



# Isolation and molecular characterization of GP5 glycoprotein gene of *Betaarterivirus suis* 2 from Mizoram, India

Fatema Akter<sup>1</sup> · Parimal Roychoudhury<sup>1</sup> · Tapan Kumar Dutta<sup>1</sup> · Prasant Kumar Subudhi<sup>1</sup> · Sanjeev Kumar<sup>1</sup> · Jagan Mohanarao Gali<sup>2</sup> · Parthasarathi Behera<sup>2</sup> · Yengkhom Damodar Singh<sup>3</sup>

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**Abstract** Porcine reproductive and respiratory syndrome (PRRS) is a serious swine disease causing great economic impact worldwide. The emergence of highly pathogenic strains in Asian countries is associated with large scale mortality in all age groups of pigs besides the classical presentation of severe respiratory distress, pneumonia, and a series of reproductive disorders in sows, like late-term abortion, premature farrowing, and an increased number of stillborn piglets. The present study was designed with the aim of isolation and characterization of the *Betaarterivirus suis* 2 from outbreaks in Mizoram in primary porcine alveolar macrophage and subsequently characterized the GP5 gene sequence of the isolate in terms of phylogenetic analysis and deduce amino acid sequence comparison. Virus propagation was performed in the porcine alveolar macrophage (PAM) primary cell culture and confirmed by immunoperoxidase test, FAT, and nested RT-PCR. The full-length GP5 gene (603nt) was amplified from the isolate and subsequently cloned and sequenced (MN928985). Phylogenetic analysis and sequence comparison of the present isolate was found to have similarity 98.7–98.8% with Myanmar HP-PRRS strains, 98–98.5% with Vietnam strains, 98.2–98.3% with China strains, indicating a close

lineage with highly pathogenic PRRS strains. In deduced amino acid sequence analysis, one mutation was found in the primary neutralizing epitope (PNE) at position <sup>39</sup>L → I<sup>39</sup> and one more mutation was also found in the decoy epitope (DCE) at position <sup>30</sup>N → D<sup>30</sup>. The amino acid at this position is an N-linked glycosylation site, and mutation of the N-linked glycosylation is an immune escaped strategy adopted by this virus causing a persistent infection in the natural host.

**Keywords** Porcine reproductive and respiratory syndrome · *Betaarterivirus suis* 2 · Isolation · GP5 glycoprotein · Molecular characterization

## Introduction

Porcine reproductive and respiratory syndrome (PRRS) is a major systemic viral infection of pigs and is recognized as an important viral disease of swine causing severe economic losses of pig husbandry worldwide. Since its first occurrence in 1987 in North America and 1991 in Europe, the disease has spread to Southeast Asian countries [16] and was introduced to India in 2013 in Mizoram [29]. The emergence of highly pathogenic strain and its rapid spread in Asian countries become a major concern in pig husbandry practice in this region as diseased animals exhibit severe respiratory distress, pneumonia, and a series of reproductive disorders in sows, like late-term abortion, premature farrowing, an increased number of stillborn piglets including high mortality by highly pathogenic strains [1–3, 32–34]. The disease is caused by *Betaarterivirus suis* 1 (European type) and *Betaarterivirus suis* 2 (North American type) of the subgenus *Eurpobartevirus* and *Ampobartevirus* respectively under the genus

✉ Fatema Akter  
dr.fatemaakter16@gmail.com

<sup>1</sup> Department of Veterinary Microbiology, College of Veterinary Sciences and Animal Husbandry, CAU, Aizawl, Mizoram, India

<sup>2</sup> Department of Veterinary Physiology and Biochemistry, College of Veterinary Sciences and Animal Husbandry, CAU, Aizawl, Mizoram, India

<sup>3</sup> Department of Veterinary Pathology, College of Veterinary Sciences and Animal Husbandry, CAU, Aizawl, Mizoram, India

*Betaarterivirus* of the subfamily *Variarterivirinae* of the family *Arteriviridae* in the order *Nidovirales*. However, both species are genetically diverse (60% homology) [23] and the North American type is mostly circulating in this region. The virus is having a selective tropism for porcine alveolar macrophages (PAM) expressing CD163 and sialoadhesin virus receptors and can be best isolated in primary PAM culture [1, 33, 34] as well as MARC-145 cells expressing the receptors [18, 33, 34]. The virus genome is around 15.1–15.5 kb having ten overlapping open reading frames (ORF) [7]. Immunogenic glycoprotein GP5 coded by ORF5 is around 603 nt length (MK315208) has been targeted for the development of recombinant vaccines [10, 25]. ORF5 sequence is used for phylogenetic study and virus characterization [5]. Since its first outbreaks in 2013 in Mizoram, subsequent years in 2015, 2016, and 2018 outbreaks of the disease have been reported [29, 30, 42]. This study was designed to isolate the virus from an outbreak in Mizoram, India, and to study the GP5 gene sequence.

