Research Article

# Genomic Analysis of Nucleocapsid and Fusion Genes of Small Ruminant Morbillivirus in Pakistan

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**Abstract** | Small ruminant morbillivirus (SRMV) is the most contagious viral disease of sheep and goats. The present study compared the select genetic markers of the vaccine and field strains originating from the field outbreaks for phylogenetic analysis, nucleotide and deduced amino acid sequence analyses, and a comparison of three-dimensional protein structures. The N gene ORF was 1578 nucleotide long with a single open reading frame encoding 526 amino acids. Similarly, the ORF of F gene was 1641 nucleotide long which encoded 547 amino acids. Upon phylogenetic analysis of the country isolates with the vaccine strains, it was revealed that isolates were clustered within lineage IV (Asian Lineage) closer to Indian isolates. On the other hand, a number of substitutions were observed at sites considered important for the structural and functional integrity of the N and F protein. All of the conserved regions were same regardless of the gene while the substitutions were seen at different residues other than the conserved domains. However, the analysis based upon the three-dimensional protein structures revealed that the study isolates showed mutation at C-terminus region of N protein. Such a study helps to improve our understanding towards evolution of emerging and re-emerging strains of SRMVs in the country.

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## Introduction

Peste des petits ruminants (PPR) caused by small ruminant morbillivirus (SRMV) previously called as PPRV is a highly contagious, transboundry, often fatal viral disease of small domestic ruminants such as sheep (Ovisaries), and goats (Capra aegagrushircus) but can infect wild ruminants also (Couacy-Hymann et al., 2005; Munir et al., 2012). This disease is characterized by high rise of fever (pyrexia), oculo-nasal discharges, enteritis, diarrhoea, abortion, erosive stomatitis and bronchopneumonia followed by either death or recovery from the disease (Munir et al., 2013; Balamurugan et al., 2014). SRMV belongs to the genus Morbillivirus in the family of Paramyxoviridae with genome length of 15,948 nucleotides along with single stranded, non-segmented RNA virus with negative polarity (Gibbs et al., 1979). The genome encodes for six structural proteins, nucleocapsid (N), phosphoprotein (P), matrix (M), fusion (F), haemaggulutinin (H) and the large polymerase proteins (L) and two non-structural proteins (V and C) in the order 3`-N-P(C/V)-M-F-H-L-5` (Bailey et al., 2005). Based upon N and F gene sequences, the virus has been classified into four distinct lineages (Lineage I-IV). First case of PPR was reported from West Africa (Ivory Coast) during 1942 (Gargadennec and Lalanne, 1942) and later on from other parts of

Africa, Asia and the Middle East (Balamurugan et al., 2014; Muthuchelvan et al., 2015; Parida et al., 2015).

Pakistan is an agro-livestock based country in which livestock plays a major role in the national economy. According to livestock survey of Pakistan year 2017-18 there are 30.5 million sheep and 74.1 million goats are present which are continuously facing several infectious and non-infectious diseases throughout the year. Among infectious, the SRMV is the leading threat to the small ruminants since first reported in 1991 from that time the disease became endemic in Pakistan (Amjad et al., 1996). Despite available disease control strategies, a number of outbreaks have been reported with subsequent economic losses to the farmers in Pakistan. The used vaccine is Nig75/1 which belongs to lineage II while currently prevailing isolates from the Middle East countries are grouped in lineage IV which provides foundation for the construction of novel vaccine as has been practiced in India (Singh et al., 2009; Saravanan et al., 2010; Singh, 2011). Therefore, the present study was designed to determine the genetic make-up of the country field isolates which causing outbreaks in central Punjab, Pakistan. Moreover, the comparison of the two most commonly used vaccines (named as Nig75/1 and Sungari 96) are also compared with the country isolates. In this context here we report the nucleocapsid and fusion gene sequences of Nig75/1, Sungari 96 and its comparative analysis with the country isolates. The present work not only aid in disease diagnosis of vaccine related outbreaks but also devises better control planning in future.

## Materials and Methods

### *Sample collection*

In the present study, swab samples were collected from small domestic animals (sheep and goats) brought by their owners to the civil veterinary hospitals at different districts of Punjab such as Lahore, Faisalabad and Layyah. Total of 18 samples including nasal, oral and rectal swab samples were screened for the SRMV detection through RT-PCR.

### *RNA extraction and RT-PCR*

Total RNA was extracted with the help of commercially available kits for the genome extraction such as RNeasy QIAmp Viral RNA Mini kit Qiagen, Germany. After the genome extraction it was subjected for the amplification of the two genes such as nucleocapsid and fusion genes. Reverse transcription was performed and the PCR cycling conditions were as described previously (Shaila et al., 1996; Forsyth and Barrett, 1995). Briefly, the genome was subjected to 30 cycles of amplification (denaturation at 95 °C, annealing of primers for 1 minute at 50°C and the extension for 2 minutes at 72 °C) by using 10 picomol of the each forward and reverse primers and 1.25 U of Taq DNA polymerase (Promega, USA).

