Journal of Animal and Veterinary Advances 11 (13): 2316-2322, 2012
ISSN: 1680-5593

Construction and Characterization of an Expression Plasmid Containing Peste des Petits Ruminants Virus Phosphoprotein Gene

J.J. Zhai, Y.X. Dou, X.N. Luo and X.P. Cai
State Key Laboratory of Veterinary Etiological Biology,
Key Laboratory of Veterinary Parasitology of Gansu Province,
Lanzhou Veterinary Research Institute,
Chinese Academy of Agricultural Sciences, 730046 Lanzhou, China

Abstract: The Phosphoprotein (P) of genus *Morbillivirus* in the family Paramyxoviridae is an essential component of the viral RNA Polymerase complex (P-L) and is thought to mediate the interaction of the large protein (L) with the viral nucleocapsid template for transcription and replication. To date, the precise role of Peste des Petits Ruminants Virus (PPRV) P is unclear. To establish its function in the present study, the encoding sequence of PPRV P was obtained by RT-PCR and then inserted into the multiple cloning site of pcDNA3.1 vector. The constructed recombinant plasmid was successfully transfected into the Vero cell as confirmed by indirect immunofluorescence assay, SDS-PAGE and Western blotting. Then, the transfected cells were inoculated by PPRV. The results showed that the constructed plasmid which was designated pcDNA 3.1/P could be expressed in Vero cells. After inoculation with PPRV, Cytopathic Effects (CPE) have been detected in the transfected cells by plasmid pcDNA3.1/P a day earlier than in cells of the control group. In conclusion, the results showed that the phosphoprotein did accelerate the virus replication; this would be very useful for further study of P protein functions during PPRV transcription and replication.

Key words: Peste des petits ruminants virus, phosphoprotein, expression plasmid, functions, replication, China

INTRODUCTION

Peste des Petits Ruminants (PPR) is an acute highly contagious viral disease of small ruminants caused by Peste des Petits Ruminants Virus (PPRV) (Balamurugan *et al*., 2006) and is notifiable to the Office International des Epizooties. PPR has a widespread distribution that spans West and Central Africa, Arabia, the Middle East and Southern Asia (Dhar *et al*., 2002; Ozkul *et al*., 2002; Kwiatek *et al*., 2007; Kerur *et al*., 2008). The occurrence of the disease is associated with high morbidity and mortality in susceptible animals (Abu-Elzein *et al*., 1990). PPRV is single-stranded, negative-sense RNA virus that belongs to the genus *Morbillivirus* within the family Paramyxoviridae (Kwiatek *et al*., 2010). The genome encodes eight proteins: Nucleocapsid protein (N), Phosphorprotein (P), Matrix protein (M), Fusion protein (F), Hemagglutinin protein (H), polymerase protein (L) and two non-structural proteins, C and V (Bailey *et al*., 2005; Muthuchelvan *et al*., 2006; Yadav *et al*., 2009).

The P is an essential component of the viral replication machinery among the genus *Morbillivirus* (Fuentes *et al*., 2010) which include human measles virus, Rinderpest Virus (RPV), PPRV, canine and phocid distemper virus. The P in association with the polymerase protein L. plays important roles in replication and transcription of the negative strand genome (Chattopadhyay and Shaila, 2004; Saikia *et al*., 2008). In addition, a common feature of paramyxoviruses *P* gene is the synthesis of two virally encoded nonstructural proteins C and V by post-transcriptional processing (Mahapatra *et al*., 2003). C protein is generated from the translation of a different Open Reading Frame (ORF) and generation of V protein requires the insertion of an additional non-templated G residue after the run of three G residues at position 751 in a conserved editing site. V protein is thought to attenuate viral mRNA synthesis and to affect viral pathogenicity (Escoffier *et al*., 1999; Wakasa *et al*., 2000). In addition, the *P* gene of RPV is a major determinant of species-specific pathogenicity (Yoneda *et al*., 2004).

Corresponding Author: X.P. Cai, State Key Laboratory of Veterinary Etiological Biology, Key Laboratory of Veterinary Parasitology of Gansu Province, Xujiaping Road, Yanchangbu, Lanzhou, 730046 Gansu, China

The *P* gene of PPRV is 1655 nucleotide long starts with the conserved gene start of morbilliviruses (AGGR) and ends with TTACAAAAAA followed by the intergenic trinucleotide CTT at the P/M intergenic junction. Similarly, the trinucleotide CTT was also present at N/P intergenic region. The P ORF starts at position 60 and ends at 1589, leaving 66 nucleotides at the 3' end (Muthuchelvan *et al*., 2006).

