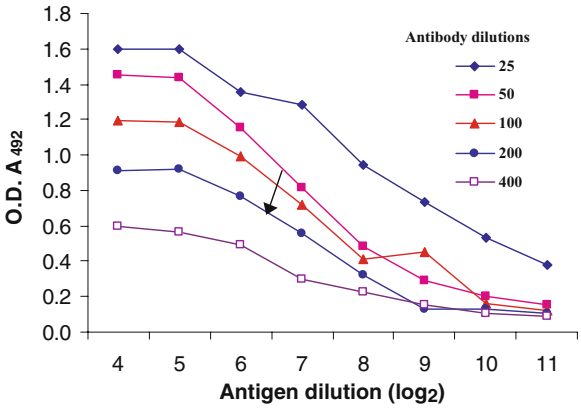

Figure 1. Reactivity of serially diluted positive serum with PPR antigen. Head of arrow indicates 75% absorbance (A_{492}) of the plateau, which corresponds to optimal working dilution of the antigen and antibody

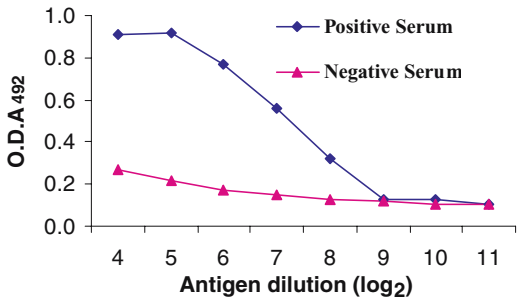

Figure 2. Reactivity of positive and negative sera at 1:200 dilution with serially diluted PPR antigen. The highest dilution of antibody that exhibited maximum difference between positive and negative samples (P/N differential) was used further for large-scale testing of serum samples

value difference over negative serum at the same dilution as the optimal working dilution of antigen and antibody in the ELISA

The 1:100 dilution of antigen and 1:200 dilution of standard positive serum, which corresponded to approximately 75% absorbance (A_{492}) of the plateau, could clearly differentiate positive and negative sera and showed maximum positive–negative (P/N) differential (Figure 2), and was arbitrarily chosen as the working dilution. Thus, 1:100 and 1:200 dilution of antigen and serum, respectively, were considered best suited for further testing and evaluation of the assay. Dilution of 1:200 standard positive serum (VNT titre $\geq$ 1:128) was found to give an OD value of 0.7 with a 1:100 dilution of purified viral antigen (75% absorbance plateau). The healthy/negative serum of goat/sheep (VNT titre $\leq$ 1:2) gave an OD value of 0.15 at the same dilution. Negative serum, even at a low dilution of 1:25, showed low OD (0.31) in the titration when compared to positive sera at 1:200 dilution, indicating the specificity of the test.

After optimization of the test reagents and protocols, samples of known status were used to determine cut-off values. For this purpose, 382 PPR-negative serum samples (goat and sheep) were tested and had a mean OD of 0.185 with a standard deviation (SD) of 0.079. As performance of the serological assays is reported to be improved by adding 2 to 3 times the standard deviations to the mean OD values of the negative controls (Jacobson, 1998), the cut-off value was adjusted to 0.345 (mean + 2 S.D.) or 0.37 (2 times mean). In this, we opted for a slightly higher cut-off value (2 times standard negative serum samples mean in each plate) to increase the specificity of the test without greatly compromising the sensitivity of the assay.

To confirm that the binding of the antibody to PPR antigen was specific, twofold serial dilution of a strong positive PPR serum were tested in indirect ELISA by dilution in known PPR-negative goat serum (titre $\leq$ 1:2) and tested at 1:200 working dilution in ELISA. The OD values decreased with the reduction in the quantity of anti-PPR virus antibody; with the strong positive serum dilution 1:6400 gave positive reaction (Figure 3) and the negative serum, even neat, gave negative reaction (OD value 0.22) in indirect ELISA, indicating the specificity. This shows that the binding is specific and is due to antibodies against PPR virus in the test sample.