### *Purification of PCR products and sequencing*

The PCR amplicons were checked for its correct length in 1% agarose gel. The corrected amplicons were purified with the help of kit named as Wizard® SV Gel and PCR Clean-Up System Promega, Co., Madison, WI, USA. The purified amplicons were now subjected for the sequencing. The method which was used for the sequencing was Sanger sequencing method from commercially available facility provided by vendor of ABI of Malaysia in Pakistan. Both *N* and *F* genes would be sequenced in both forward and reverse directions.

### *Sequence analysis and phylogenetic tree construction*

The sequenced fragments of the genome were assembled using the Genius software 8.1.6 version. The portions overlapping and the primer sequences were eluted from the data appropriately. After that, it would be BLAST for matching of database and submitted to the GenBank for the designation of accession number. SRMV-related sequences with complete *N* and *F* genes were retrieved from GenBank, NCBI database under the accession numbers KY967608, KY967609 and KY967610. For the sake of comparison the following sequences of vaccines were also retrieved from NCBI; (GenBank Accession number X74443 for Nigeria 75/1 and XF727981 for Sungari 96). The phylogenetic tree was constructed on MEGA after the alignment in BioEdit by using the Clustal W multiple alignments. The tree was constructed using the neighbour joining method and the number of bootstrap value was 1000.

### *Comparative residue analysis*

The present study isolates were compared to the query references vaccines strains in order to know the differences at amino acids and nucleotides. After the comparison of nucleotides, we would convert them into amino acids in order to evaluate the positions of amino acids in comparison with the available query vaccine strain. With the help of available options in

the BioEdit we would convert these visual details into graphical view.

### *Three dimensional protein analyses*

Three dimensional structures of the antigenic epitopes of the study as well as vaccine strains (N and F) were made in I-TASSER (Iterative Threading Assembly Refinement) which was available online after converting them into amino acids available from ExPASy tools. The predicted protein functions such as ligand binding sites, enzyme commission numbers and active site residues were also analysed. By using PyMol software, we did comparative alignment and explain the mutations at both conserved and significant motifs. Further observations and images of residues were carried out and captured through PyMol software. The comparison between vaccines strains with query study isolate was also done in this present study.

## Results and Discussion

### *Phylogenetic analysis*

The tree for complete phylogenetic analysis was constructed by using complete ORF of *N* (1578 nucleotide) gene along with other complete ORF of *N* (1578 nucleotide) genes from different country isolates reported since the outbreaks of this disease. As, the first time complete genomic analysis was assessed from Pakistan so, it was revealed that our country study isolates (KY967608-10) belong to lineage IV since 1991 and it made a separate cluster which was closer to Indian Revati isolates (FJ750559) as shown in Figure 1A.

