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Genetic Evolutionary Aspects of Nucleoprotein (N) and Fusion (F) Gene of Circulating Peste Des Petits Ruminants (PPR) Virus in Bangladesh

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ABSTRACT

This study aimed to understand the genetic evolutionary aspects of the circulating PPR Virus in Bangladesh using sequence and phylogenetic analysis. 18 samples of N gene and 7 samples of F gene of PPRV isolates were sequenced from a commercial source (Macrogen, Korea). CLC sequence viewer 8 and ExPasy Protein translation tools were used for analysis. The PPRV isolates from the years 2008 and 2015 in Bangladesh were used for comparative genetic analysis with study isolates. The N gene of PPRV (18 isolates) nucleotide analysis revealed that the nucleotide divergence highest was 3-6% and only 1-3% when aligned with BD-2008/MG581412 and BD-2015/OK274192 isolates, respectively. The mutation of N gene of PPRV is slow but continuously evolved. The F gene of PPRV (7 isolates) nucleotide sequence analysis revealed that the nucleotide divergence was only 1-2% when aligned with both (MG581412/2008) and (UQW80114/2015), which remained unchanged from 2008 to 2021. The amino acids divergence of N gene and F gene were 0-11% and 0-2%, respectively. The F gene is relatively more conserved than the N gene of PPRV. The highest homology of N gene sequences with China isolates (98.19%) and the F gene sequences with India isolates (99.29%), respectively, and genetically belong to Lineage IV. The similarity of the studied N gene (94-99%) and F gene isolates (98-99%) among other Bangladeshi isolates made quite a distinct and separate subcluster. The knowledge from such studies will aid in attaining the goal of managing and eradicating the PPR disease from the country.

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INTRODUCTION

The World Organization for Animal Health (OIE) has designated Peste des Petits ruminants (PPR) as a notifiable, economically significant transboundary viral disease that is acute and highly contagious in small ruminants (Dubie et al., 2022). PPR virus (PPRV) is single-stranded non-segmented RNA virus under the genus Morbillivirus, family Paramyxoviridae (Balamurugan et al., 2023). It has a 15,948-bp RNA genome that encodes for six structural proteins: a nucleoprotein (N), a viral RNA-dependent polymerase (L), an RNA-polymerase phosphoprotein co-factor (P), a matrix protein (M), a fusion protein (F), and a hemagglutinin protein (H), as well as two non-structural proteins (C and V proteins) (Rahman et al., 2023). PPRV is divided into four genetic lineages: I, II, III, and IV. Lineages I and II are found in Western Africa, Lineage III in Eastern Africa and the Middle East, while Lineage IV is extensively distributed throughout Asia and parts of the Middle East (Alidadi et al., 2021). More than two decades PPR virus has been prevalent in Bangladesh and circulating PPRVs belong to Lineage IV (Rahman et al., 2018). So much research has been carried out on clinical investigation and sero-monitoring of PPR diseases but limited research on genome sequence and phylogenetic analysis of PPRV mainly based on nucleotide (N) and fusion (F) genes. Over one decade ago, research stated the genetic character based on F and N genes of the PPRV strains is evolving continuously (Rahman et al., 2016). Few researchers worked on PPRV genetic analysis such as using N gene (Clarke et al., 2018), using N and H gene (Rahman et al., 2018) and using both F and H gene (Rahman et al., 2023) for phylogenetic analysis of PPRV in Bangladesh. However, among the genes, F and N gene-based RT-PCR approaches were most suited for detecting the PPRV and analyzing its lineages (Pandey et al., 2020). The successful eradication of PPRV from the country required continued disease monitoring in an endemic area (Baynard et al., 2010). Considering the above facts, this study aimed at genome sequence analysis of the N and F genes of circulating PPRV compared with previous Bangladeshi isolates to identify the evolutionary aspects of PPRV. This study also conducted phylogenetic analysis of the circulating PPR virus to discover any co-circulation of other lineages of PPRV in Bangladesh.

