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SHORT COMMUNICATION

Comparative sequence analysis of double stranded RNA binding protein encoding gene of parapoxviruses from Indian camels

G. Nagarajan *, Shelesh Kumar Swami, Shyam Singh Dahiya, G. Sivakumar, F.C. Tuteja, S.D. Narnaware, S.C. Mehta, Raghvendar Singh, N.V. Patil

National Research Centre on Camel, Post Bag No. 7, Jorbeer, Bikaner 334 001, Rajasthan, India

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ABSTRACT

The dsRNA binding protein (RBP) encoding gene of parapoxviruses (PPVs) from the Dromedary camels, inhabitating different geographical region of Rajasthan, India were amplified by polymerase chain reaction using the primers of pseudocowpoxvirus (PCPV) from Finnish reindeer and cloned into pGEM-T for sequence analysis. Analysis of RBP encoding gene revealed that PPV DNA from Bikaner shared 98.3% and 76.6% sequence identity at the amino acid level, with Pali and Udaipur PPV DNA, respectively. Reference strains of *Bovine papular* stomatitis virus (BPSV) and PCPV (reindeer PCPV and human PCPV) shared 52.8% and 86.9% amino acid identity with RBP gene of camel PPVs from Bikaner, respectively. But different strains of orf virus (ORFV) from different geographical areas of the world shared 69.5–71.7% amino acid identity with RBP gene of camel PPVs from Bikaner. These findings indicate that the camel PPVs described are closely related to bovine PPV (PCPV) in comparison to caprine and ovine PPV (ORFV).

Introduction

Pseudocowpox virus (PCPV, previously known as parapoxvirus bovis II) is one of the two parapoxviruses (PPVs) of cattle, along with *Bovine papular stomatitis virus* (BPSV, previously known as parapoxvirus bovis I). Two other virus species, namely *orf virus* of sheep and goats (ORFV, previously known as parapoxvirus ovis) and *parapoxvirus of red deer in New Zealand* (NZPV), complete the genus *parapoxvirus* within the subfamily *Chordopoxvirinae* of the family *Poxviridae* [1]. Parapoxviruses are epitheliotropic viruses identified throughout the world as causing nonsystemic, vesicular, and eruptive skin disease in domestic and wild mammals, especially ruminants [2]. Individual PPV species usually display a narrow host range yet are occasionally transmitted to human beings, causing localised lesions on the hands [3].

In Indian subcontinent, contagious ecthyma is a major exanthematous skin infection of the Dromedary camels (*Camelus dromedarius*) and is caused by pseudocowpoxvirus [4]. This disease usually occurs during and immediately after monsoon season in Indian camels.

* Corresponding author. Tel.: +91 151 2230183; fax: +91 151 2231213.
E-mail address: camelnag@yahoo.com (G. Nagarajan).

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It has become apparent that the survival of poxviruses in the presence of an active immune response is caused in large part, if not solely, by the expression of virus virulence genes that interfere with host immune and inflammatory response effector molecules. Many of these are viral orthologues of host cellular genes that have been acquired and modified by the viruses. The protein products of these genes, in general, target effector molecules of the early phase of the host antiviral inflammatory and immune response, including interferons, complement and the cytokines interleukin-lβ and tumour necrosis factor-alpha [5].

Recently, several putative immune-modulating virulence genes have been discovered within the reindeer PCPV genome. These include viral homologues of ovine vascular endothelial growth factor, interleukin-10 (IL-10) interferon-resistance gene [6]. Interferon-resistance gene is otherwise called as double stranded (ds) RNA binding protein gene (RBP) as this gene encoded proteins inhibit PKR (dsRNA dependent kinase) by competing with the enzyme for dsRNA binding and acting as a decoy for eIF-2 respectively [7].

The interferon-resistance gene (ORF 020) is an orthologue of vaccinia virus (VACV) E3L, which is essential for the broad host-range of VACV *in vitro* and affects virulence *in vivo* [8]. Due to the variation in the N-terminal domains of E3L orthologue of ORFV and BPSV, Delhon and his team [9] suggested that this domain might have a role to play in host range and pathogenesis. But Hautaniemi and his team [6] reported that analysis of the variation between different PPV species does not clearly support a role in host range determination as there was no greater identity between BPSV and PCPV 020 proteins than between them and the corresponding ORFV proteins.