## Materials and methods

### Virus isolation

Isolation of the virus was performed using archival tissue samples preserved in the Department of Veterinary Microbiology from the previous outbreaks of PRRS in Mizoram, India reported by Zohlimpuia et al. [42]. The archival samples used are lung and spleen tissues of dead piglets which were collected in the 2016 PRRS outbreak from Lawngtlai and Saiha districts of Mizoram. One isolate (MZ/5/2016) was used for molecular characterization in our present study. Porcine alveolar macrophage (PAM) primary cell culture was prepared from bronchoalveolar lavage from a 3 months old pig as per the method described by Mayer and Lam, 1984 [20]. These terminally differentiated PAM cells were used for virus isolation.

### Virus detection

After 72 h of incubation at 37°C in a CO<sub>2</sub> incubator, the infected PAM cells were processed for virus growth detection. Growth of the virus was detected by immunoperoxidase test (IPT) using rabbit anti GP5 antibody (Cat. No. bs-4504R, Bioss Antibodies) and DAB nickel substrate (Ref. No. SK-4100, Vector Laboratories). Further fluorescent antibody test (FAT) using a rabbit anti GP5 antibody (Cat. No. bs-4504R, Bioss Antibodies) and anti-rabbit FITC conjugate (Cat. No. FTC2, Genei) was performed following the method described by OIE, 2018 [24]. A nested reverse transcriptase-polymerase chain

reaction (RT-PCR) was carried out to detect the virus by molecular method [9].

### Virus titration

One of the positive isolates was passaged in PAM cells twice and then virus titer was determined. A five-fold dilution of the virus isolate was prepared in the RPMI-1640 media without added serum and inoculated in a 96 well cell culture plate (Cat. No. 260836, Thermo Scientific) keeping 5 replica. Then the plate was incubated for 3 days and processed for IPT staining as described by OIE, 2018 [24]. TCID<sub>50</sub> value was determined by the Spearman-Kärber method.

### GP5 gene sequence analysis

RNA extraction was carried out from the virus isolate using the QIAamp Viral RNA extraction kit (Cat. No. 52904 QIAGEN) as per the manufacturer's protocol. The cDNA was synthesized using the Maxima H Minus First Strand kit (Cat. No. K1651, Thermo Scientific) as per the manufacturer's protocol. The full-length GP5 gene was amplified using published primers [40] (Table 1) and was cloned in a TA cloning vector and sequenced by outsourcing. A reaction mixture of 25 µl containing 2.5 µl of 10X Taq buffer with 20 mM MgCl<sub>2</sub>, 2 µl of 10 mM dNTPs, 1 µl of each of 10 pM forward and reverse primers, 0.2 µl of 5 U/µl Taq polymerase, and 2 µl of cDNA as template was prepared. Cycling condition was carried out as an initial denaturation at 94°C for 2 min, and 30 cycles of denaturation at 94 °C for 30 s, annealing at 56.1 °C for 45 s and extension at 72°C for 30 s. A final extension was set at 72 °C for 10 min. The amplified product was verified on 1.5% agarose gel electrophoresis and was gel eluted using GeneJET Gel Extraction Kit (Cat. No. K0691, Thermo Scientific) as per the manufacturer's protocol. The purified product was used for cloning using InsTAclone PCR Cloning Kit (Cat. No. K1213, Thermo Scientific) as per the manufacturer's protocol. The gel eluted PCR product was ligated with pTZ57R/T cloning vector and transformation was performed in competent DH5α (mutant *Escherichia coli*). The transformed cells were plated in LB ampicillin and incubated for 18 h at 37 °C. The clone was confirmed by colony PCR and subsequently sequenced by outsourcing. Phylogenetic analysis was carried out based on the full-length GP5 sequence using MEGA9 software. The phylogenetic tree was generated by using the Neighbor-Joining method, keeping bootstrap consensus from 1000 replicates. Deduced amino acid sequence of GP5 was compared using BioEdit software, with sequences of previous outbreaks and prototype and vaccine virus sequences retrieved from GenBank.