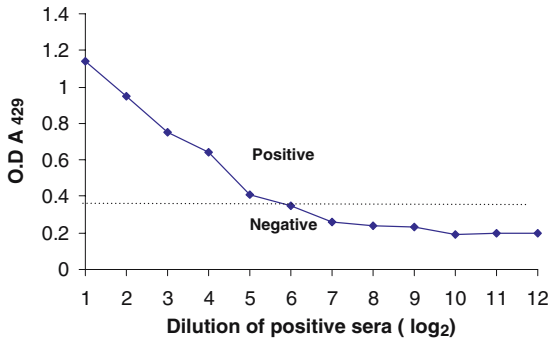

Figure 3. Reactivity of a convalescent serum at different dilutions in negative serum with the antigen. Dotted line shows positive/negative cut-off. OD value decreases with decrease in antibody concentration

The assay included standard controls (conjugate, strong positive, weak positive and negative control) to assess the quality of the assay, to ensure accuracy and to eliminate any plate-to-plate variation (Jacobson, 1998). The mean OD values of negative, strong positive and weak positive goat serum samples were 0.19 ± 0.07, 1.13 ± 0.09 and 0.75 ± 0.08, respectively. However, the sheep control panel samples showed little variation: 0.15 ± 0.05, 0.95 ± 0.08 and 0.49 ± 0.07, respectively. This could be due to the cross-reactivity of the anti-goat–HRPO conjugate with sheep antibodies, which is not complete. However, the overall mean OD values of samples were 0.174 ± 0.05, 1.02 ± 0.16 and 0.70 ± 0.18, respectively. The mean OD value of blank control reaction (reaction between convalescent goat PPR antibody and conjugate in the absence of virus–antigen) and conjugate control without antibody were 0.14 ± 0.05 and 0.121 ± 0.02, respectively. The OD values of all the standards used in this assay did not show much variation between the plates, indicating the accuracy of the assay. Obi and colleagues (1990) standardized indirect ELISA for titration of hyperimmune serum raised in rabbit using the Vero cell-adapted purified Nigerian PPR virus isolate and showed that 1:100 dilution of antiserum gave a positive signal only with the homologous PPR virus, although there were some cross-reactions with rinderpest, canine distemper and measles viruses.

In the present study, the performance of indirect ELISA in terms of relative sensitivity and specificity was compared with that of c-ELISA and VNT using a two-sided contingency table. Out of 1091 goat serum samples tested, 409 samples were found to be positive by indirect ELISA and compared very well with c-ELISA, with a high degree of specificity (94.39%) and sensitivity (91.09%). Similarly, out of 453 sheep serum samples tested, 105 samples were found to be positive and showed specificity (96.73%) and sensitivity (89.74%) compared with c-ELISA. The overall specificity and sensitivity of indirect ELISA were 95.09% and 90.81% and 100% and 80% when compared with c-ELISA and VNT, respectively (Tables I and II). The lower sensitivity of 80% when compared to VNT is due to the limited number of samples analysed. The low sensitivity of indirect ELISA is best explained by the maxim ‘diagnostic sensitivity of a test is inversely proportional to reduction in the specificity’ (Jacobson, 1998).

TABLE I
Relative specificity and sensitivity of indirect- ELISA compared with c-ELISA based on 1544 laboratory and field goat and sheep serum samples

	Indirect ELISA		
c-ELISA	Positive	Negative	Total
Positive	514	52	566
Negative	48	930	978
Total	562	982	1544

Relative specificity of indirect ELISA = 930 of 978, or 95.09%
Relative sensitivity of indirect ELISA = 514 of 566, or 90.81%

TABLE II
Relative specificity and sensitivity of indirect-ELISA compared with VNT based on 658 laboratory goats and sheep serum samples

	Indirect ELISA		
VNT	Positive	Negative	Total
Positive	216	54	270
Negative	0	388	388
Total	216	442	658

Relative specificity of indirect ELISA = 388 of 388, or 100%
Relative sensitivity of indirect ELISA = 216 of 270, or 80%

Serum samples collected periodically from three goats vaccinated with an experimental PPR vaccine were also tested in an indirect ELISA. Employing indirect ELISA, a steady increase in the antibody titre was observed after the first week in all three goats, which crossed the cut-off value (0.37) in the second week post vaccination and was maintained at this level throughout the observation period of 3 years (Figure 4). These serum samples were also tested by c-ELISA (Singh *et al.*, 2004); an increase in antibody titre was observed after one week and it was maintained at the same level throughout the observation period (unpublished data). For an effective control programme for any infectious disease, its detailed epidemiological characteristics must be studied by extensive clinical and serological surveillance before launch of the control programme. The test clearly differentiates infected from uninfected population and compared very well with c-ELISA. Using ELISA, large numbers of serum samples can be screened rapidly and economically. The indirect ELISA described here is an ideal alternative to c-ELISA for seroepidemiological surveys of PPR virus antibodies in small ruminants.