Till date, there is no published data related to the information about interferon-resistance gene of camel PPVs. Keeping this in view, the objective of the present study was to amplify interferon-resistance gene of camel PPVs from the skin scabs of the Dromedary camels (*C. dromedarius*) suspected to be infected with contagious ecthyma by polymerase chain reaction (PCR) and subsequent cloning of the PCR amplified DNA fragments into the vector for sequence analysis and to find out their relatedness with the other PPVs available in the NCBI database.

Material and methods

During the epidemiological survey conducted in the last week of July 2010 at various camel inhabitating areas of Rajasthan, India, it was observed that the camel calves (either sex around 6 months of age) of a herd (two males and three females) belonging to the camel keepers dwelling in Khod village, Pali district, Rajasthan state, India were showing the exanthematous skin lesions around the facial region and were suspected to be infected with contagious ecthyma. In the same year, in the last week of August at National Research Centre on Camel (NRCC) herd, Bikaner, Rajasthan, India, camel calves aged between 6 months and 2 years of either sex were also showing similar kind of lesions suspected for contagious ecthyma (total 30 animals). During mid August 2011, camel calves of below 1 year of age of either sex in a camel herd (four males and six females) at Jagthi village of Udaipur district, Rajasthan state, India were also exhibiting symptoms suspected for contagious ecthyma. Scab materials were collected from three (from each geographical area) severely affected animals and stored at −20 °C. All animal experiments were performed according to protocols approved by the institutional committee for use and care of animals (Animal ethical clearance No. 354/ÇPCSEA, National Research Centre on Camel, Bikaner, India).

Total genomic DNA was extracted from collected skin scabs using AxyPrep Multisource Genomic DNA Miniprep kit (Geneaxy Scientific Pvt. Ltd.) according to the manufacturer's instructions. As per our previous report [4], the nucleotide sequences of the envelope gene amplified from PPV DNA of camel skin scabs suspected for contagious ecthyma is found to be closely related to PCPV. Therefore, in the present study, nucleotide primers were designed using the coding sequences of dsRNA binding protein (RBP) encoding gene of pseudocowpoxvirus isolate from Finnish reindeer (GenBank Accession No. GQ329669); forward primer RBPF: 5′tta gaa gct gat gcc gca g ttg tcg atg agg 3′, reverse primer RBPR: 5′atg gcc agc gac tgc gct tcc ctg atc ctc 3′. PCR amplification of RBP encoding gene was performed using the following thermal profiles: initial denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 60 °C for 1 min, extension at 72 °C for 1 min, and final extension at 72 °C for 10 min. The PCR-amplified products were checked by electrophoresis on a 1% agarose gel. After purification of the amplified products from the low melting point agarose gel by phenol extraction followed by ethanol precipitation, the fragments were cloned into pGEM-T Easy Vector (Promega). The ligated mixtures were then used for transformation into *Escherichia coli* DH 5α [10]. Positive clones were identified by colony PCR using gene-specific primers and restriction analysis with EcoRI.

Sequencing of the PCR amplified DNA fragments (three from each geographical area) in both directions was carried out in an automated DNA sequencer at sequencing facility of Delhi University (South Campus), Delhi. Sequences of the virus isolates from Bikaner, Pali and Udaipur were submitted to NCBI GenBank and assigned the accession numbers JN712917, JQ388235 and JQ388236, respectively. Nucleotide identity, amino acid identity and comparison of the sequences with published sequences of members of PPVs available in the GenBank database were carried out using the computer software BioEdit version 7.0.9. These sequences were compared in Clustal X [11] and a phylogenetic tree was constructed based on the amino acid sequences by the neighbour-joining method using Mega 4(Molecular Evolutionary genetics Analysis software with bootstrap values calculated for 1000 replicates [12]. The open reading frame (ORF), translation of nucleotide sequences to amino acid sequences and functional motifs such as asn-glycosylation and myristylation of the gene products were predicted by using the computer software Generunner version 3.05 (hastings Software Inc. Hastings, NY, USA; http://www.generunner.net).

Results and discussion

The clinical signs of pox, contagious ecthyma and papillomatosis of camel are similar and indistinguishable [13] upon the clinical inspection. Despite the usefulness of electron microscopy, the methods of PCR, sequencing and restriction fragment length polymorphism (RFLP) would be more useful for genetic characterisation and classification of parapoxviruses, especially when the virus cannot be isolated [14].