**Table 1** Primer sequences used in the present study

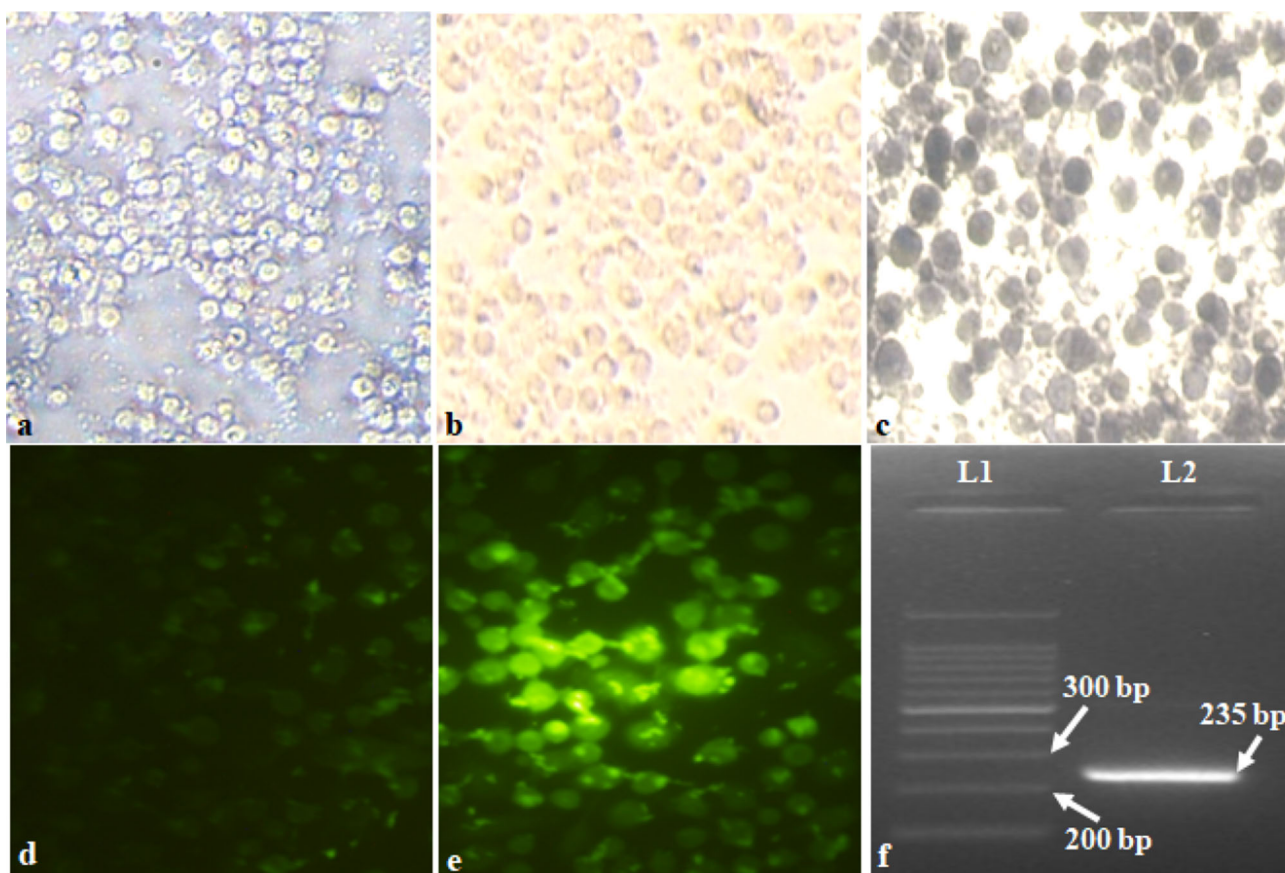
Primer name	Sequence	Amplicon size	Use	References
US OUT F	TCGTGTTGGGTGGCAGAAAAGC	484 bp	Detection of <i>Betaarterivirus suid 2</i> by nested RT-PCR	Christopher-Hennings et al. [30]
US OUT R	GCCATTCACCACACATTCTTCC			
US IN F	CCAGATGCTGGGTAAGATCATC	235 bp		
US IN R	CAGTGTAAGTATCCTCCCTGA			
GP5 Forward	CGGGAATTCATGTTGGGGAAATGC	603 bp	Amplification of full-length GP5 gene	Xia et al. [42]
GP5 Reverse	ATACTCGAGCTAGAGACGACCCCATG			

## Results and discussion

### Virus isolation

After 24 h incubation, terminally differentiated healthy PAM cells attaching to the surface could be observed

(Fig. 1a). Isolation of the virus was detected by immunoperoxidase test, fluorescent antibody test as well as by nested RT-PCR. Primary porcine alveolar macrophage (PAM) cell culture is a standard system for the isolation of *Betaarterivirus suid 2*. This virus can be isolated in very few cell types of porcine and monkey origin like MARC-



**Fig. 1** Isolation and detection of *Betaarterivirus suid 2*. **a** Harvested Healthy PAM cells (400X). **b** Control PAM cells after IPT staining (400X). **c** Infected PAM cells after IPT staining showing the color of the substrate (gray-black) (400X). **d** Control PAM cells after FAT

staining (400X). **e** Infected PAM cells after FAT staining showing apple-green fluorescence (400X). **f** Positive amplicon of 235 bp of target product observed after nested PCR