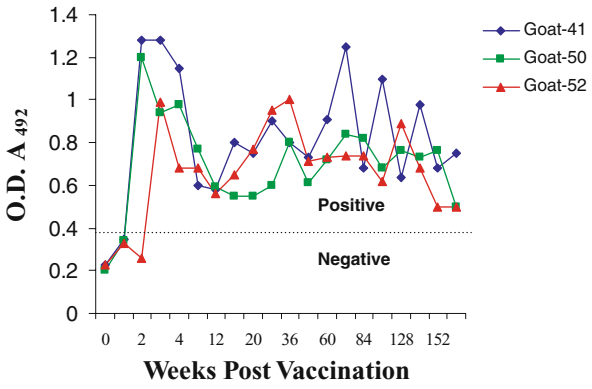

Figure 4. Seromonitoring of PPR-vaccinated animals (three goats) using indirect-ELISA over a period of 3 years. These goats received a single dose of vaccine. Dotted line shows negative/positive cut-off

ACKNOWLEDGEMENTS

The authors are grateful to the Director, Indian Veterinary Research Institute, for providing the necessary facilities to carry out this work. The help rendered by Dr C.C. Satheesh in data analysis is gratefully acknowledged. This study was partly funded by National Agricultural Technology Programme (NATP) launched by ICAR, Government of India, New Delhi, India.

REFERENCES

Abu-Elzein, E.M.E., Hassanien, M.M., Al-Afaleq AI, Abd-Elhadi, M.A. and Housain, F.M.I., 1990. Isolation of Peste-des-petits-ruminants virus from goats in Saudi Arabia. *The Veterinary Record*, **127**, 309–310

Anderson, J., McKay, J.A. and Butcher, R.N., 1991. The use of monoclonal antibodies in competition ELISA for detection of antibodies to rinderpest and peste des petits ruminants viruses. In: *The Sero-monitoring of rinderpest throughout Africa: Phase I*, (IAEA, Vienna), 43–53

Bandyopadhyay, S.K., Singh, R.P. and Chandra, U., 1999. Efficacy of the micro-method of the assessment of neutralizing antibodies following vaccination/infection with rinderpest virus. *Indian Journal of Animal Sciences*, **69**, 82–84

Dhar, P., Sreenivasa, B.P., Barrett, T., Corteyn, M., Singh, R.P. and Bandyopadhyay, S.K., 2002. Recent epidemilogy of peste-des-petits-ruminants virus (PPRV). *Veterinary Microbiology*, **88**, 153–159

Diallo, A., Barrett, T., Barbron, M., Shaila, M.S. and Lefevre, P.C., 1989. Differentiation of rinderpest and Peste-des-petits-ruminants viruses using specific cDNA clones. *Journal of Virological Methods*, **23**, 127–136

Durojaiye, O.A., 1982. Precipitating antibody in sera of goats naturally affected with peste des petits ruminants. *Tropical Animal Health Production*, **14**, 98–100

Durojaiye, O.A., 1984. Detection of the antigen of peste des petits ruminants virus in tissues by indirect immunoflurescence technique. *Nigerian Veterinary Journal*, **13**, 77–80

Durojaiye, O.A. and Taylor, W.P., 1984. Application of counter current immunoelectro-osmophoresis to the serology of peste des petits ruminants. *Revue d1 Elevage et de Médecine Vétérinaire des Pays Tropicaux*, **37**, 272–276

Gargadennec, L. and Lalanne, A., 1942. La peste des petits ruminants. *Bulletin des Services Zootechniques et des Epizooties de l'Afrique Occidentale Francaise*, **5**, 16–21

Gibbs, E.P., Taylor, W.P., Lawman, M.J.P. and Bryant, J., 1979. Classification of peste des petits ruminants virus as the fourth member of the genus Morbillivirus. *Intervirology*, **11**, 268–274

Govindarajan, R., Koteeswaran, A., Venugopalan, A.T., Shyam, G., Shaouna, S., Shaila, M.S. and Ramachandran, S., 1997. Isolation of pestes des petits ruminants virus from an outbreak in Indian buffalo (*Bubalus bubalis*). *The Veterinary Record*, **141**, 573–574

Ismail, T.M., Yamanaka, M.K., Saliki, J.T., EL-Kholy, A., Mebus, C. and Yilma, T., 1995. Cloning and expression of the nucleoprotein of Peste-des-petits-ruminants virus in baculovirus for use in serological diagnosis. *Virology*, **20**, 776–778

Jacobson, R.H., 1998. Validation of serological assays for diagnosis of infectious diseases. *Revue Scientifique et Technique de l'Office International des Epizooties*, **17**, 469–486

Jeggo, M.H. and Anderson, J., 1992. FAOIAEA external quality assurance programme for the FAOIAEA competitive ELISA results in 1992. In: *The Sero-monitoring of Rinderpest throughout Africa: Phase II*, (IAEA, Vienna), 9–21

Jones, L., Giavedoni, L., Saliki, J.T., Brown, C., Mebus, C. and Yilma, T., 1993. Protection of goats against peste-des-petits-ruminants with a vaccinia virus double recombinant expressing the F and H genes of rinderpest virus. *Vaccine*, **11**, 961–964