While, the P of all paramyxoviruses are essential for viral RNA synthesis and are heavily phosphorylated (hence the name P protein), the precise role of PPRV P remains unclear. It has been proposed that phosphorylation of P protein plays a critical role in Morbillivirus RNA synthesis. During the course of paramyxoviruses replication, RNA is tightly encapsidated by L to form N-RNA complex (Finch and Gibbs, 1970; Lamb and Choppin, 1976; Saikia *et al*., 2008). The N-RNA serves as a template for both mRNA synthesis and genome replication by the viral RNA dependent RNA polymerase consisting of L and P. In the process, P interacts with the N protein maintaining it in a form that is competent to support efficient RNA encapsidation during replication (Masters and Banerjee, 1988; Curran *et al*., 1994). It also interacts with the L protein, stabilizes it against proteolytic degradation (Curran *et al*., 1994; Canter and Perrault, 1996; Chattopadhyay and Shaila, 2004) and places the polymerase complex on N-RNA template as L alone is unable to interact with the N-RNA. This interaction depends on phosphorylation of P protein; otherwise they are incapable of interacting with each other. But, conclusive evidence supporting this notion is lacking in PPRV. Furthermore, a previous study has shown that bacterially expressed P protein is un-phosphorylated (Kaushik and Shaila, 2004), so the purpose of this study was to construct a eukaryotic expression plasmid containing PPRV *P* gene and then to study the function of phosphoprotein during the virus replication. This research would form a basis for further studies, especially for the function and mechanism of P in the course of PPRV transcription and replication.

MATERIALS AND METHODS

Amplification of the *P* gene: According to the complete genome of PPRV Nigeria 75/1 strain (Gen Bank Accession No. X 74443), PCR primers were designed using the software Oligo 6.0 and synthesized by Sangon Company (Shanghai, China): Forward primer 5'-CGGCTAGCGCCA CCATGGCAGAAGAACAAG-3' (NheI site underlined, Kozak consensus sequence in italics); Reverse primer 5'-ATTTGGGCCCATGTTGATTATAGGATGTG-3' (ApaI site underlined). PPRV Nigeria 75/1 vaccine strain (provided by Professor Hai-bing Zhi from China Institute of Veterinary Drugs Control) was used to infect Vero cells (an African green monkey kidney cell line) (obtained from the Institute of Biochemistry and Cell Biology, SIBS CAS). The virus RNA was extracted from infected cells using the Rneasy Mini kit according to the manufacturer's instructions (Qiagen, Hilden, Germany) and retro-transcribed with the PrimeScript II 1st Strand cDNA Synthesis kit (Takara, Beijing, China). cDNA was amplified with PrimeStar® HS DNA Polymerase (Takara) using the before mentioned primers with the following reaction conditions: pre-degeneration at 96°C for 5 min then 98°C for 10 sec, 60°C for 15 sec and 72°C for 2 min and 30 cycles. The PCR products were subjected to electrophoresis in 1% agarose gel and a 1.53 kb DNA fragment was retrieved.

Construction of the pcDNA3.1/P vector and its sequencing: The pcDNA3.1 vector (Clontech, USA) and retrieved DNA fragment were digested by NheI/ApaI endonucleases, respectively. The PCR fragment of *P* gene and the pcDNA3.1 vector were mixed at a 3:1 ratio and ligated at 4°C. The ligated product was transformed into *Escherichia coli* DH5a and the transformed bacteria were screened by Kan+plate. Plasmid extracted from the screened bacteria was identified by NheI/ApaI double digestion. The positive recombinant was sequenced by Sangon Company and the correct plasmid was named pcDNA3.1/P.