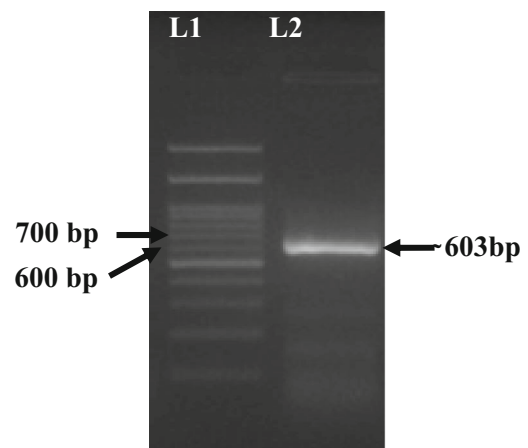
145. In our present study, we could isolate this virus in PAM cells and after two passages virus titer of  $10^{5.55}$  TCID<sub>50</sub>/ml could be achieved. PAM cells have been used extensively for the isolation of *Betaarterivirus suid 2* in different outbreaks [1, 33]. However, a cell line derived from MA-104 cells expressing high and stable porcine CD163 named MARC-145 is a very suitable cell line for isolation of the North American genotype of the virus and is also used for vaccine production [18, 33, 34, 39]. A newly derived cell line SJPL from monkey origin was found to be permissive for this virus and reported to be an additional tool to study in vitro pathogenesis [27].

### Detection of *Betaarterivirus suid 2*

Virus isolation was detected by immunoperoxidase test and infected cells appeared gray-black color indicating virus infected cells (Fig. 1c) than compared with no color development in uninfected control cells (Fig. 1b). Virus growth was also confirmed by indirect fluorescent antibody test and a distinct apple-green fluorescence of infected cells (Fig. 1e) could be seen under a fluorescent microscope (Olympus, Model no. IX51) whereas uninfected control cells did not produce any fluorescence (Fig. 1d). Further virus growth was detected by molecular detection by reverse transcriptase nested polymerase chain reaction (RT-nPCR) test and targeted amplification of 235 bp product could be detected from the sample (Fig. 1f). Detection of this virus in a cell culture system can be done by indirect immunoperoxidase test as well as by fluorescent antibody test, which are standard tests for the detection of viral infection in cell culture. Viral antigen expressed on the surface of infected cells by non-cytopathic or mild cytopathic viruses can be detected by these tests as well as can be used for virus titration assay [11, 35, 37]. In viral pathogenicity studies, an immunoperoxidase test is a powerful tool for in situ detection of the virus in infected tissues [13]. FAT is a highly sensitive assay for the accurate detection of virus-laden cells. The test can detect even when a fewer number of infected cells are present in the monolayer [1, 6, 19, 36]. Molecular detection can be done by OIE recommended RT-PCR protocol which is considered to be a standard procedure for confirming the virus nucleic acid in the sample. This assay can detect both *Betaarterivirus suid 1* and *2* viruses and has been reported in many literatures [9, 8, 17]. Nested PCR reactions are very sensitive and specific and can detect even from the sample with a low virus load [21]. However, the test cannot differentiate the genotypes of the virus.

### Molecular characterization of GP5 gene of *Betaarterivirus suid 2*

Positive amplification of full-length GP5 of 603 bp was observed after electrophoresis (Fig. 2) and the present isolate was cloned and sequenced and also annotated and submitted to the GenBank and accession number received is MN928985 for the present sequence. Phylogenetic analysis was carried out by comparing with GP5 sequences of previous outbreaks in India as well as different outbreaks of south-east Asian countries including China, prototype VR-2332, and Lelystad Virus (LV) strains and vaccine strains (Table 2). The present isolate was placed within a cluster of Indian isolates of 2016 and 2018 recorded outbreaks of Mizoram, India (Fig. 3). Sequence similarity within this cluster was ranged between 96.6 and 100%. However, the sequence of the first outbreak of PRRS in India during 2013 was found phylogenetically placed in a different clade within the same cluster. Sequence similarity with 2013 isolates was found to be 97.7–98.2%. When compared with the highly pathogenic strains from other south-east Asian countries present sequence showed a phylogenetic similarity with all those reported sequences. Sequence similarity of the present isolate was found 98.7–98.8% with Myanmar HP-PRRS strains, 98–98.5% with Vietnam strain, 98.2–98.3% with China strains (Table 3). A phylogenetic cluster of present isolate within the NA strains and a close similarity with the highly pathogenic strains indicates the present isolate is a highly pathogenic strain of *Betaarterivirus suid 2* (North American genotype). All the strains of *Betaarterivirus suid 1* were forming a separate branch in the phylogenetic tree and are also phylogenetically diverse from NA strains with a homology percent of 62.3%. The deduced amino acid sequence of GP5 protein of the present isolate was compared with other sequences of genotype 2 from the