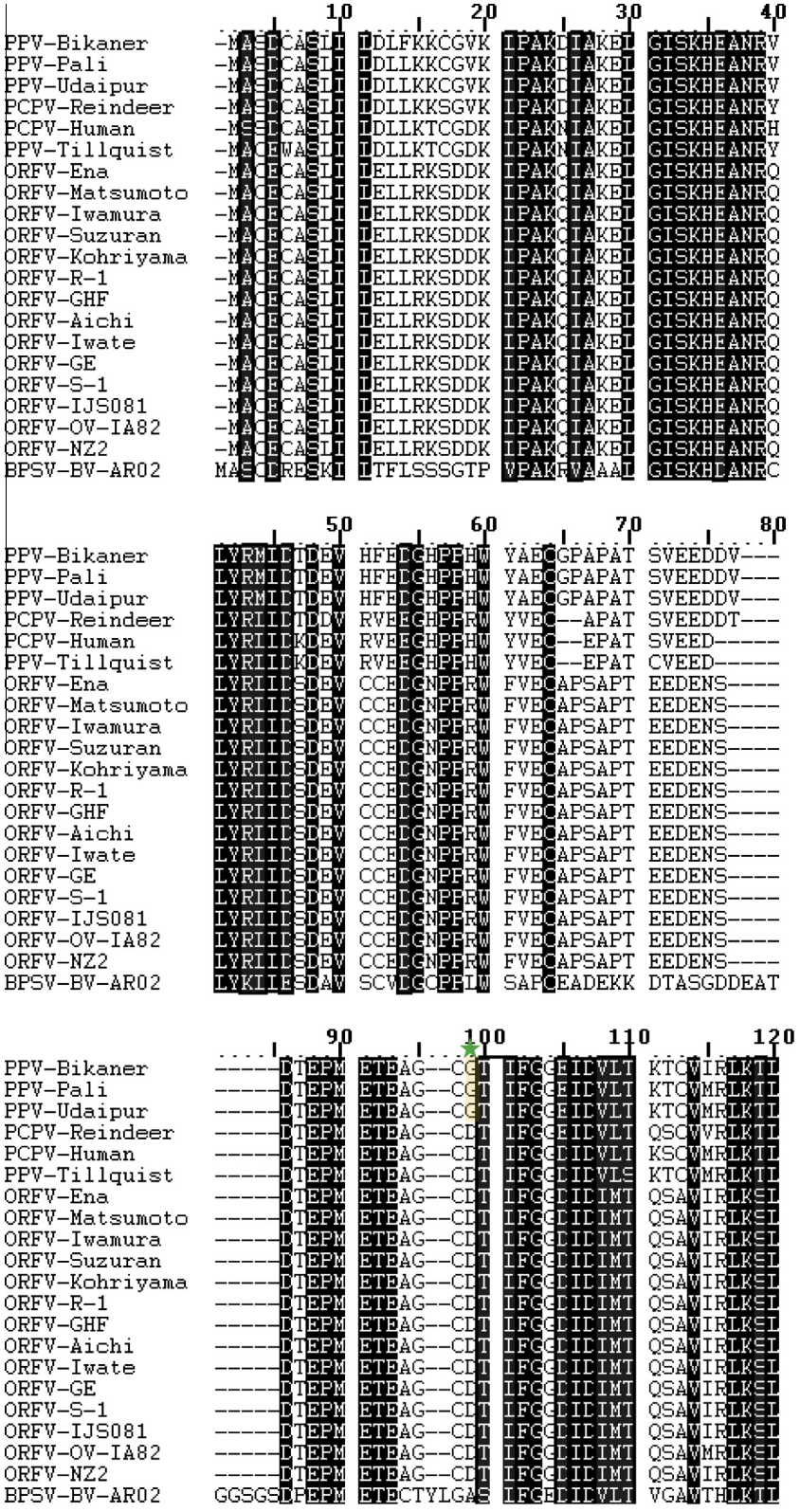

Fig. 1 Alignment of amino acid sequences of RBP encoding gene of camel PPV-Bikaner with other parapoxviruses using the software BioEdit Version 7.0.9. Star indicates the position of myristylation motif in camel PPVs. Triangle denotes the position of asn-glycosylation motif in camel PPVs. Arrow denotes the position of amino acid residues at the carboxy terminal domain of the E3L protein of camel PPVs and ORFV required for dsRNA binding. Shaded areas indicate the conserved amino acids in the protein described.