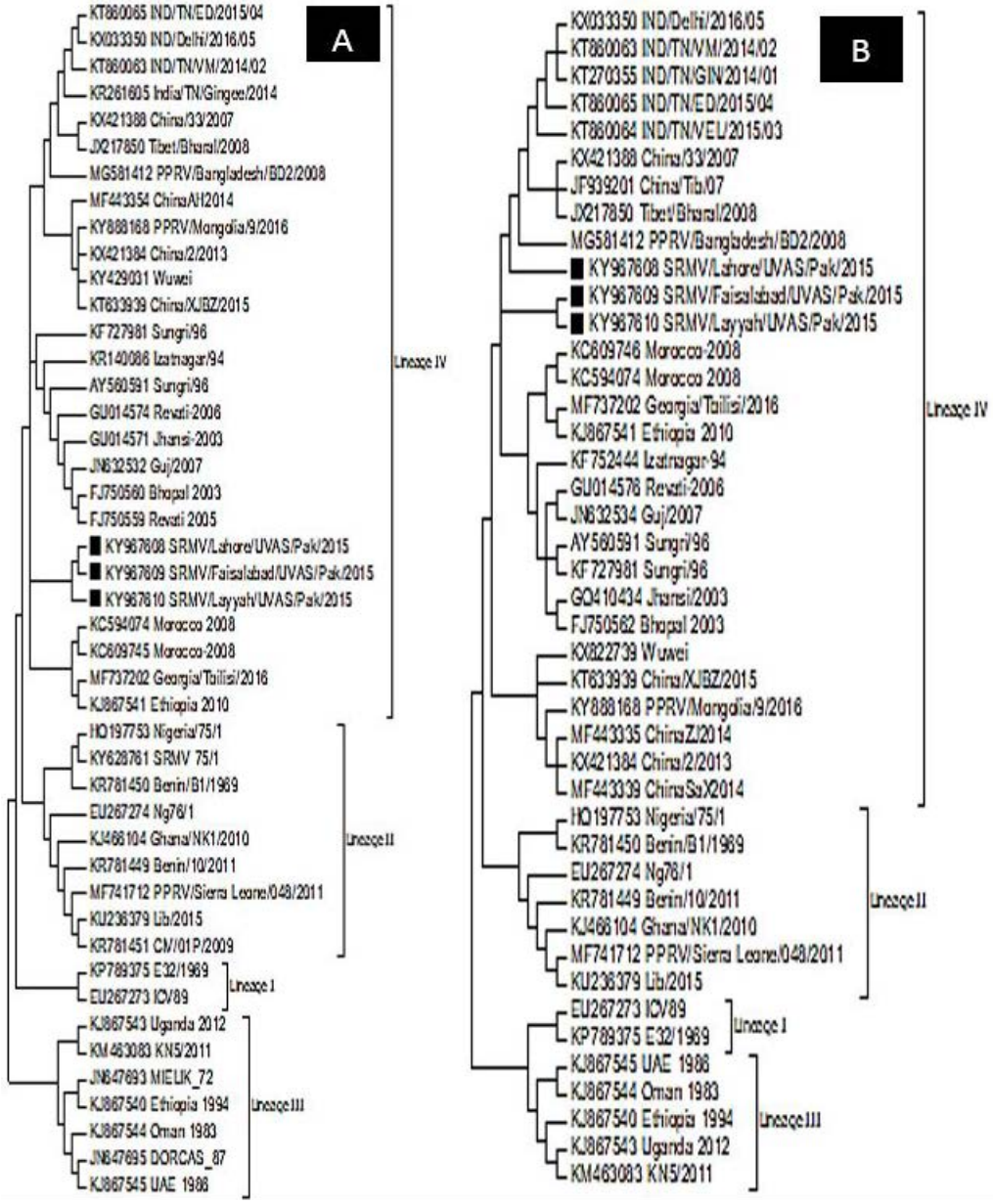


**Figure 1:** *Complete phylogenetic analysis of N (A) and F gene (B) of study isolates along with other lineages isolates.*

Similarly, another tree was constructed but this time using complete ORF of *F* (1641 nucleotide) gene along with our study isolates with the ORF of *F* (1641 nucleotide) genes from other different country isolates. It was revealed that our study isolates now this time made a separate cluster within lineage IV in which Lahore isolate (KY967608) made a separate cluster along with Bangladesh (MG581412), China (KX421388) and Indian isolates (KX033350) as shown in Figure 1B.

### *Residue analysis of N gene according to Nig75/1*

There were five tryptophan (W) amino acids were observed at position 146, 175, 196, 215 and 333 in all of the studied isolates (KY967608-10) together with the vaccine (Nig 75/1) and all the isolates from each lineage (I, II, III and IV). Similarly, one cysteine (C) amino acid residue at position 255 was also observed in all of the isolates of SRMV. RNA-binding activity of N protein was due to the amino acids at positions 324–338 (FSAGAYPLLWSYAMG), which was also observed in all the isolates of SRMV. By examining the N protein, it is revealed that the nuclear export and nuclear localizing signals have been identified both in morbilliviruses as well as in all SRMV isolates. The NES motif with the sequence of LLKSLALF was observed at the residue position of 4 to 11, whereas the sequence for NLS, TGVLISML, was estimated at 70 to 77 residue position.

Beyond these conserved positions in all SRMV strains, the regions at residue number 46(I→T), 443(P→S) and 459(P→S) showed substitutions to other amino acids in our study isolates only, in case of comparison with Nigerian vaccine strain. Interestingly, at amino acid residue number 140(S→G), 160(A→P), 401(T→A), 407(V→A), 437(E→G), 453(R→Q) and 525(S→G) expressed mutations in our study isolates as well as to all the isolates (China and India) which were representing lineage IV strains. These whole substitutions were presented in Figure 2.

### *Residue analysis of F gene according to Sungari 96*

Upon sequence analysis regarding the F protein, the sequence for the cleavage site (GRRTRR) was analysed at nucleotide position of 103 to 108. Other than the cleavage site, there are three more glycosylation sites represented by G1, G2 and G3 at amino acid position of 25 to 27 (NLS), 57 to 59 (NIT) and 63 to 65 (NCT) respectively. This observation was not only seen in our studied isolates bur also in the vaccine strains and the isolates from each of the lineages at similar positions. Furthermore, the structure such as Leucine zipper, whose position was 459 to 480, was also found consistent in position in all of the SRMV strains.

Beyond these positions, the points where the substitutions of amino acids were observed in our study isolates such as Faisalabad and Layyah were at residue number 13(F→L) and 16(A→V). These types of substitutions were neither seen in the isolate representing Lahore nor in the isolates belonging to the lineage IV isolates such as India and China. However, a single mutation was observed in the isolate of Lahore, at the residue number 14(P→L), which was not seen in any other isolates under studied. Interestingly, the substitution in Lahore isolate was similar to the isolate from India was at residue number of 276(I→M). Although the substitution of amino acids was seen regardless of the lineages were at the residue number 11(Y→F), 209(T→I), 304(T→A) and 372(G→S). On the other hand, 299(S→T) and 486(V→A) showed substitution in our query study isolates as well as in the lineage IV isolates as shown in Figure 3.