**Fig. 2** Full-length GP5 amplification of *Betaarterivirus suid 2*

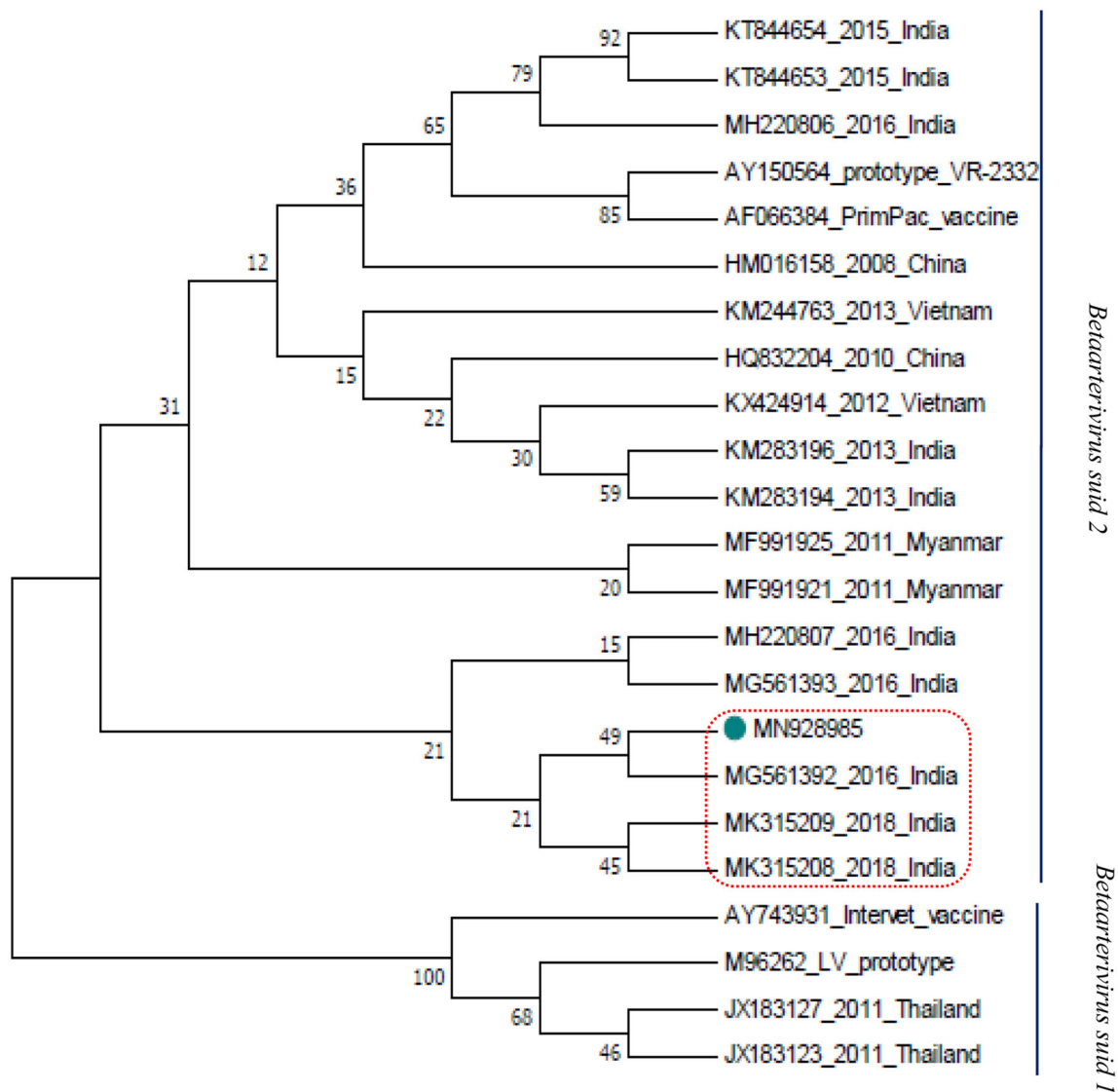


**Table 2** Sequence used for analysis of the present isolate

Accession number	Country	Year	Strain/isolate	Genotype
MN928985	India	2016	MZ/5/2016	<i>Betaarterivirus suid 2</i>
AY150564	North America	2002	VR-2332	Prototype of <i>Betaarterivirus suid 2</i>
M96262	Netherlands	2000	Lelystad virus	Prototype of <i>Betaarterivirus suid 1</i>
KM283195	India	2013	PRRS/MZ/AZ/50/13	<i>Betaarterivirus suid 2</i>
KM283196	India	2013	PRRS/MZ/AZ/49/13	<i>Betaarterivirus suid 2</i>
KT844653	India	2015	PRRS/MZ/IND/5/15	<i>Betaarterivirus suid 2</i>
KT844654	India	2015	PRRS/MZ/IND/2/15	<i>Betaarterivirus suid 2</i>
MG561392	India	2016	MZ/1/2016	<i>Betaarterivirus suid 2</i>
MG561393	India	2016	MZ/2/2016	<i>Betaarterivirus suid 2</i>
MK315208	India	2018	PRRSV/MZ/IND/399A/18	<i>Betaarterivirus suid 2</i>
MK315209	India	2018	PRRS/MZ/IND/DBT1/18	<i>Betaarterivirus suid 2</i>
MH220806	India	2016	ML-8	<i>Betaarterivirus suid 2</i>
MH220807	India	2016	ML-9	<i>Betaarterivirus suid 2</i>
MF991921	Myanmar	2011	HP/MYANMAR/1908AM/2011	<i>Betaarterivirus suid 2</i>
MF991925	Myanmar	2011	HP/MYANMAR0411AM/2011	<i>Betaarterivirus suid 2</i>
KX424914	Vietnam	2012	HUA/HP5	<i>Betaarterivirus suid 2</i>
KM244763	Vietnam	2013	MN1	<i>Betaarterivirus suid 2</i>
HQ832204	China	2010	HUBWH10	<i>Betaarterivirus suid 2</i>
JQ663553	China	2010	10-10HEB-3	<i>Betaarterivirus suid 2</i>
JX183123	Thailand	2011	SCP0311EU/1	<i>Betaarterivirus suid 1</i>
JX183127	Thailand	2011	FDT0111EU2/1 (clone)	<i>Betaarterivirus suid 1</i>
AY743931	Italy	2004	Intervet vaccine strain	<i>Betaarterivirus suid 1</i>
AF066384	USA	1998	PrimePac PRRS vaccine acronym: PRRS virus	<i>Betaarterivirus suid 2</i>

GenBank. The deduced amino acid sequence showed a total of 26 mutations in the signal peptide domain including decoy epitope (DCE), ectodomain including primary neutralizing epitope (PNE) (Table 4), transmembrane domain, and endodomain region. There are five N-linked (Asparagine-linked) glycosylation sites in the amino acid position viz. N30, N32/ N33/ N34, N35, N44, N51 of the GP5 glycoprotein of *Betaarterivirus suid 2* [22, 38]. The first potential N-linked glycosylation site of this virus is N32/N33/N34, the second and third potential N-linked glycosylation sites are N44 and N51 respectively reported by Music and Gagnon, 2010 [22]. They also mentioned a fourth potential N-linked glycosylation site at position N30 among few NA strains such as VR-2332 