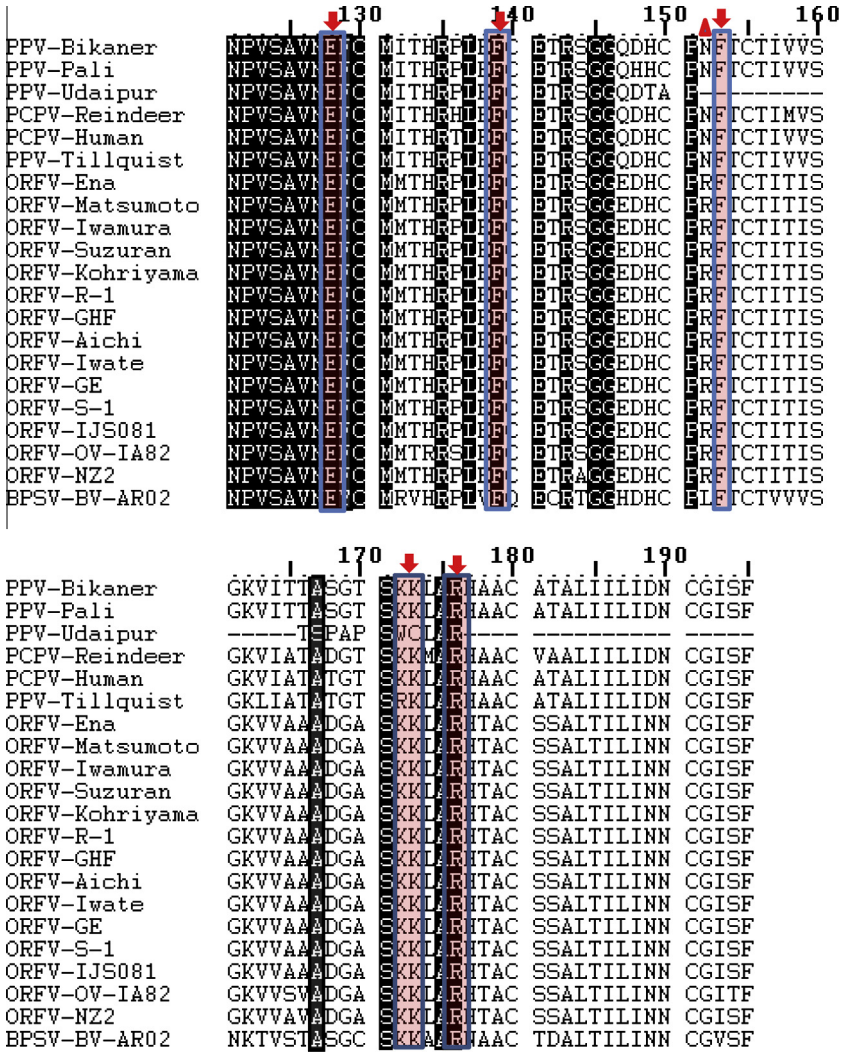

Fig. 1 (*continued*)

All vertebrate poxviruses encode orthologues of vaccinia virus (VACV) E3L, with the exception of the avipoxviruses and molluscum contagiosum virus. It will be interesting to study the role of the VACV E3L orthologues in the biology of the viruses that naturally express them [15]. As a preliminary step related to the aforementioned statement, the present study describes the baseline information about VACV E3L orthologue of camel PPVs.

The complete nucleotide sequences of RBP encoding gene of camel PPVs from three different geographical areas of Rajasthan state (Bikaner, Pali and Udaipur), India were analysed for the first time. These sequences of RBP encoding gene and their comparison to corresponding amino acid sequences from seventeen other PPV sequences are shown in Fig. 1.