### *Structural comparison of N gene with Nig75/1*

The changed amino acid sequences were highlighted and present them in different styles such as spheres, sticks, lines and mesh. The following images were obtained as analysed through PyMol software. The enzyme commission number for both studied isolates and the vaccine strains were similar to each other (3.2.1.63). There was no active site residue in both vaccine (Nig 75/1) and under study isolates. There were about twenty ligand binding sites at residue number 191, 201, 204, 205, 208, 211, 212, 274, 279, 280, 281, 285, 342, 347, 368, 369, 373, 374, 375 and 378 were observed in the reference Nigerian isolate, while only one ligand binding site was shifted in our studied isolate (373→371). Besides this, there were about nineteen substitutions were recorded in our studied isolate by comparing it with the Nigerian isolates (vaccine) given as 168(P→A), 148(G→S), 427(A→T), 433(A→V), 451(G→D), 457(A→T), 463(G→E), 469(S→P), 474(E→G), 477(R→T), 479(Q→R), 482(P→S), 484(R→K), 485(S→P), 495(L→P), 501(P→Q), 505(V→L), 510(G→S) and 555(G→S) as depicted in Figure 4.

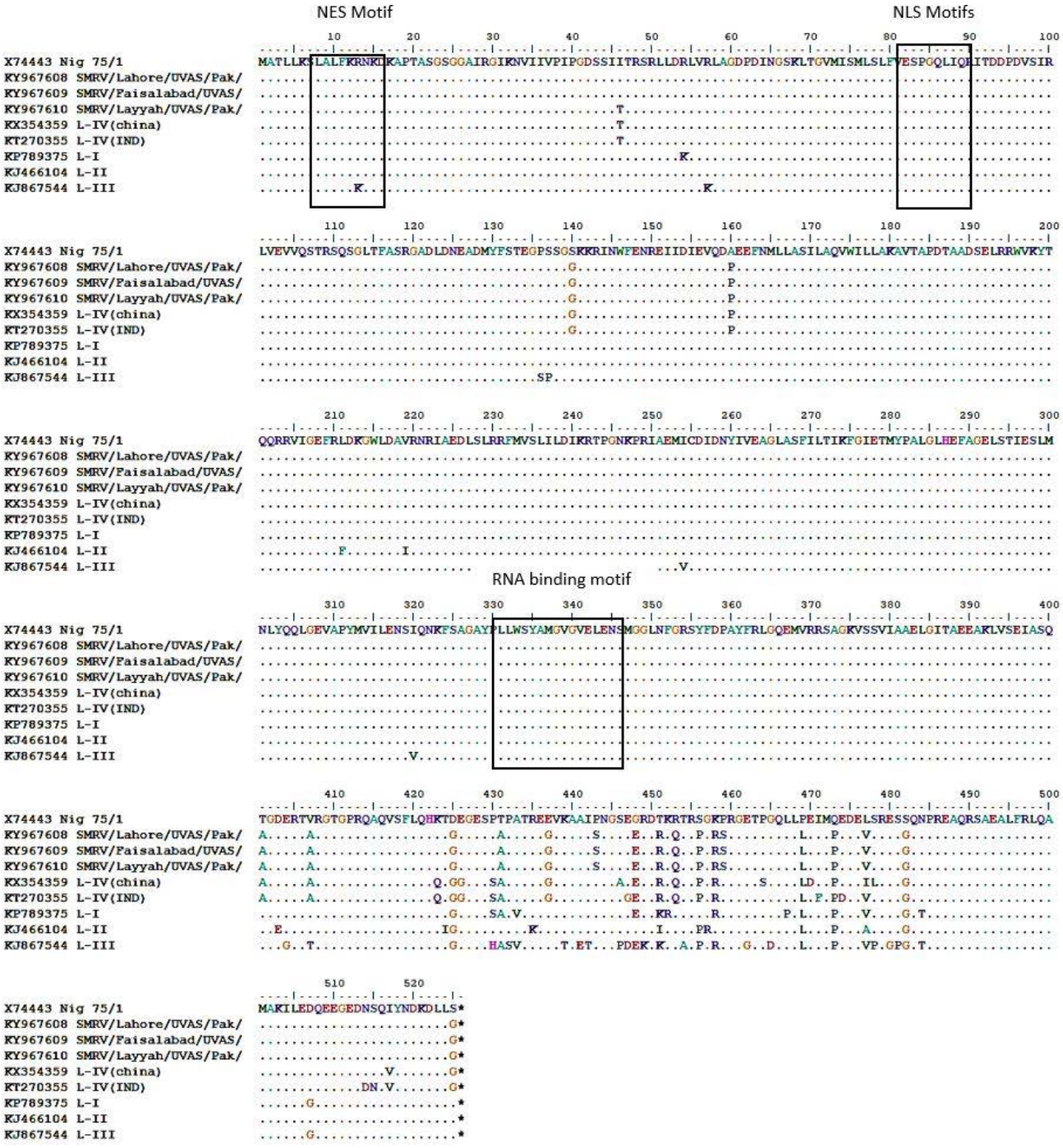


**Figure 2:** *Residue analysis for N gene of our study isolates along with N gene of Nig75/1 (vaccine) and representatives of all lineages.*