METHODOLOGY

Collection of PPRV sequence data

This study used eighteen PPRV isolates for genetic and phylogenetic analysis. PPRV isolates were detected by conventional RT-PCR (reverse transcriptase polymerase reaction) in clinical cases and slaughtered goat samples from January 2019 to March 2021. N gene-based primers (forward PPRV NF, GCTCTGTGATTGCGGCTGAGC and reverse PPRV NR, CCTGGTCCTCCAGAATCTTGGCC) and F gene-based primers (forward PPRVF1b, AGTACAAAAGATTGCTGATCACAGT and reverse PPRVF2b, GGGTCTCGAAGGCTAGGCCCGAATA) were used during RT-PCR reaction described detailed in our previous studies (Sultana et al., 2022). 18 PPRV N genes (partial) and 7 PPRV F genes (partial) constitute 402bp and 448bp, respectively, and were sequenced in both directions (forward and reverse) from commercial suppliers (Macrogen Inc., Korea). These raw PPRV sequences were aligned and edited using BioEdit 7.0.5. Sequenced isolates were submitted to GenBank and are now available with accession numbers. Table 1 shows detailed background data of PPR virus isolates between 2019- 2021.

Analysis of N and F gene of field PPRV

This sequence data was primarily detected by the Basic Local Alignment Search Tool (BLAST), which then downloaded the highest percent identity PPRV gene isolates from various countries and years from the National Centre for Biotechnology Information (NCBI) gene bank. The studied N and F gene sequences of PPRV (2019-2021) were used for phylogenetic, nucleotide, and amino acid substitution analysis. Previous

Bangladeshi PPRV isolates from 2008 (MG581412/2008) and 2015 (OK274192/2015) were used for N gene nucleotide and amino acids sequence analysis with the study isolates. Similarly, 2008 (MG581412/2008) and 2015 (UQW80114/2015) PPRV isolates were used for F gene nucleotide and amino acids analysis with the study isolates. CLC Sequence Viewer 8 software was used to construct multiple alignments of the sequences using UPGMA, Kimura 80, and Maximum Likelihood (MJ) tests for phylogenetic analysis. The phylogenetic tree reliability was evaluated by 100 replication of the Bootstrap values (Tamura et al., 2013). ExPasy protein translation tools were used to carry out the amino acid analysis of the N and F genes of the PPRV.

RESULTS

Nucleotide sequence analysis of PPRV

N gene sequence of PPRV

The nucleotide sequence analysis of N gene sequences of 18 isolates in BLAST- Nucleotide search revealed that the studied isolates had the highest nucleotide percent identity ranges 96.73%- 98.19% with China isolates (KX4213888/2007) and then 93.83%- 96.54% with India isolates (MN369543/2013). Aligned the studied isolates with over 10 years ago previous Bangladeshi isolates, 2008 (MG581412/2008) revealed nucleotide percent identity ranges from 94%-97%, and only 11-17 number of nucleotide substitutions occurred in studied isolates. In this alignment, the highest nucleotide (17) and lowest nucleotide (11) substitutions were found in OM158233 and MZ028630, respectively. Aligned the studied isolates with 5 years ago previous Bangladeshi isolates, 2015 (OK274192/2015) revealed nucleotide percent identity ranges from 97%-99%. Only 1-10 number of nucleotide substitutions were seen in the studied isolates. In this comparison, the highest nucleotide (10) substitution was found in MW525118 and the lowest nucleotide (only 01) substitution was found in 5 isolates in MZ028633- MZ028637. From this nucleotide analysis, it was found that the nucleotide divergence that occurred highest was 3-6% when aligned with one decade ago PPRV isolates (BD-2008, MG581412), and a small amount of only 1-3% nucleotide divergence was observed when aligned with BD-2015, OK274192 isolates. Therefore, this analysis shows that the mutation N gene of PPRV is slow but continuously evolved.

F gene sequence of PPRV

Similarly, the nucleotide sequence analysis of F gene sequences of 7 PPRV isolates searched in BLAST-Nucleotide search revealed that the studied isolates highest nucleotide percent identity ranges from 98.39%-99.29% with India isolates (KX860026/2004) and then similarity with the China isolates (KX421388/2007) ranges from 97.54%-98.59%. The F gene of 7 studied isolates aligned with Bangladeshi isolates, 2008 (MG581412/2008) revealed that nucleotide percent identity ranges from 98%-99% and only 3-8 number of nucleotide substitutions occurred in the F gene of studied isolates. In this comparison, the highest nucleotide (8) substitution was seen in 2 isolates (OM992365, OM992367) and lowest nucleotide (3) substitution were found in 1 isolates (OM992369). Aligned with Bangladeshi isolates, 2015 (UQW80114/2015) revealed that nucleotide percent identity ranges from 98%-99%, and only 2-10 nucleotide substitutions occurred in studied isolates. In this comparison, the highest nucleotide (10) substitution in 01 isolate (OM992365) and lowest nucleotide (2) substitution were found in 03 isolates (OM992366, OM992370-71). The F gene of 7 isolates nucleotide sequence analysis revealed that the nucleotide divergence occurred only 1-2% and nucleotide homogeneity percent identity was the same 98-99% when aligned with both (MG581412/2008) and (UQW80114/2015), which remained unchanged from 2008 to 2021. The F gene is relatively more conserved than the N gene of PPRV.