and Quebec IAF Klop. N30 comes under the decoy epitope (DCE) which comprises four amino acids at position <sup>27</sup>VLAN<sup>30</sup> in the NA prototype VR-2332. DCE delays the neutralizing antibody response induced by primary neutralizing epitope (PNE)<sup>20</sup>. In our study we could observe the present isolate does not have N-linked glycosylation at position N30, rather the asparagine (N) has been substituted by aspartic acid (D) (<sup>30</sup>N → D<sup>30</sup>). This change was not observed in the viruses of the 2013, 2015, and 2018 outbreaks in

Mizoram. The substitution of amino acid of the present isolate was observed within the PNE in position 39 where leucine (L) has been substituted by isoleucine (I) (<sup>39</sup>L → I<sup>39</sup>) when compared with prototype strain. The same change has also been observed from Mizoram and Meghalaya isolates. In the present study, some random amino acid substitutions in the signal peptide, ectodomain, transmembrane domain, and endodomain were also observed when compared with prototype VR-2332. Amino acid substitutions observed in the position <sup>3</sup>E → G<sup>3</sup>, <sup>9</sup>G → C<sup>9</sup>, <sup>10</sup>C → S<sup>10</sup>, <sup>16</sup>S → F<sup>16</sup>, <sup>23</sup>F → S<sup>23</sup>, <sup>24</sup>C → Y<sup>24</sup>, <sup>25</sup>F → L<sup>25</sup> within the signal peptide; <sup>34</sup>D → S<sup>34</sup>, <sup>35</sup>S → N<sup>35</sup>, <sup>58</sup>N → Q<sup>58</sup> within the ectodomain; <sup>66</sup>S → T<sup>66</sup>, <sup>92</sup>A → G<sup>92</sup>, <sup>94</sup>V → A<sup>94</sup>, <sup>101</sup>F → Y<sup>101</sup>, <sup>102</sup>V → Y<sup>102</sup>, <sup>121</sup>T → I<sup>121</sup>, <sup>127</sup>F → L<sup>127</sup> within the transmembrane domain; <sup>137</sup>A → S<sup>137</sup>, <sup>161</sup>I → V<sup>161</sup>, <sup>164</sup>R → G<sup>164</sup>, <sup>170</sup>E → G<sup>170</sup>, <sup>185</sup>V → A<sup>185</sup>, <sup>189</sup>I → L<sup>189</sup>, <sup>200</sup>P → L<sup>200</sup> within the endodomain. The similar amino acid substitutions of other isolates of 2013, 2015, and 2016 outbreaks of Mizoram were also reported by Rajkhowa et al. [30] at the position <sup>3</sup>E → G<sup>3</sup>, <sup>9</sup>G → C<sup>9</sup>, <sup>10</sup>C → S<sup>10</sup>, <sup>16</sup>S → F<sup>16</sup>, <sup>23</sup>F → S<sup>23</sup>, <sup>24</sup>C → Y<sup>24</sup>, <sup>25</sup>F → L<sup>25</sup>, <sup>34</sup>D → S<sup>34</sup>, <sup>35</sup>S → N<sup>35</sup>, <sup>58</sup>N → Q<sup>58</sup>, <sup>66</sup>S → T<sup>66</sup>,