### *Structural comparison of F gene with Sungari 96*

Similar analysis was also carried out for *F* gene of Sungari isolate. The enzyme commission number for the studied isolate was 6.1.1.5. For the Sungari's isolate *F* gene the enzyme commission number was at 2.3.1.86. There was no active site residue for both of the isolates (Sungari and study isolate). There was also similarity in the ligand binding sites in both vaccine and study isolates (207 and 211). On the other hand, there was about nine substitutions were occurred in our query study isolates as compare to Sungari (vaccine) which was given as 11(S→F), 13(F→Y), 15(L→F), 18(V→A), 219(I→T), 309(T→S), 314(A→T), 384(S→G) and 498(A→V) as shown in Figure 5.

In this study, a total of three confirmed isolates of SRMV originating from domestic small ruminants from different districts of Punjab were used to be analysed for determining the genetic make-up of the two most important antigenic sites including the nucleocapsid and fusion regions of SRMV. As the mutation rate is found to be higher in RNA containing viruses so that the plan of study is to evaluate the substitutions at specific region with its consequences in the form of novel genotype which evade the immune system or cause failure in the control strategies.

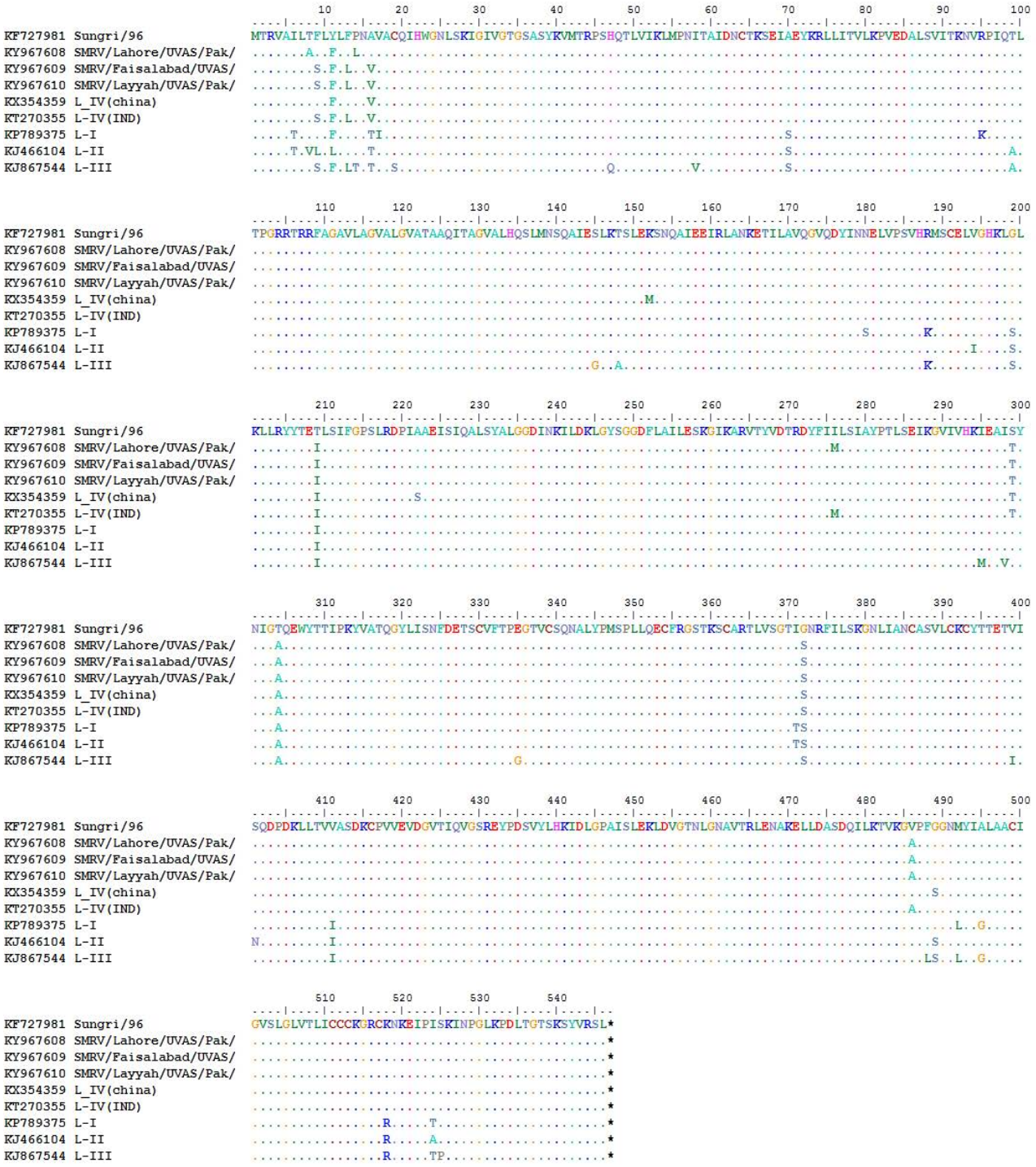


**Figure 3:** *Residue analysis for F gene of our study isolates along with F gene of Sungari 96 (vaccine) and representatives of all lineages.*