Table 1. GenBank accession number of N and F gene sequences (partial) of PPRV in goats with detailed history

Isolate ID	Date of collection	Name of district in Mymensingh division	Host information	Sex and Age	Organ	GenBank Accession Number for N gene	GenBank Accession Number for F gene
PPRV/24LN	24-Jan-2019	Mymensingh	PPR suspected dead goat	Female and 1.6 yrs	Lungs	MW525117	-
PPRV/26AL	26-Feb-2019	Mymensingh	PPR suspected dead goat	Female and 2 yrs	Lungs	MW525118	OM992369
PPRV/26DS	26-Feb-2019	Mymensingh	PPR suspected dead goat	Female and 1 yr	Spleen	MW525119	OM992370
PPRV/14L	14-Mar-2019	Mymensingh	Goats at slaughter house	Female and 1.5 yr	Spleen	MW444788	-
PPRV/18AL	18-Mar-2019	Mymensingh	Goats at slaughter house	Male and 7 months	Lungs	MZ028630	-
PPRV/18BL	18-Mar-2019	Mymensingh	Goats at slaughter house	Female and 6 months	Lungs	MZ028631	-
PPRV/26EL	26-Feb-2019	Mymensingh	Goats at slaughter house	Female and 2 yrs	Lungs	MZ028632	-
PPRV/16S	16-Nov-2019	Mymensingh	Clinically suspected goat	Female and 9 months	Spleen	MZ028633	-
PPRV/21S	17-Nov-2019	Mymensingh	Goats of slaughter house	Male and 1.4 yrs	Spleen	MZ028634	-
PPRV/26BS	25-Nov-2019	Mymensingh	Goats at slaughter house	Female and 2 yrs	Spleen	MZ028635	-
PPRV/31S	04-Dec-2019	Mymensingh	Goats at slaughter house	Female and 1.5 yrs	Spleen	MZ028636	OM992371
PPRV/40S	18-Dec-2019	Mymensingh	Clinically suspected goat	Male and 1.3 yrs	Spleen	MZ028637	-
PPRV/7L	17-Feb-2020	Mymensingh	Clinically suspected goat	Male and 1 yr	Lungs	MZ028638	-
PPRV/9L	22-Feb-2020	Mymensingh	Goats at slaughter house	Male and 1 yr	Lungs	MZ028639	-
PPRV/1LN	05-Jan-2021	Netrokona	Goats at slaughter house	Female and 9 months	Lungs	OM158230	OM992365
PPRV/3SP	11-Jan-2021	Netrokona	Goats at slaughter house	Female and 1yr	Spleen	OM158231	OM992366
PPRV/4LN	15-Jan-2021	Sherpur	Goats at slaughter house	Male and 1.5 yr	Lungs	OM158232	OM992367
PPRV/J4SP	18-Jan-2021	Jamalpur	Goats at slaughter house	Female and 10 months	Spleen	OM158233	OM992368

Amino acid analysis of PPRV

Amino acid analysis of N gene of studied isolates with 2008 and 2015 PPRV Bangladeshi isolate

This study shows N gene-based amino acids percent identities and the number of amino acid substitutions of 18 studied isolates compared with previous Bangladeshi PPRV isolates MG581412/2008 and OK274192/2015. Aligned with MG581412/2008, it was observed that amino acids percent identity was 89%-95% and divergence was 5-11% are shown in Table 2. Only 6-10 amino acid substations occurred in the N gene of PPRV-studied isolates in this alignment. However, in this comparison K(Lysine), S(Serine), R(Arginine) and D(Aspertic acid) were substituted by R(Arginine), N(Asparagine), Q(Glutamine) and E(Glutamic acid), respectively were seen in all 18 studied isolates (100%). T(Therionine) and I(Isoleucine) were substituted by A(Alanine) and F(Phenylalanine) respectively in 17 studied isolates (94%), whereas V(Valine) was converted to A(Alanine) in 10 studied isolates (55.5%). Also, G(Glycine), P(Proline), M(Methionine) and L(Leucine) were substituted by A(Alanine), L(Leucine), L(Leucine) and P(Proline) were seen in 1 isolate ease (5.6%). Aligned with OK274192/2015, it was observed that 96%-100% of amino acids percent identity and divergence was 0-3%. Only 1-4 amino acid substitutions occurred in 13 studied isolates, and 5 samples appeared at 100% identical are provided in Table 2. However, in this comparison, it was revealed that A(Alanine) was substituted by V(Valine) in 9 studied isolates(69.23%), E(Glutamic acid) was substituted by D(Aspertic acid) in 4 studied isolates(30.76%) and Q(Glutamine) was substituted by H(Histidine) in 3 studied isolates(23.07%), whereas T(Therionine), L(Leucine) and G(Glycine) was substituted by N(Asparagine), P(Proline) and A(Alanine) respectively in 2 studied isolates (15.38%). Also, I(Isoleucine), P(Proline), M(Methionine) and L(Leucine) were substituted by N(Asparagine), L(Leucine), L(Leucine) and F(Phenylalanine) were seen in 1 isolate ease (7.69%). A limited extent of amino acid substitution happened in the partial N gene of PPRV-studied isolates from 2008 to 2021 that provide evolutionary history