**Fig. 3** Phylogenetic tree generated on the basis of full-length GP5 (603nt) of present isolate when compared with sequences of previous outbreaks in India and other countries (both type 1 and type 2 strains) including vaccine strains using MEGA9 software

<sup>92</sup>A → G<sup>92</sup>, <sup>94</sup>V → A<sup>94</sup>, <sup>101</sup>F → Y<sup>101</sup>, <sup>102</sup>V → Y<sup>102</sup>, <sup>121</sup>T → I<sup>121</sup>, <sup>127</sup>F → L<sup>127</sup>, <sup>137</sup>A → S<sup>137</sup>, <sup>161</sup>I → V<sup>161</sup>, <sup>164</sup>R → G<sup>164</sup>, <sup>170</sup>E → G<sup>170</sup>, <sup>185</sup>V → A<sup>185</sup>, <sup>189</sup>I → L<sup>189</sup>, <sup>200</sup>P → L<sup>200</sup>. However, some changes were not observed in our present isolate at the position <sup>6</sup>L → L/S<sup>6</sup>, <sup>11</sup>C → C/Y<sup>11</sup>, <sup>34</sup>D → N<sup>34</sup>, <sup>58</sup>N → K<sup>58</sup>. GP5 gene (ORF5) is the most commonly targeted gene for the phylogenetic analysis of this virus strain. Since ORF5 is the most variable region containing both neutralizing and non-neutralizing epitopes, N-linked glycosylation sites, apoptotic cell death induced region; so phylogenetic differences are more prominent in this region [12, 14, 26]. The close similarity of Indian strains and their relation with other highly pathogenic strains were also reported by Rajkhowa et al. [28];

Zohlimpua et al. [42]; Chaithra et al. [4]. If we observe the timeline of the highly pathogenic strain of *Betaarterivirus suid 2* outbreaks in the south-east Asian region then it can be assumed that the virus has entered Mizoram from neighboring Myanmar. Close sequence identity within the Mizoram outbreaks was also recorded by Rajkhowa et al. [28] where a sequence similarity of 99.4–100% was observed within 2015 outbreaks and in comparison to the 2013 outbreak, it was reported 96.15–97.35%. Similarly compared with highly pathogenic 10 HEB-3 strain and 2013 outbreak was 98.33–99.17%. In another observation by Zohlimpua et al. [42] reported 98.3–100% similarity among the four isolates of the 2016 outbreak in Mizoram, 98.2–99.7% similarity with Meghalaya isolates. He also

**Table 3** Sequence homology study of present isolate with other published isolates

Percent identity with published sequences	GenBank published sequences
88.3	AY150564 prototype VR-2332
97.7	KM283194 2013 Mizoram, Pig
98.2	KM283196 2013 Mizoram, Pig
96.2	KT844653 2015 Mizoram, Pig
95.9	KT844654 2015 Mizoram, Pig
100	MG561392 Mizoram 2016, Pig lung Lwangtlai
99.2	MG561393 Mizoram 2016, Pig lung Saiha
98.8	MK315208 Mizoram 2018
98.3	MK315209 Mizoram 2018
96.6	MH220806 Meghalaya 2016, Pig serum
99	MH220807 Meghalaya 2016, Pig serum
98.8	MF991921 Myanmar 2011, highly pathogenic strain, serum, wild boar
98.7	MF991925 Myanmar 2011, highly pathogenic strain, serum, wild boar
98.5	KX424914 Vietnam 2012, NA, wild boar
98	KM244763 Vietnam 2013, highly pathogenic strain, Pig
98.2	HM016158 China 2008, highly pathogenic strain, Pig
98.3	HQ832204 China 2010, NA, Pig
89.8	AF066384 USA, PrimePac PRRS Vaccine
63.3	M96262 Lelystad virus, Prototype <i>Betaarterivirus suid 1</i>
63.3	AY743931 Italy, Intervet Vaccine
62.5	JX183123 Thailand 2011, <i>Betaarterivirus suid 1</i> , Pig
62.4	JX183127 Thailand 2011, <i>Betaarterivirus suid 1</i> , Pig