Based upon partial phylogenetic analysis of N gene along with the study isolates has revealed that the outcome is consistent of these circulating strains of SRMV in the country with the similar observations as reported earlier from Pakistan (Balamurugan et al., 2010; Munir et al., 2012; Muniraju et al., 2014). This analysis also depicted that the current study isolates categorized under lineage IV since 1991, of the first case of SRMV reported in Pakistan. The query isolates made a separate cluster which was closer to the isolates has been previously reported during 2011-2012 from Multan and Okara respectively (Abubakar et al., 2018; Anees et al., 2013; Munir et al., 2012). This was the analysis based upon the partial gene as far there was no previously reported studies for the complete genes of SRMV from Pakistan. Therefore, we included here for the first time for the complete genomic characterization and the complete gene (N

and F) sequencing of SRMV from Pakistan. When the similar analysis was made for complete genes either N or F revealed the different findings, as reported earlier that the phylogenetic analysis was more reliable based upon N gene as it was specific for the distinction between lineages and also within a lineage isolate. The complete phylogenetic tree analysis revealed that the study isolates made a cluster which was closer to the Indian isolates as similar to the geographic location of the study areas. While, astonishing results were observed when the analysis was done on the basis of F gene. The one study isolate (Lahore) made a cluster with Bangladesh isolates and rest of the study isolates made another cluster which was closer but a separate to the isolates from Lahore (Muniraju et al., 2014).

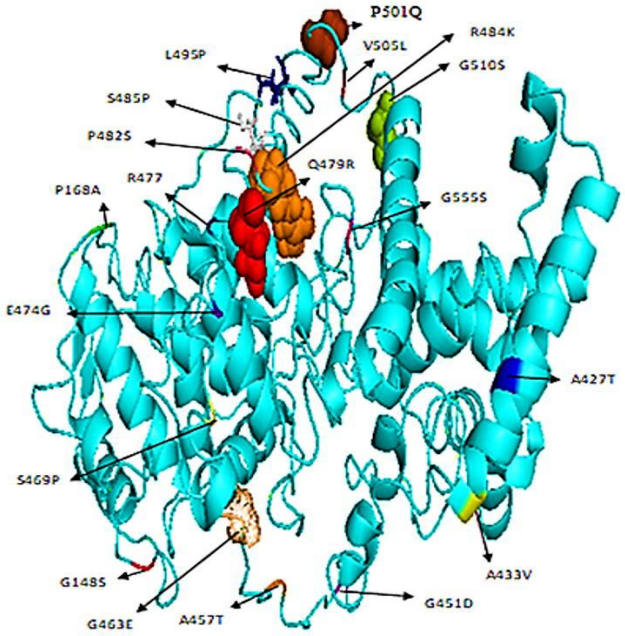


**Figure 4:** *Structural comparison of N gene of study isolates together with the Nigerian isolate (vaccine). The regions with different colours and shapes are showing substitutions to the backbone structure.*

Comparative residue analyses of SRMV isolates were carried out with the similar isolates sequences retrieved from the GenBank at nucleotide and amino acid levels, each of them representing their lineages of classification system. This comparison of residue was also done with the commercially available used vaccines. By considering the conserved regions in N gene, it was revealed that the entire five tryptophan and one cysteine residue was at similar position. Such findings were in the agreement of the previous studies which reported that the all tryptophan and one cysteine residue were at similar positions in all SRMV Nigerian isolates (Diallo et al., 1987). Conversely, when the analysis was done with the Sungari isolate as a reference strain, it was observed that one tryptophan at position 196 was substituted into cysteine amino acid. Fortunately, this substitution was observed in Sungari isolate only while all of the lineages including the study isolates having conserved tryptophan instead of any other substitution. In addition to these, there are many other significant conserved motifs among N gene such as, nuclear export signals (NES), nuclear localizing signals (NLS) and the RNA binding motifs. With the name indicates that NES are involved in the transportation of N protein from the nucleus to the cytoplasm and the other motif (NLS) is important for localizing the protein to move from the cytoplasm to the nucleus. Similarly, the RNA binding motifs are essential for the genomic interactions. All of these significant motifs were found to be conserved in all of the study isolates and among all lineages strains (Balamurugan et al., 2010). Besides these regions, the most of mutations were observed in the C-terminus that is end of the protein either considering Nigeria or Sungari as a reference strain for the nucleocapsid protein (Abubakar et al., 2018).