Amino acid analysis of F gene of studied isolates with 2008 and 2015 PPRV Bangladeshi isolates

In this study, the F gene-based amino acids percent identities and the number of amino acids substitution of 7 PPRV studied isolates compared with previous Bangladeshi isolates**,** MG581412/2008 and UQW80114/2015. The amino acid substitutions remained unchanged in the F gene of PPRV during the period from 2008 to 2021, as shown in Table 3. From this analysis, it was observed that amino acids L(Leucine), I(Isoleucine), L(Leucine) were substituted by I(Isoleucine), L(Leucine), F(Phenylalanine) in 1 studied isolate (14.28%) whereas R (Arginine) was substituted by K(Lysine) in 1 studied isolate and S(Serine) was substituted by I(Isoleucine) in 1 studied isolate (14.28%) and other 4 F gene PPRV isolates appeared at 100% identical. It was concluded that for more than one decade, the F gene of PPRV remained unchanged, indicating that the F gene poses a conserved region and that mutation of amino acids was very very slow or rare. It takes a long time for mutation.

Both N and F gene sequence analyses were done on 7 PPRV isolates; only one PPRV isolates id no. PPRV/31S (N gene Acc. MZ028636 and F gene Acc. No. OM992371) lacked nucleotide and amino acid substitution in the last 6 years.

There were mutations at the nucleotide level both at the N gene and F gene of PPRV, but effective mutation was seen in the N gene of PPRV. Based on amino acid sequence analysis, the F gene was conserved compared to the N gene of PPRV from 2008 to 2021.

Table 2. Amino acids percent identities and amino acids substitution of N gene of 18 study isolates compared with Bangladesh-2008-MG581412 and Bangladesh-2015-OK274192

GeneBank AC. No. N gene sequence	Amino acids percent identities with Bangladesh-2008-MG581412	Amino acids of Bangladesh-2008-MG581412	Amino acids substitution in study isolates	Amino acids percent identities with Bangladesh-2015-OK274192	Amino acids in Bangladesh-2015-OK274192	Amino acids substitution in study isolates
MW444788	125/132 (94%)	T, K, S, R, T, D, R	A,R,N,I,N, E, Q	129/132 (98%)	A,R,T	V,I,N
MW525117	81/91 (89%)	G,V,E,G,K,S,R, D, I, R	A, A, D,A,R,N,W,E,F,Q	87/91 (96%)	G,E,G,R	A,D,A,W
MW525118	126/133 (95%)	T,K,S,D,I,R,A	A,R,N,E,F,Q,V	131/133 (98%)	A,A	V,V
MW525119	124/133 (93%)	T,K,S,E,T,P,D,I,R	A,R,N,Q,N,L,E,F,Q	129/133 (97%)	A,E,T,P	V,Q,N,L
MZ028630	118/124 (95%)	T,K,S,D,I,R	A,R,N,E,F,Q	123/124 (99%)	A	V
MZ028631	119/125 (95%)	T,K,S,D,I,R	A,R,N,E,F,Q	124/125 (99%)	A	V
MZ028632	119/125 (95%)	T,K,S,D,I,R	A,R,N,E,F,Q	124/125 (99%)	A	V
MZ028633	121/128 (95%)	T,V,K,S,D,I,R	A,A,R,N,E,F,Q	128/128 (100%)	-	-
MZ028634	117/124 (94%)	T,V,K,S,D,I,R	A,A,R,N,E,F,Q	124/124 (100%)	-	-
MZ028635	119/126 (94%)	T,V,K,S,D,I,R	A,A,R,N,E,F,Q	126/126 (100%)	-	-
MZ028636	121/128 (95%)	T,V,K,S,D,I,R	A,A,R,N,E,F,Q	128/128 (100%)	-	-
MZ028637	119/126 (94%)	T,V,K,S,D,I,R	A,A,R,N,E,F,Q	126/126 (100%)	-	-
MZ028638	103/113 (91%)	T,Q,V,E,K,S,L,D,I,R	A,H,A,D,R,N,P,E,F,Q	110/113 (97%)	Q,E,L	H,D,P
MZ028639	114/124 (92%)	T,Q,V,E,K,S,L,D,I,R	A,H,A,D,R,N,P,E,F,Q	121/124 (98%)	Q,E,L	H,D,P
OM158230	119/128 (93%)	I,T,V,K,S,D,I,M,R	N,A,A,R,N.E,F.L,Q	126/128 (98%)	I,M	N,L
OM158231	117/123 (95%)	T,KS,D,I,R	A,R,N,E,F,Q	122/123 (99%)	A	V
OM158232	119/125 (95%)	T,K,S,D,I,R	A,R,N,E,F,Q	124/125 (99%)	A	V
OM158233	113/123 (92%)	T,Q,V,E,K,S,L,D,I,R	A,H,A,D,R,N,P,E,F,Q	120/123 (98%)	Q,E,L	H,D,F