The open reading frame (ORF) of RBP encoding gene of Bikaner and Pali PPVs is 555 bp encoding a polypeptide of 19.9 kDa whereas the full length of Udaipur PPVs is 554 bp only containing the deletion of one cytosine residue at position 418. Due to one nucleotide deletion, RBP encoding gene of camel PPV from Udaipur resulted in the formation of truncated polypeptide of 16.5 kDa. The ORF of both Bikaner and Pali PPVs has one asn-glycosylation motif at position 141 (marked with triangle symbol at the position of 152 in the Fig. 1). One myristylation motif is present in all the three camel PPVs described at position 88 (marked with star symbol at the position of 99 in the Fig. 1). Both asn – glycosylation and myristylation motifs are absent in all the ORFV strains analysed in this study. Ho and Shuman [16] reported that there are six amino acid residues in the carboxy terminal domain of VACV E3L protein, being essential for the binding of dsRNA. Subsequently, it was found that ORFV protein **(OV20.0L)** also consists of the six amino acid residues of carboxy terminal domain essential for its binding to dsRNA. The six amino acid residues include-one E(glutamic acid, two F(phenyl alanine), two K(lysine) and one R(arginine) (marked with arrow symbol in the Fig. 1)[17]. As the case of OV20.0L protein, the six amino acid residues in the carboxy terminal domain are conserved in the E3L protein of two camel PPVs (Bikaner and Pali PPVs) Due to the mutation in Udaipur PPV, out of six, only three amino acid residues (one glutamic acid, one phenyl alanine and one arginine) essential to the binding of dsRNA are retained (Fig. 1).

Sequence analysis revealed that RBP encoding gene of camel PPV from Bikaner shared 98.3% and 76.6% sequence

Table 1 Parapoxviruses (PPVs) and their percent nucleotide and amino acid identity with camel PPVs of Bikaner.

S. no.	Virus isolate	Host	Country and year	NCBI accession no.	% Identity		References
					Nucleotide	Amino acid	
1	PPV-camel, Bikaner	Dromedary camel	India, 2010	JN712917	–	–	This report
2	PPV-camel, Pali	Dromedary camel	India, 2010	JQ388235	99.2	98.3	This report
3	PPV-camel, Udaipur	Dromedary camel	India, 2011	JQ388236	99.0	76.6	This report
4	PCPV-F00.120R	Reindeer	Finland, 2010	GQ329669	91.1	86.9	[6]
5	PCPV-VR634	Human	New Zealand, 2010	GQ329670	91.3	86.9	[6]
6	Tillquist parapoxvirus	Human	Not available	AY278212	91.1	84.7	[19]
7	ORFV-Ena	Not available	Not available	AB522803	75.9	71.7	[14]
8	ORFV-Matsumoto	Not available	Not available	AB522802	75.9	71.7	[14]
9	ORFV-Iwamura	Not available	Not available	AB522801	75.9	71.7	[14]
10	ORFV-Suzuran	Not available	Not available	AB522800	75.9	71.7	[14]
11	ORFV-Kohriyama	Not available	Not available	AB522799	75.9	71.7	[14]
12	ORFV-R-1	Not available	Not available	AB522797	75.9	71.7	[14]
13	ORFV-GHF	Not available	Not available	AB522796	75.9	71.7	[14]
14	ORFV-Aichi	Not available	Not available	AB522795	75.9	71.7	[14]
15	ORFV-Iwate	Not available	Japan,1970	AB499038	75.9	71.7	[14]
16	ORFV-GE	Japanese Serow	Japan,2007	AB499037	75.9	71.7	[14]
17	ORFV-S-1	Japanese Serow	Japan,1985	AB492086	75.9	71.7	[14]
18	ORFV-IJS081	Japanese Serow	Japan, 2008	AB492085	75.9	71.7	[14]
19	ORFV-OV-IA82	Sheep	USA, 2004	AY386263	75.9	69.5	[9]
20	ORFV-NZ2	Not available	New Zealand	DQ184476	75.4	71.1	[20]
21	BPSV-BV-AR02	Calf	USA	NC005337	60.0	52.8	[9]