Likewise, F protein also contains significant motifs such as cleavage site (Meyer and Diallo, 1995), signal peptide and leucine zipper structures. The virulency of the virus is highly dependent upon the cleavage site, the signal peptide for the signalling and for the adaptation of virus in the environment. To maintain the tertiary structure of viral protein the leucine zipper structure needed to be conserved. The glycine residue is very crucial for membrane fusion activity (Rajak et al., 2005), where replacement of this amino acid residue can affect its fusion to the host cells (Qin et al., 2012). All of the significant motifs were found to be conserved in all of the SRMV isolates. On the other hand, most of the mutations were seen equally distributed among the entire length of fusion protein.

Since the similarity between our study isolates were about 99% so that, only one isolate was used for three dimensional structure prediction and subsequent comparative residue analysis with the vaccine strains. Significant substitutions were observed regarding the enzyme commission number and ligand binding sites. The creation of new active site residues is enough for escape mutant and subsequent vaccine failure. Nigerian isolate belongs to lineage II and Sungari strain from linage IV but still our study virus is not in accordance to these strains.

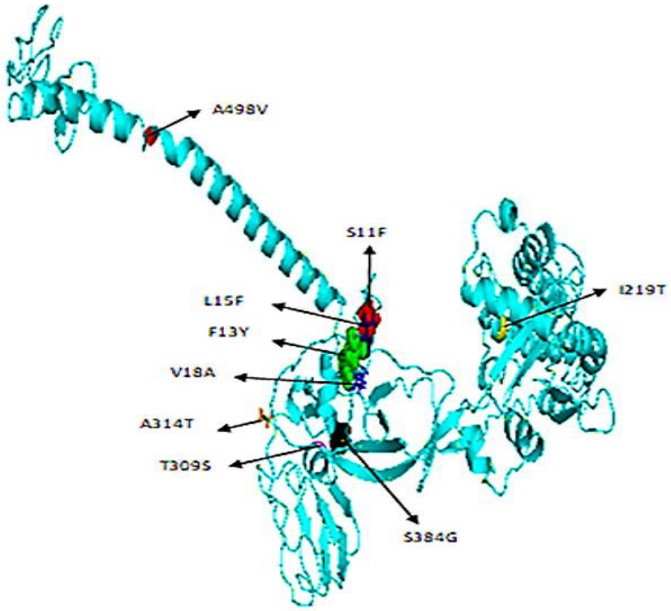


**Figure 5:** *Structural comparison of F gene of study isolates together with the Sungari isolates (vaccine). The regions with different colours and shapes are showing substitutions to the backbone structure.*

## Conclusions and Recommendations

Despite Pakistan is endemic for SRMV where outbreaks are continuously occurring, and there is limited information about the genetic nature of SRMV. The sequences reported here along with complete sub-genomic analysis (Nucleocapsid and Fusion) will shed new light upon the circulating strains of SRMV in Pakistan. Such understanding plays an important role for devising future control plans regarding the eradication of the disease as aimed by Food and Agriculture Organization (FAO). Currently, the vaccine used in Pakistan against SRMV is Nigeria 75/1which is originated from West African countries such as Nigeria and classified into Lineage II of the classification system. On the other hand, our country field originated isolates are categorized under Lineage IV which is contradictory from the vaccine isolate. Genetic characterization of SRMV is reported, which will certainly help to improve our understanding towards evolution of emerging and re-emerging strains of SRMVs in the country. With the further acknowledgement regarding the genomic characterization of SRMV will provide us the new insight about the construction of novel vaccine from filed originated domestic isolates as similarly been practiced in the neighbour country like, India.

## Authors Contribution

HMIJ and MA conceived the main idea. MA drafted the skeleton of the paper. HMIJ did the final checking and approved the manuscript.

### *Conflict of interest*

The authors have declared no conflict of interest.

## References

Abubakar, M., A.B. Zahur, K. Naeem, M.A. Khan and S. Qureshi. 2018. Field and molecular epidemiology of Peste des Petits ruminants in Pakistan. Pakistan J. Zool. 50(2): 401-797. https://doi.org/10.17582/journal.pjz/2018.50.2.559.566

Anees, M., M.Z. Shabbir, K. Muhammad, J. Nazir, M.A.B. Shabbir, J.J. Wensman and M. Munir. 2013. Genetic analysis of peste des petits ruminants virus from Pakistan. BMC Vet. Res. 9(1): 60. https://doi.org/10.1186/1746-6148-9-60

Amjad, H., Q.U. Islam, M. Forsyth, T. Barret and P.B. Rossitter. 1996. Peste des petits ruminants in goats in Pakistan. Vet. Rec., 139(5): 118–119. https://doi.org/10.1136/vr.139.5.118

Balamurugan, V., A. Sen, G. Venkatesan, V. Yadav, V. Bhanuprakash and R. Singh. 2010. Isolation and identification of virulent peste des petits ruminants viruses from PPR outbreaks in India. Trop. Anim. Health Prod. 42(6): 1043-1046. https://doi.org/10.1007/s11250-010-9527-0

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. Virus Res. 110: 119–124. https://doi.org/10.1016/j.virusres.2005.01.013