Table 3. Amino acids percent identities and amino acids substitution of F gene of 7 study isolates comparison with Bangladesh-2008-MG581412 and Bangladesh-2015-UQW80114

GeneBank AC. No. F gene sequence	**Amino acids percent identities with Bangladesh-2008-MG581412**	**Amino acids of Bangladesh-2008-MG581412**	**Amino acids substitution of study isolates**	**Amino acids percent identities with Bangladesh-**2015-UQW80114	**Amino acids of Bangladesh** 2015_	**Amino acids substitution of study isolates**
OM992365	145/148 (98%)	L,I,L	I,L,F	145/148 (98%)	L,I,L	I,F,F
OM992366	140/140 (100%)	-	-	140/140 (100%)	-	-
OM992367	145/146 (99%)	R	K	145/146 (99%)	R	K
OM992368	143/144 (99%)	S	I	143/144 (99%)	S	I
OM992369	141/141 (100%)	-	-	141/141 (100%)	-	-
OM992370	140/140 (100%)	-	-	140/140 (100%)	-	-
OM992371	132/132 (100%)	-	-	132/132 (100%)	-	-

Phylogenetic analysis

The phylogenetic tree was constructed based on the partial N gene sequences of 18 PPRV isolates, compared with other representative downloaded PPRV isolates belonging to Lineages I-IV. The study isolates nucleotide had homology (96.73%- 98.19%) with other Asian and African isolates like India, China, Dubai, Iran, Pakistan, Turkey, Sudan and Mongolia belonging to the lineage IV are provided in Figure 1. The N gene studied isolates had homology 94%-99% with other downloaded Bangladeshi PPRV isolates. Phylogenetic analysis revealed that the study isolates made a separate cluster with Bangladeshi isolates and also segregated into separate subclusters among the study isolates. The cluster of study isolates was very close to other previous Bangladeshi PPRV isolates. This study revealed that the partial N gene of PPRV was evolving continuously. Similarly, the phylogenetic tree was constructed based on the F gene sequences of studied 7 PPRV isolates with other representatives downloaded PPRV belonging to Lineages I-IV are provided in Figure 2. The study isolates nucleotide had homology (97.54%-99.29%) with other Asian and African isolates like India, China, Dubai, and Pakistan belonging to the lineage IV. The F gene studied isolates had homology 98%-99% with other downloaded Bangladeshi PPRV isolates. The F gene of the studied isolates (07) made a separate cluster and was also divided into separate subclusters among the study isolates. The cluster of study isolates was very close to other previous Bangladeshi PPRV isolates.