observed 98–99% sequence similarity with the highly pathogenic strains of the 2011 outbreak in Myanmar. Amino acid substitution at DCE was also reported by Yu et al. [41] and Hong et al. [15] from China. N-linked glycan of GP5 ectodomain is important for the biological activity of the protein. Modification of this glycosylation site is one way of altering the receptor binding site, membrane fusion, and entry and exit process from the infected cells. Studies on enveloped viruses revealed that glycosylation of viral envelope protein is a major mechanism of immune escape as well as viral persistence or minimize the virus-neutralizing antibody response [2, 31]. Ansari et al. [2] reported that the loss of glycan residues in positions N34, N44, N51 enhances the immunogenicity of the nearby neutralizing epitope. The PNE within GP5 of NA prototype VR-2332 comprises 9 amino acids ranging from position 37 to 45 (<sup>37</sup>SHLQLIYNL<sup>45</sup>). Substitution within PNE was also observed among HP-PRRS strains in China (JXA1, HUN4, JXwn06) which was assumed to be due to large scale vaccination with modified live vaccines (MLV) in China.

This type of alteration in neutralizing epitope maybe because of the immune escape strategy adapted by the virus as mentioned by Hong et al. [15].

## Conclusion

In conclusion, based on the complete gene sequence of *Betaarterivirus suid 2* GP5 gene analysis of one isolate of 2016 outbreak in Mizoram revealed that the isolate belonged to the highly pathogenic strain which is circulating in South East Asian countries. Our phylogenetic analysis indicates that 95.9–100% sequence similarity with previous outbreaks in Mizoram and 98.7–98.8% with neighboring Myanmar isolates. Furthermore, deduced amino acid sequence analysis reveals a mutation in the primary neutralizing epitope (PNE) as well as an amino acid substitution in decoy epitope which has not been reported earlier from India. Periodical sequence analysis of

**Table 4** Amino acid sequence of decoy epitope (DCE) and primary neutralizing epitope (PNE) of GP5

Accession no	Decoy epitope (DCE) ( <sup>27</sup> VLAN <sup>30</sup> )				Primary Neutralizing Epitope (PNE) ( <sup>37</sup> SHQLIYNL <sup>45</sup> )								
	27	28	29	30	37	38	39	40	41	42	43	44	45
AY150564 VR-2332	V	L	A	N	S	H	L	Q	L	I	Y	N	L
MN928985 (Present study) MZ/5/2016 Mizoram 2016	V	L	A	D	S	H	I	Q	L	I	Y	N	L
KM283196 Mizoram 2013	V	L	A	N	S	H	I	Q	L	I	Y	N	L
KT844653 Mizoram 2015	V	L	A	N	S	H	I	Q	L	I	Y	N	L
KT844654 Mizoram 2015	V	L	A	N	S	H	I	Q	L	I	Y	N	L
MG561392 Mizoram 2016	V	L	A	D	S	H	I	Q	L	I	Y	N	L
MG561393 Mizoram 2016	V	L	A	N	S	H	I	Q	L	I	Y	N	L
MH220806 Meghalaya 2016	V	L	A	N	S	H	I	Q	L	I	Y	N	L
MH220807 Meghalaya 2016	V	L	A	N	S	H	I	Q	L	I	Y	N	L
MK315208 Mizoram 2018	V	L	A	N	S	H	I	Q	L	I	Y	N	L
MK315209 Mizoram 2018	V	L	V	N	S	H	I	Q	L	I	Y	N	L
MF991921 Myanmar 2011	V	L	A	N	S	H	I	Q	L	I	Y	N	L
MF991925 Myanmar 2011	V	L	A	N	S	H	I	Q	L	I	Y	N	L
KX424914 Vietnam 2012	V	L	A	N	S	H	I	Q	L	I	Y	N	L
KM244763 Vietnam 2013	V	L	A	N	S	H	I	Q	L	I	Y	N	L
HM016158 China 2008	V	L	A	N	S	H	I	Q	L	I	Y	N	L
HQ832204 China 2010	V	L	A	N	S	H	I	Q	L	I	Y	N	L
AF066384 PrimPac Vaccine	V	L	V	N	S	H	L	Q	L	I	Y	N	L

the virus associated with the outbreak is necessary to understand the disease status in this region.

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