Balamurugan, V., D. Hemadri, M.R. Gajendragad, R.K. Singh and H. Rahman. 2014c. Diagnosis and control of Peste des petits ruminants: a comprehensive review. Virus Dis. 25(1): 39–56. https://doi.org/10.1007/s13337-013-0188-2

Couacy-Hymann, E., C. Bodjo, T. Danho, G. Libeau and A. Diallo. 2005. Surveillance of wildlife as a tool for monitoring Rinderpest and peste des petits ruminants in West Africa. Rev. Sci. Tech. 24: 869–877. https://doi.org/10.20506/rst.24.3.1615

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(7): 2033-2038. https://doi.org/10.1099/0022-1317-68-

7-2033

Forsyth, M. and T. Barrett. 1995. Detection and differentiation of Rinderpest and peste des petits ruminants viruses in diagnostic and experimental samples by polymerase chain reaction using P and F gene-specific primers. Virus Res. 39: 151–163. https://doi.org/10.1016/0168-1702(95)00076-3

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

Gibbs, E.P., W.P. Taylor, M.J. Lawman and J. Bryant. 1979. Classification of peste des petits ruminants virus as the fourth member of the genus Morbillivirus. Intervirology. 11: 268–274. https://doi.org/10.1159/000149044

Munir, M., S. Zohari and M. Berg. 2012. Molecular Biology and Pathogenesis of Peste des Petits Ruminants Virus, 1st edn. Springer, Germany. https://doi.org/10.1007/978-3-642-31451-3_1

Munir, M., S. Zohari and M. Berg. 2013. Molecular biology and pathogenesis of peste-des-petits-ruminants virus. Chapter 2: 23-32. https://doi.org/10.1007/978-3-642-31451-3_2

Muthuchelvan, D., K.K. Rajak, M.A. Ramakrishnan, D. Choudhary, S. Bhadouriya, P. Saravanan, A.B. Pandey and R.K. Singh. 2015. Peste- Des-Petits-Ruminants: An Indian Perspective. Adv. Anim. Vet. Sci., 3(8): 422 -429. https://doi.org/10.14737/journal.aavs/2015/3.8.422.429

Muniraju, M., M. Munir, A.R. Parthiban, A.C. Banyard, J. Bao, Z. Wang, C. Ayebazibwe, G. Ayelet, M. El-Harrak and M. Mahapatra. 2014. Molecular evolution of peste des petits ruminants virus. Emerg. Infect. Dis. 20(12): 2023. https://doi.org/10.3201/eid2012.140684

Meyer, G. and A. Diallo. 1995. The nucleotide sequence of the fusion protein gene of the peste des petits ruminants virus: the long untranslated region in the 5′-end of the F-protein gene of morbilliviruses seems to be specific to each virus. Virus Res. 37(1): 23-35. https://doi.org/10.1016/0168-1702(95)00013-G

Parida, S., M. Muniraju, M. Mahapatra, D. Muthuchelvan, H. Buczkowski and A.C. Banyard. 2015. Peste des petitis ruminants. Vet. Microbiol. 181(1-2): 90-106. https://doi.org/10.1016/j.vetmic.2015.08.009

Qin, J., H. Huang, Y. Ruan, X. Hou, S. Yang, C. Wang, G. Huang, T. Wang, N. Feng, Y. Gao and X. Xia. 2012. A novel recombinant Peste des petits ruminants-canine adenovirus vaccine elicits long-lasting neutralizing antibody response against PPR in goats. PLoS One. 7(5): e37170. https://doi.org/10.1371/journal.pone.0037170

Rajak, K., B. Sreenivasa, M. Hosamani, R. Singh, S. Singh, R. Singh and S. Bandyopadhyay. 2005. Experimental studies on immunosuppressive effects of peste des petits ruminants (PPR) virus in goats. Comp. Immunol. Microbiol. Infect. Dis. 28(4): 287-296. https://doi.org/10.1016/j.cimid.2005.08.002

Saravanan, P., V. Balamurugan, A. Sen, B.P. Sreenivasa, R.P. Singh, S.K. Bandyopadhyay and R.K. Singh. 2010. Long term immune response of goats to a Vero cell adapted live attenuated homologous PPR vaccine. Indian Vet. J. 87: 1–3.

Shaila, M.S., D. Shamaki, M.A. Forsyth, A. Diallo, L. Goatley, R.P. Kitching and T. Barrett. 1996. Geographic distribution of peste des petits ruminants viruses. Virus Res. 43: 149–153. https://doi.org/10.1016/0168-1702(96)01312-3

Singh, R.K., V. Balamurugan, V. Bhanuprakash, A. Sen, P. Saravanan and M.P. Yadav. 2009. Possible control and eradication of Peste des petits ruminants from India: technical aspects. Vet. Ital. 45: 449–462.

Singh, R.P., 2011. Control strategies for Peste des petits ruminants in small ruminants of India. Rev. Sci. Tech. Off. Int. Epizoot. 30(3): 879-887. https://doi.org/10.20506/rst.30.3.2079