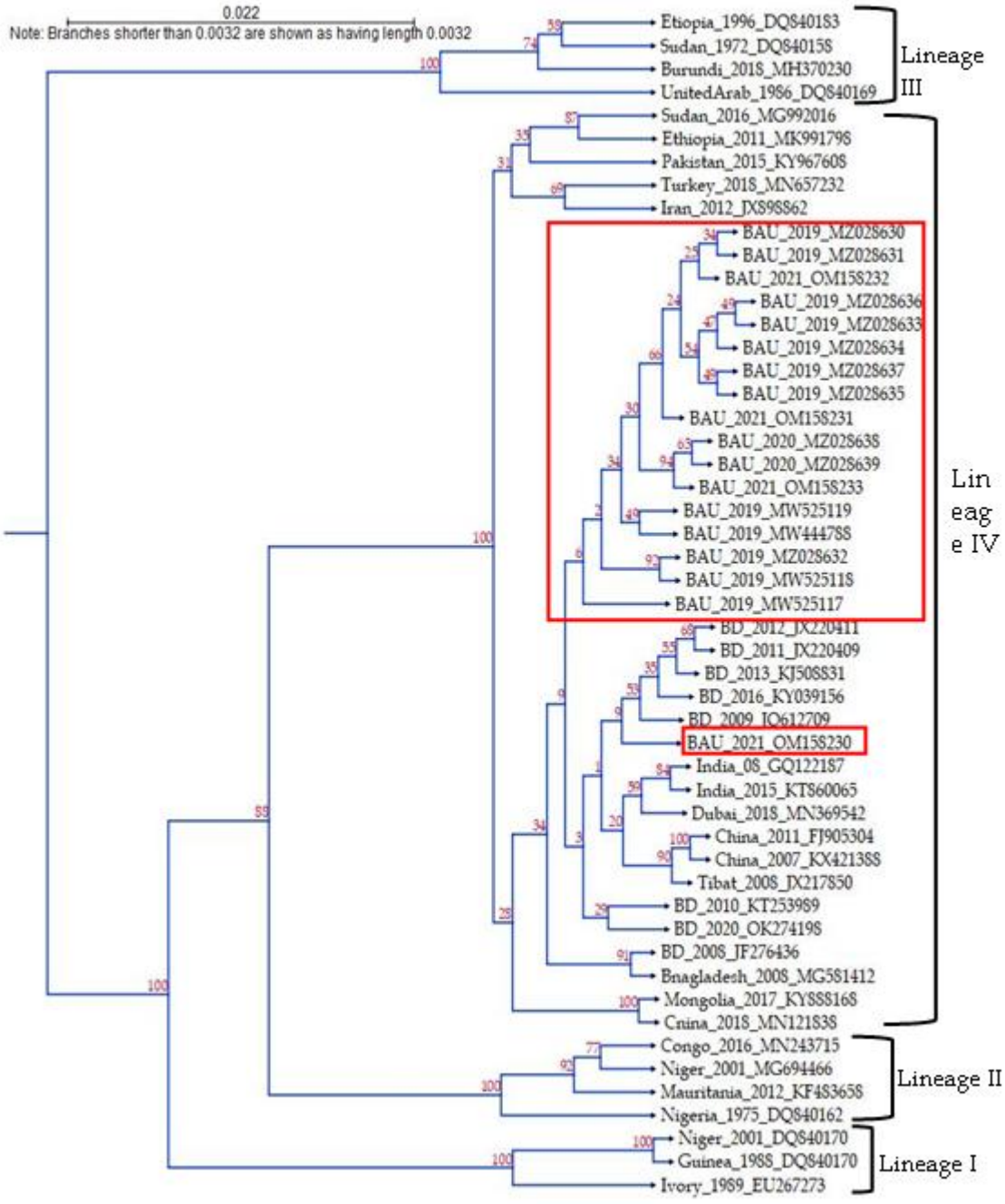

Figure 1. The phylogenetic relationship is based on the partial N gene sequences of studied PPRV isolates compared with other representative PPRVs belonging to Lineages I-IV. The 18 studied N gene isolates made a separate subcluster with other Bangladeshi and Asian isolates belonging to lineage IV.