Transfection of Vero cells with pcDNA 3.1/P: Vero cells were cultured in Dulbecco's Minimal Essential Medium (DMEM) (Hyclone, USA) supplemented with 10% Fetal Calf Serum (FCS; Hyclone), 100 U mL^{-1} penicillin, 100 mg mL^{-1} streptomycin and 2 mM L-glutamine at 37°C in 5% CO_2. Vero cells were cultured in DMEM supplemented with 8% FCS. Recombinant plasmids were purified from *E. coli* DH5a using the Plasmid Mini kit (Qiagen). Plasmid DNA was diluted to the required concentration in Phosphate Buffered Saline (PBS) (Hyclone). After cells were grown to reach 80% confluency on 6 well tissue culture plates, they were transfected with 2 μg recombinant plasmid in 10 μL Lipofectamine™ 2000 (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. The transfected cells were harvested at 48 h post-transfection for analysis of the P protein presence.

Detection of phosphoprotein mRNA expression by Reverse Transcription-Polymerase Chain Reaction (RT-PCR): Levels of the mRNA transcripts encoding *P* and *β-actin* (housekeeping gene, internal control) genes were determined by RT-PCR, β-actin forward primer: 5'-GTGAGAAGATGACCCAGAT-3'; β-actin reverse primer: 5'-TTGA AGGTAGTTTCGTGAAT-3'.

Total RNA was extracted using Trizol reagent (Promega, USA). Single stranded cDNAs were synthesized from total RNA using cDNA synthesis kit (Takara). RT-PCR was carried out with specific primers for *P* gene. The PCR products were separated by 1.0% agarose gel electrophoresis with a Division of Synoptics Ltd. (Syngene, UK).

Detection of phosphoprotein expression with indirect immunofluorescence assay: Vero cells were grown on glass coverslips in 6 well plates. Cells were grown to reach 80% confluency before transfection. At 48 h post-transfection, transfected cells were fixed in ice-cold acetone for 15 min at -20°C. After washing 5 times with PBS, coverslips were incubated with a 1:200 dilution of polyclonal mouse anti-P immunoglobulin (produced in the State Key Laboratory of Veterinary Etiological Biology, LVRI, CAAS) (Zhai *et al*., 2010) for 1 h at 37°C. After washing with PBS 5 times, goat anti-mouse IgG-FITC antibody (1:128) (Sigma-Aldrich, St Louis, MO, USA) was applied onto the samples and incubated for 1 h at 37°C. After washing with PBS 5 times, the slides were visualized with an SZ61 microscope (Olympus, Japan).

Detection of phosphoprotein expression with Western Blotting (WB): About 8 h after transfection, cells were washed 3 times using cold PBS (Hyclone). Total cell extracts were prepared by lysis in cold RIPA buffer (Sigma) and centrifuged for 5 min at 10,000 g to remove cell debris. Protein concentrations were determined using the BCA Protein Assay kit (Thermo Scientific Pierce, USA). Equal amount of samples (10 μg lane^{-1}) were separated by 12% SDS-PAGE and electrically transferred to a Polyvinylidene Fluoride membrane (Hybond-P; Amersham Biosciences, Uppsala, Sweden). Mouse anti-PPRV-P serum (produced at State Key Laboratory of Veterinary Etiological Biology, Lanzhou, China) and monoclonal anti-actin antibody (Sigma-Aldrich) was diluted to 1:400 and secondary HRP-conjugated anti-mouse antibody (Sigma-Aldrich) was diluted to 1,8000 and was stained with Diaminobenzidine (DAB) Staining kit (Beijing CellChip Biotechnology Co., Ltd., China).

Inoculation of PPRV and observation of Cytopathic Effect (CPE): Identification of the preliminary function of phosphoprotein at 48 h post-transfection was done. Vero cells were washed 3 times using 2 mL PBS (Hyclone) and then 100 Plaque Forming Units (PFU) of PPRV Nigeria 75/1 strain were inoculated into 6 well plates and incubated in DMEM containing 2% FCS at 37°C, 5% CO_2. A group of non-transfected cells was inoculated as positive control and another group of non-transfected cells was left without inoculation as negative control. At 0-7 days after inoculation, CPE were visualized with an SZ61 microscope (Olympus, Japan).