Libeau, G., Prehaud, C., Lancelot, R., Colas, F., Guerre, L., Bishop, D.H. and Diallo, A., 1995. Development of a competitive ELISA for detecting antibodies to the peste des petits ruminants virus using a recombinant nucleoprotein. *Research in Veterinary Science*, **58**, 50–55

McCullough, K.C., Sheshberadaran, H., Norrby, E., Obi, T.U. and Crowther, J.R., 1986. Monoclonal antibodies against morbilliviruses. *Revue Scientifique et Technique de l'Office International des Epizooties*, **5**, 411–427

Mondal, A.K., Chottopadhyay, A.P., Sarkar, S.D., Saha, G.R. and Bhowmik, M.K., 1995. Report on epizootiological and clinicopathological observations on peste des petits ruminants (PPR) of goats in west Bengal. *Indian Journal of Animal Health*, **34**, 145–148

Nanda, Y.P., Chatterjee, A., Purohit, A.K., Diallo, A., Innui, K., Sharma, R.N., Libeau, G., Thevasagayam, J.A., Bruning, A., Kiching, R.P., Anderson, J., Barrett, T. and Taylor, W.P., 1996. The isolation of peste des petits ruminants virus from Northern India. *Veterinary Microbiology*, **51**, 207–216

Obi, T.U., McCullough, K.C. and Taylor, W.P., 1990. The production of peste des petits ruminants hyperimmune sera in rabbits and their application in virus diagnosis. *Journal of Veterinary Medicine*, **37**, 345–352

Obi, T.U. and Patrick, D., 1984. The detection of peste des petits ruminants (PPR) virus antigen by agar gel precipitation test and counter-immunoelectrophoresis. *Journal of Hygiene*, **93**, 579–586

Rossiter, P.B., Jessett, D.M. and Taylor, W.P., 1985. Microneutralisation systems for use with different strains of peste des petits ruminants virus and rinderpest virus. *Tropical Animal Health Production*, **17**, 75–81

Saliki, J.T., Libeau, G., House, J.A., Mebus, C. A. and Dubovi, E. J., 1993. A monoclonal antibody-based blocking enzyme-linked immunosorbent assay for specific detection and titration of peste-des-petits-ruminants virus antibody in caprine and ovine sera. *Journal of Clinical Microbiology*, **31**, 1075–1082

Shaila, M.S., Purushothaman, V., Bhavasar, D., Venugopal, K. and Venkatesan, R.A., 1989. Peste-des-petits-ruminants of sheep in India. *The Veterinary Record*, **125**, 602

Shaila, M.S., Shamaki, D., Forsyth, M.A., Diallo, A., Groatley, L., Kitching, R.P. and Barrett, T., 1996. Geographic distribution and epidemiology of Peste-des-petits-ruminants viruses. *Virus Research*, **43**, 149–153

Singh, R.P., Sreenivasa, B.P., Dhar, P., Roy, R.N. and Bandyopadhyay, S.K., 2000. Development and evaluation of a monoclonal antibody based competitive enzyme-linked immunosorbent assay for the detection of the rinderpest virus antibodies. *Revue Scientifique et Technique de l'Office International des Epizooties*, **19**, 754–763

Singh, R.P., Sreenivasa, B.P., Dhar, P., Shah, L.C. and Bandyopadhyay, S.K., 2004. Development of a monoclonal antibody based competitive ELISA for detection and titration of antibodies to Peste-des-petits-ruminants (PPR) virus. *Veterinary Microbiology*, **98**, 3–15

Sreenivasa, B.P., Dhar, P., Singh, R.P. and Bandyopadhyay, S.K., 2000. Evaluation of an indigenously developed homologous live attenuated cell culture vaccine against Peste-des-petits-ruminants infection of small ruminants. *Proceedings of XX Annual Conference of Indian Association of Veterinary Microbiologists, Immunologists and Specialists in Infectious Diseases (IAVMI)*, Pantnagar, Uttaranchal, India, 84

Taylor, W.P., 1984. The distribution and epidemiology of peste des petits ruminants. *Preventive Veterinary Medicine*, **2**,157–166

Van Regenmortel, M.H.V., Fauquet, C. M. and Bishop, D.H.L., 2000. Virus taxonomy. *Seventh Report of the International Committee on Taxonomy of Viruses* (Academic Press, San Diego)

Wright, P.F., 1998. International standards for test methods and reference sera for diagnostic tests for antibody detection. *Revue Scientifique et Technique de l'Office International des Epizooties*, **17**(2), 527–533

(Accepted: 18 October 2005; Published online: 23 December 2006)