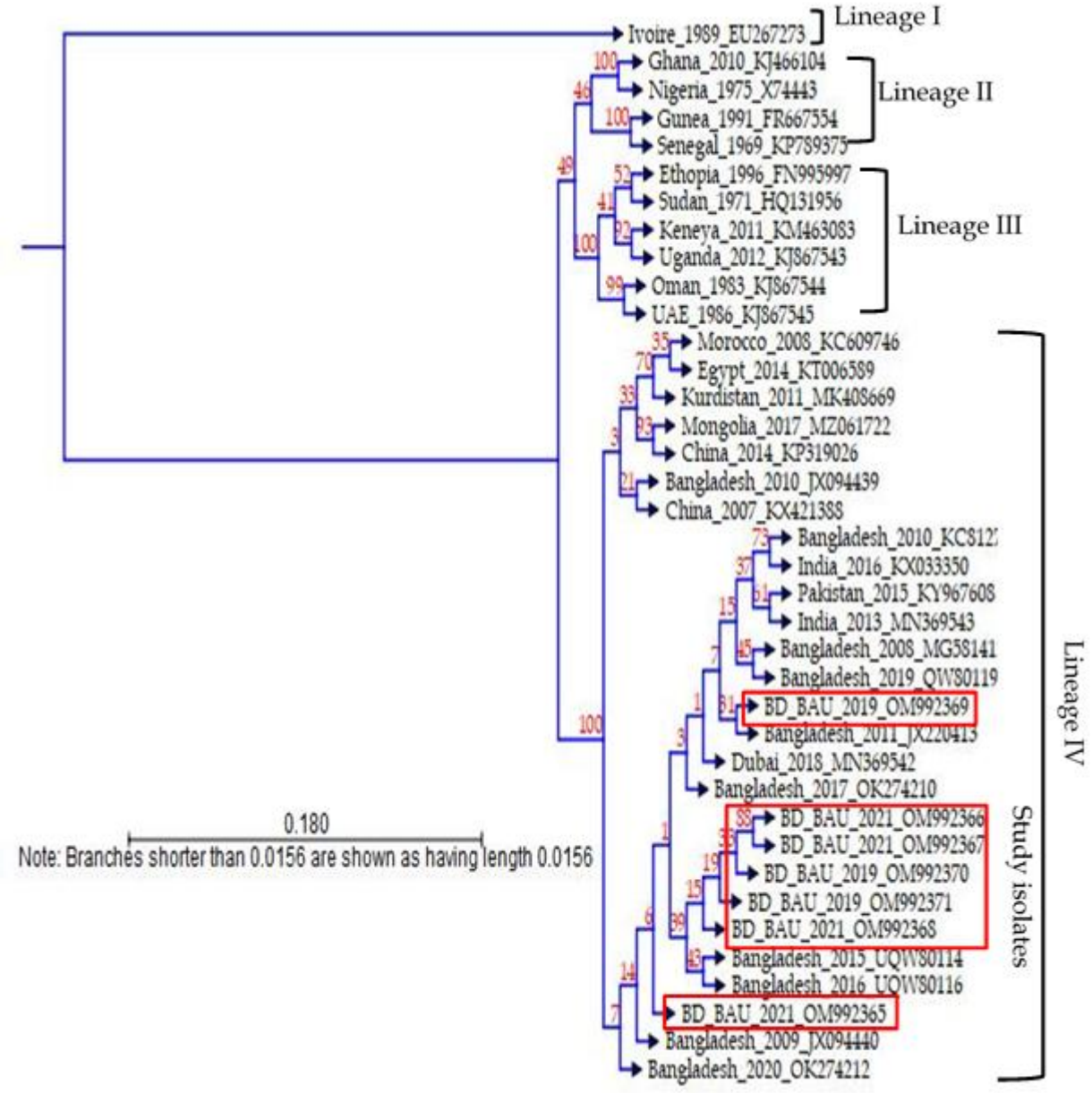

Figure 2. The phylogenetic relationship is based on the partial F gene sequences of studied PPRV compared with other representative PPRVs belonging to Lineages I-IV. The studied F gene isolates made a separate subcluster with other Bangladeshi and Asian isolates belonging to lineage IV.

DISCUSSION

The phylogeny of morbilliviruses shows that PPRV's ancestor may be older than rinderpest or measles, which have been circulating since ancient times (Dux et al., 2020). There is just one serotype of PPRV, and vaccinations are widely accessible to protect against all circulating strains (Hodgson et al. 2018). The evolutionary history of PPRV (Mahapatra et al. 2021), the dynamics of PPR emergence in China (Liu et al. 2018) and the molecular epidemiology of PPRV spillover into wildlife in Mongolia (Benfield et al. 2021) have all been elucidated by PPRV phylogenomic studies. PPRV genome analysis have aided in understanding the formation and attenuation process of PPR vaccination strains (Kwiatek et al. 2022). The necessity of collecting additional PPRV partial and whole genome data to support epidemiologically informed control tactics has increased because to the increasing focus of PPR global eradication efforts on episystems, which are areas of extensive transboundary PPR circulation (FAO, WOAH 2022). For this reason, we took this study for analysing the genome of circulating PPR virus with previous Bangladeshi and other Asian PPRV isolates.