RESULTS AND DISCUSSION

Construction of pcDNA3.1/P vector and sequence analysis: The electrophoresis detection result showed that the whole ORF of *P* gene was amplified which was accorded with the expectation. To verify plasmids extracted from the screened bacteria, they were identified by NheI/ApaI double digestion and electrophoresis on 1% agarose gel; two fragments of about 5.4 and 1.5 kb were detected. The fragment of 5.4 kb was in accord with pcDNA3.1 and the other fragment of 1.5 kb was in accord with PPRV *P* gene by RT-PCR. This demonstrated that the PPR *VP* gene was inserted into pcDNA3.1 (Fig. 1 and 2) and the resulting plasmid was named

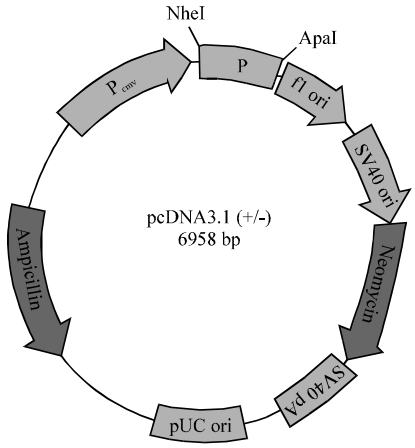

Fig. 1: Physical map of recombinant

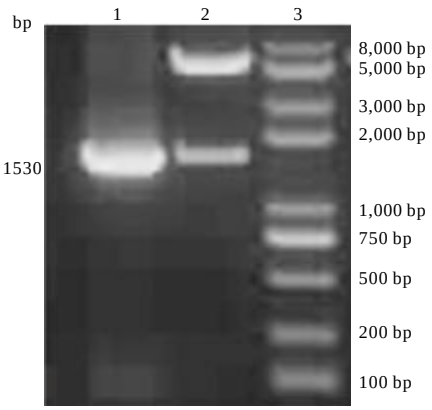

Fig. 2: Identification of recombinant plasmid by PCR and enzyme digestion. Lane 1: Marker DL 2000; Lane 2: PCR product of pcDNA3.1/P; Lane 3: Recombinant plasmid by enzyme digestion

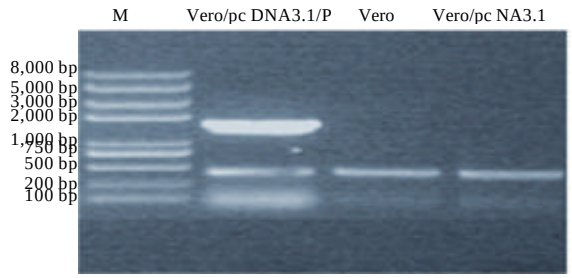

Fig. 3: The *P* gene was successfully transcribed in transfected cells with pcDNA3.1/P which could express P when compared with the cells transfected with plasmid pcDNA 3.1 and non-transfected cells

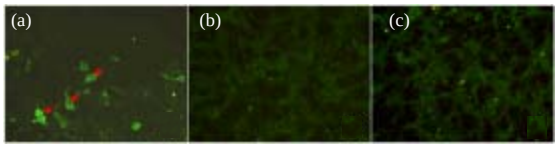

Fig. 4: Vero cells successfully transfected and the targeted protein successfully transfected and the targeted protein expressed in the cell

pcDNA3.1/P. At the same time, BLAST search analysis revealed that the amplified gene sequence had 100% homology with the referenced sequence.

Phosphoprotein expression in Vero cells: The level of mRNA in the cell reflects the level of transcription, therefore the mRNA level of the P in the transfected cells was analysed by RT-PCR. RT-PCR results (Fig. 3) demonstrated that the *P* gene was successfully transcribed in transfected cells with pcDNA3.1/P which could express P when compared with the cells transfected with plasmid pcDNA3.1 and non-transfected cells. In order to detect phosphoprotein expression, indirect immunofluorescence results confirmed that Vero cells were successfully transfected and the targeted protein can be expressed in the cell (Fig. 4). In addition for further confirmation of protein expression, SDS-PAGE and WB were applied (Fig. 5 and 6). In lysates of cells transfected with plasmid pcDNA3.1/P, a protein of about 80 kDa reacted with anti-PPRV-P specific serum in WB. There was no protein that corresponded to this in cell lysates from control cells (Fig. 7).