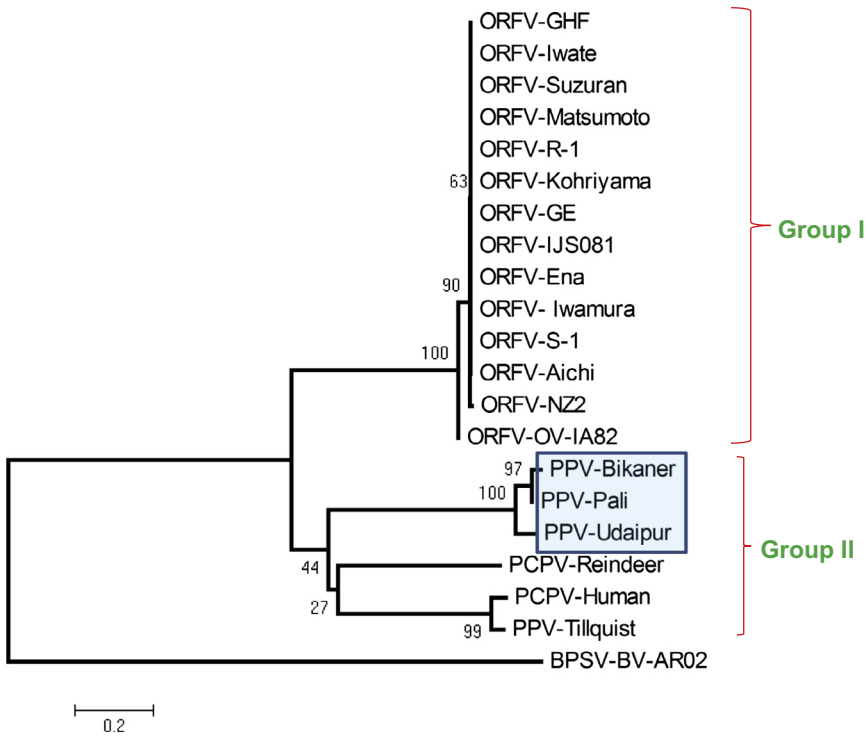

Fig. 2 Phylogenetic tree based on amino acid sequences of RBP encoding gene from different parapoxviruses, constructed by the neighbour-joining method using Mega 4(Molecular Evolutionary genetics Analysis software with bootstrap values calculated for 1000 replicates. Horizontal distances are proportional to the genetic distances. Vertical distances are arbitrary. The numbers at each branch represent bootstrap values (1000 replicates).

identity at the amino acid level, with Pali and Udaipur PPVs, respectively. BPSV reference strain exhibited 52.8% identity, where as reindeer PCPV and human reference strain PCPV shared 86.9% amino acid identity with RBP encoding gene of camel PPVs from Bikaner. All the different strains of ORFV from different geographical areas of the world shared 71.7% amino acid identity with RBP encoding gene of camel PPVs from Bikaner. But the reference strains of ORFV, i.e., OV-IA82 and ORFV-NZ2 shared 69.5% and 71.7% sequence identity, respectively at the amino acid level, with Bikaner

PPVs. (Table 1). As per our earlier report related to the sequence analysis of IL-10 from camel PPV [18], the results of the present study also suggest that the cameline PPVs are closely related to bovine PPV (PCPV) when compared to caprine and ovine PPV (ORFV) and could further support the view that contagious ecthyma in camels is caused by a virus from cattle but not from sheep and goats.

As the amino acid sequences in comparison to the nucleotide sequences of any gene gives more realistic picture of its biological function, a phylogenetic tree therefore constructed using amino acid sequences of the RBP encoding gene of various parapoxviruses revealed that the camel PPVs from Bikaner, Pali and Udaipur clustered with other parapoxviruses published earlier, supported by high bootstrap values (Fig. 2). All the three camel PPVs grouped with reindeer PCPV, reference strain PCPV and Tillquist PPV, where as ORFV from different regions of the world clustered together forming another group. In this phylogenetic tree, BPSV reference strain was kept as the out-group.

It is recommended that extensive research work on sequence analysis and functional assays of various immunomodulatory protein genes of PPVs from the camels inhabitating different geographical areas of the world needs to be carried out for the elucidation of pathogenesis of PPVs in dromedaries in comparison to other PPVs circulating among other farm animal species.

Conclusions

The RBP encoding gene of camel PPVs from Bikaner and Pali contains an open reading frame of 555 bp encoding 184 amino acid polypeptide whereas the size of RBP encoding gene of Udaipur PPVs is 554 bp only possessing the deletion of one cytosine residue at position 418. Because of one nucleotide deletion, RBP encoding gene of Udaipur PPV resulted in the formation of truncated polypeptide. Similar to OV20.0L protein of ORFV, the six amino acid residues in the carboxy terminal domain needed for the binding of dsRNA are conserved in the camel PPVs from Bikaner and Pali.

Conflict of interest

The authors have declared no conflict of interest.

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