PPR has been endemic in various Asian nations for a long period, including India, where the disease was first formally documented in 1987 but may have been present for much longer (Baron et al. 2016). Later, it was found in Bangladesh in 1993 (Islam *et al.*, 2001), Pakistan in 1994 and Nepal in 1995 (Dhar *et al.*, 2002). PPRV isolates from Bangladesh and India have molecular evidence of transboundary transmission (Clarke *et al.*, 2018). Previous studies reported the PPRV endemic in goats in Mymensingh and Netrokona (Nooruzzaman *et al.*, 2021). In this study, PPRV suspected samples were collected from the Mymensingh division which has a border connection with India. The phylogenetic analysis N gene and F gene of PPRV isolates belongs to the Lineage IV and genetically close to Indian isolates (Clarke *et al.*, 2018). Still now, the other lineages of PPRV (LI, LII, LIII) were not detected in this area, this observation may enable in immunization against PPRV and designing control strategies.

The maximum divergence of 2.2% was reported in the F gene across Bangladeshi PPRV isolates, whereas 8.8% was observed in the N gene fragment (Rahman *et al*., 2016) and another previous study showed that 1.7% divergence was seen in N gene of Bangladeshi PPRV isolates (Clarke *et al*., 2018). This study revealed that 3-6% divergence and 1-2% divergence was observed in N gene and F gene of PPRV isolates respectively which is somewhat difference from previous studies. Nucleotide homogeneity percent identity was 98-99% and the amino acid homogeneity (98-100%) in the F gene of PPRV which was remained unchanged from 2008 to 2021. A limited extent of amino acid substitution happened (5-11%) in the N gene of PPRV-studied isolates from 2008 to 2021 that provide evolutionary history. This study observed F gene is relatively conserved than N gene of PPRV (Rahman *et al*., 2016).

Previous research showed that multiple alignments of the F gene fragment revealed that all Bangladeshi isolates were closely related to the Bhutan/2010 and China/Tibet-2007 isolates, with nucleotide identities of 99.7% and 99.4%, respectively. The F gene sequences of study isolates had the highest homology with India (KX860026/2004) ranged 98.39%-99.29%, then China isolates (KX421388/2007) ranging from 97.54%-98.59%, which slightly differ in this study. This result may support the transboundary transmission of PPRV (Clarke *et al*., 2018).

In contrast, multiple alignments of the N gene fragment revealed that Bangladeshi isolates were extremely close to China/Tibet 2007 isolates, with 94.5-98.8% homology. However, the N gene sequences of study isolates had the highest homology with China (KX4213888/2007), ranged 96.73%-98.19%, which was in line with previous studies. Mutations were observed more in N gene sequence than F gene sequence of PPRV (Rahman *et al*., 2016).

This study describes a details comparative analysis of nucleotide and amino acids of N and F gene of PPRV that is the new findings from previous studies. Aligned with MG581412/2008 and OK274192/2015, it was observed only 6-10 amino acids and only 1-4 amino acid substations respectively, occurred in the N gene of PPRV studied isolates. Alignment with MG581412/2008 and UQW80114/2015, it was observed that 1-3 amino acid were substituted in the F gene of 3 studied isolates and 4 samples appeared at 100% identical. It was concluded that over one decade the F gene of PPRV remained unchanged that indicates F gene poses conserved regions and mutation of amino acids was very slow or rare.

CONCLUSIONS

The N gene of PPRV (18 isolates) nucleotide analysis revealed that the nucleotide divergence highest was 3-6% and only 1-3% when aligned with BD-2008/MG581412 and BD-2015/OK274192 isolates, respectively. The mutation of N gene of PPRV is slow but continuously evolved. The F gene of PPRV (7 isolates) nucleotide sequence analysis revealed that the nucleotide divergence was only 1-2% when aligned with both (MG581412/2008) and (UQW80114/2015), which remained unchanged from 2008 to 2021. The amino acids divergence of N gene and F gene were 0-11% and 0-2%, respectively. The F gene is relatively more conserved than the N gene of PPRV. The highest homology of N gene sequences with China isolates (98.19%) and the F gene sequences with India isolates (99.29%), respectively, and genetically belong to Lineage IV. The knowledge from such studies will aid in attaining the goal of managing and eradicating the PPR disease, especially because the country intends to initiate control programs in many districts following the successful eradication of the Rinderpest.

COMPETING INTEREST

The authors declare no conflict of interest.

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Authors contributions: Khan MAHNA and Sultana S designed the study, generated research funds, and tuned the manuscript for submission. Sultana S, N Sultana, D Parvin and M Pervin were involved in field sample collection and processing. Sultana S did nucleotide, amino acid sequence and phylogenetic analysis. MM Islam made an intellectual effort in the drafted manuscript. Sultana S and Khan MAHNA drafted and revised the final manuscript. All authors read and approved the final submitted version of the manuscript.

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