Observation of CPE: For detection of biological activity and function, the CPE was observed after inoculation. There were no significant changes in cells of the 3 group at 2 days after inoculation. In the 3rd day after

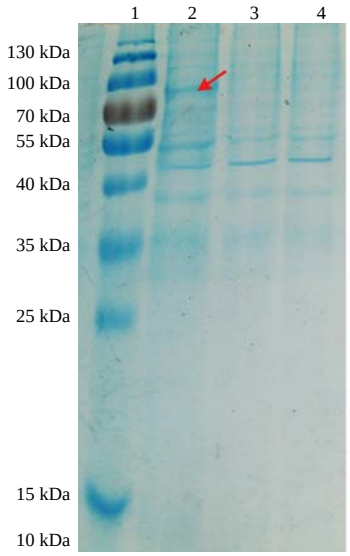

Fig. 5: For confirmation of protein expression, SDS-PAGE and WB were applied

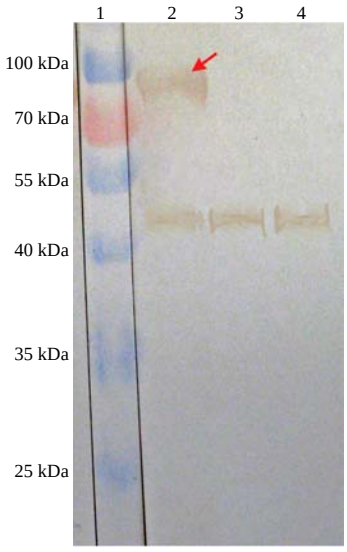

Fig. 6: In lysates of cells transfected with plasmid pcDNA3.1/P, a protein of about 80 kDa reacted with anti-PPRV-P specific serum in WB

infection, cells in the experimental group begin to clump, balloon and form syncytia while cells in the other groups have no significant changes. In the cells of positive control, on the 4th day, CPE begin to appear. On further incubation, CPE were up to 80% in the experimental group in the 5th day while in the 7th day, CPE were up to 80% in the positive control group. However, the cells in negative control group never formed CPE (Fig. 7). This demonstrated that phosphoprotein could accelerate virus replication.

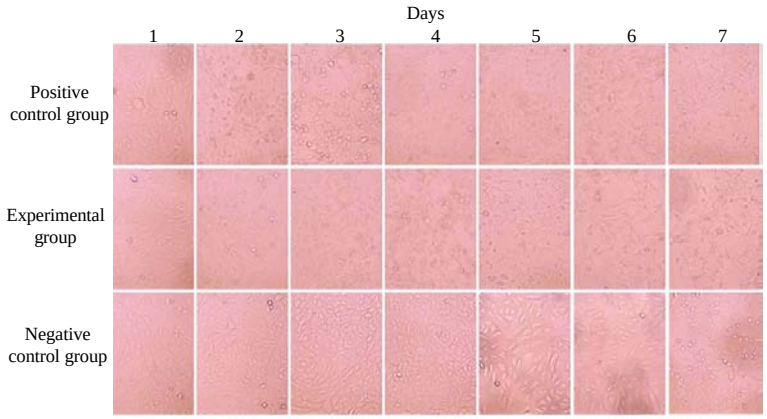

Fig. 7: The result of observed CPE in every group (20×20)

About 2 days before, the change of cells is not obvious but in the 3rd day after infection, cells in the experimental group begin to clump, balloon and form syncytia while cells in the other groups have no significant changes. For the cells of positive control, on the 4th day, CPE begin to appear. Along with the extending of time, on the 5th day, CPE were up to 80% in the experimental group while on the 7th day, CPE were up to 80% in the positive control group in this course, the cells in negative control group never formed CPE.

To contribute to the study of the complex structure and multiple functions of the PPRV P protein, a stable expression system was established. The pcDNA3.1-Vero cell system was chosen because it combines several important features:

- Plasmids bearing the SV40 origin produce components that can be reproduced within host cells which ensures stable transfer of the gene of interest
- Insertion of vectors pUC and pCMV upstream of the polyclonal site which can express the inserted gene fragment efficiently
- The neomycin resistance gene, allows cells containing this plasmid to be screened by G418. These features help to establish the cell line with stable expression of PPRV *P* gene
- The Vero cell line is the sensitive cell line for PPRV (Chaudhary *et al*., 2009) and PPRV is usually isolated and cultured in Vero cells

In the present study, the PPRV P protein from transfected cells migrated as an 80 kDa protein on SDS-PAGE and Western blot, although its calculated molecular weight was 55 kDa. By means of WB, researchers found that the protein could be identified by the specific antibody of the object protein which demonstrated that the object protein was expressed in the Vero cells. In addition, the aberrant mobility of P of negative-sense RNA viruses has been reported previously (Diallo *et al*., 1987; Emerson and Schubert, 1987; Huber *et al*., 1991). The reason is that post-translational phosphorylation of the P protein *in vivo* can significantly alter its mobility on polyacrylamide gels. In order to evaluate the activity and verify the function of the expressed object protein, after the Vero cells were infected with PPRV, researchers demonstrated CPE in the cells transfected by plasmid pcDNA3.1/P a day earlier than in the cells of the positive group and the CPE process was shortened. This is a very interesting finding as far as PPRV is concerned. It is well known that P plays important roles in replication and transcription of the negative strand genome (Chattopadhyay and Shaila, 2004; Saikia *et al*., 2008).

From the present study, it is clearly evident that the transfected cells provided phosphoprotein for PPRV replication by protein expression and the expressed object proteins could accelerate virus replication. The exact reason behind this and mechanisms involved therein, await further studies.

CONCLUSION

In this study, researchers have successfully cloned PPRV *P* gene and constructed a eukaryotic expression vector which will facilitate further research into the function of P. Moreover, Vero/P cells were successfully constructed. In addition, researcher briefly studied the function of phosphoprotein using the constructed cells; the preliminary results showed that it could accelerate the virus replication. To the best of the knowledge, this is the 1st report and is very useful for further studies, especially of the function and kinetics of P in the course of PPRV transcription and replication *in vitro*.

ACKNOWLEDGEMENTS

Researchers are grateful to all the anonymous reviewers for the amendments and comments on this manuscript. This research was supported by grants from the earmarked fund for Modern Agro-industry Technology Research System (CARS-40-10) and Special Fund for Agro-Scientific Research in the Public Interest (200803018).

REFERENCES

Abu-Elzein, E.M., M.M. Hassanien, A.I. Al-Afaleq, M.A. Abd-Elhadi and F.M. Housawi, 1990. Isolation of peste des petits ruminants from goats in Saudi Arabia. Vet. Rec., 127: 309-310.

Bailey, D., A. Banyard, P. Dash, A. Ozkul and T. Barrett, 2005. Full genome sequence of peste des petits ruminants virus, a member of the Morbillivirus genus. Vet. Res., 110: 119-124.

Balamurugan, V., A. Sen, P. Saravanan, R.P. Singh, R.K. Singh, T.J. Rasool and S.K. Bandyopadhyay, 2006. One-step multiplex RT-PCR assay for the detection of peste des petits ruminants virus in clinical samples. Vet. Res. Commun., 30: 655-666.

Canter, D.M. and J. Perrault, 1996. Stabilization of vesicular stomatitis virus L polymerase protein by P protein binding: A small deletion in the C-terminal domain of L abrogates binding. Virology, 219: 376-386.

Chattopadhyay, A. and M.S. Shaila, 2004. Rinderpest virus RNA polymerase subunits: Mapping of mutual interacting domains on the large protein L and phosphoprotein p. Virus genes., 28: 169-178.

Chaudhary, S.S., K.D. Pandey, R.P. Singh, P.C. Verma and P.K. Gupta, 2009. A vero cell derived combined vaccine against sheep pox and Peste des Petits ruminants for sheep. Vaccine, 27: 2548-2553.

Curran, J., T. Pelet and D. Kolakofsky, 1994. An acidic activation-like domain of the Sendai virus P protein is required for RNA synthesis and encapsidation. Virology, 202: 875-884.

Dhar, P., B.P. Sreenivasa, T. Barrett M. Corteyn R.P. Singh and S.K. Bandyopadhyay, 2002. Recent epidemiology of Peste des Petits Ruminants Virus (PPRV). Vet. Microbiol., 88: 153-159.

Diallo, A., T. Barrett, P.C. Lefevre and W.P. Taylor, 1987. Comparison of proteins induced in cells infected with rinderpest and peste des petits ruminants viruses. J. Gen. Virol., 68: 2033-2038.

Emerson, S.U. and M. Schubert, 1987. Location of the binding domains for the RNA polymerase L and the ribonucleocapsid template within different halves of the NS phosphoprotein of vesicular stomatitis virus. Proc. Natl. Acad. Sci. USA., 84: 5655-5659.

Escoffier, C., S. Manie, S. Vincent, C.P. Muller, M. Billeter, D. Gerlier, 1999. Nonstructural C protein is required for efficient measles virus replication in human peripheral blood cells. J. Virol., 73: 1695-1698.

Finch, J.T. and A.J. Gibbs, 1970. Observations on the structure of the nucleocapsids of some paramyxoviruses. J. Gen. Virol., 6: 141-150.

Fuentes, S.M., D. Sun, A.P. Schmitt and B. He, 2010. Phosphorylation of paramyxovirus phosphoprotein and its role in viral gene expression. Future Microbiol., 5: 9-13.

Huber, M., R. Cattaneo, P. Spielhofer, C. Orvell and E. Norrby *et al*., 1991. Measles virus phosphoprotein retains the nucleocapsid protein in the cytoplasm Virology, 185: 299-308.

Kaushik, R. and M.S. Shaila, 2004. Cellular casein kinase II-mediated phosphorylation of rinderpest virus P protein is a prerequisite for its role in replication/transcription of the genome. J. Gen. Virol., 85: 687-691.

Kerur, N., M.K. Jhala and C.G. Joshi, 2008. Genetic characterization of Indian peste des petits ruminants virus (PPRV) by sequencing and phylogenetic analysis of fusion protein and nucleoprotein gene segments. Res. Vet. Sci., 85: 176-183.

Kwiatek, O., C. Minet, C. Grillet, C. Hurard and E. Carlsson *et al*., 2007. Peste des Petits Ruminants (PPR) outbreak in Tajikistan. J. Comp. Pathol., 136: 111-119.

Kwiatek, O., D. Keita, P. Gil, J. Fernandez-Pinero, M.A.J. Clavero, E. Albina and G. Libeau, 2010. Quantitative one-step real-time RT-PCR for the fast detection of the four genotypes of PPRV. J. Virol. Methods, 165: 168-177.

Lamb, R.A. and P.W. Choppin, 1976. Synthesis of influenza virus proteins in infected cells: Translation of viral polypeptides, including three P polypeptides, from RNA produced by primary transcription. Virology, 74: 504-519.

Mahapatra, M., S. Parida, B.G. Egziabher, A. Diallo and T. Barrett, 2003. Sequence analysis of the phosphoprotein gene of peste des petits ruminants (PPR) virus: Editing of the gene transcript. Virus Res., 96: 85-98.

Masters, P.S. and A.K. Banerjee, 1988. Complex formation with vesicular stomatitis virus phosphoprotein NS prevents binding of nucleocapsid protein N to nonspecific RNA. J. Virol., 62: 2658-2664.

Muthuchelvan, D., A. Sanyal, J. Sarkar, B.P. Sreenivasa and S.K. Bandyopadhyay, 2006. Comparative nucleotide sequence analysis of the phosphoprotein gene of peste des petits ruminants vaccine virus of Indian origin. Res. Vet. Sci., 81: 158-164.

Ozkul, A., Y. Akca, F. Alkan, T. Barrett and T. Karaoglu *et al*., 2002. Prevalence, distribution and host range of *Peste des petits ruminants* virus, Turkey. Emerging Infect. Dis., 8: 708-712.

Saikia, P., M. Gopinath and M.S. Shaila, 2008. Phosphorylation status of the phosphoprotein P of rinderpest virus modulates transcription and replication of the genome. Arch. Virol., 153: 615-626.

Wakasa, C., K. Iwatsuki, K. Ohashi, K. Nakamura and C. Kai, 2000. Sequence analysis of the genes encoding the phosphoprotein of recent isolates of canine distemper virus in Japan. J. Vet. Med. Sci., 62: 97-101.

Yadav, V., V. Balamurugan, V. Bhanuprakash, A. Sen and V. Bhanot *et al*., 2009. Expression of *Peste des petits ruminants* virus nucleocapsid protein in prokaryotic system and its potential use as a diagnostic antigen or immunogen. J. Virol. Methods, 162: 56-63.

Yoneda, M., R. Miura, T. Barrett, K. Tsukiyama-Kohara and C. Kai, 2004. Rinderpest virus phosphoprotein gene is a major determinant of species-specific pathogenicity. J. Virol., 78: 6676-6681.

Zhai, J.J.D.Y., H.R. Zhang, L. Mao, X.L. Meng, Q.X. Wang, X.N. Luo and X.P. Cai, 2010. Expression, purification and polyclonal antibody preparation and preliminary application of phosphoprotein of *Peste des petits ruminants* virus. Acta Vet. Zootech. Sin., 4: 995-1000.