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2 **Running Title:** Novel FMDV Asia1 lineage BD-18 (G-IX)

3 **Keywords:** Emergence, Foot-and-Mouth Disease Virus, Serotype, Asia1, Bangladesh, Novel
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6 **Emergence of Novel Lineage of Foot-and-Mouth Disease Virus Serotype Asia1 BD-18 (G-**
7 **IX) in Bangladesh**

8 **Authors:** M. Rahmat Ali¹, A. S. M. R. U. Alam^{1,2}, M. Al Amin, Mohammad A. Siddique,
9 Munawar Sultana, M. Anwar Hossain²

10 **Affiliations:**

11 University of Dhaka, Dhaka-1000, Bangladesh (M.R. Ali, A.S.M. Alam, M.A. Amin, M.
12 Siddique, M. Sultana, M.A. Hossain)

13 ¹These authors contributed equally to this article.

14 ²Current affiliation: Jessore University of Science & Technology, Jessore-7408, Bangladesh

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16 **Abstract**

17 In 2018, a novel circulatory foot-and-mouth disease virus serotype Asia1 BD-18 (G-IX)
18 lineage containing a unique mutation has emerged in Bangladesh. This emergence may be
19 following the evolutionary roadmap of previously reported lineage. Inappropriate vaccination
20 and inefficient outbreak surveillance possibly contributed to the current episode of emergence.

21 **Text**

22 Foot-and-Mouth Disease is an endemic animal disease of transboundary importance in
23 Bangladesh (1, 2). This economically devastating, contagious disease is caused by FMD virus
24 (FMDV; *Picornaviridae* family, *Aphthovirus* genus) which exists as seven serotypes (O, A, C,
25 Asia1, SAT 1-3), each divided into topotypes, lineages, sublineages and strains (3). Among
26 them, three serotypes (O, A and Asia1) have been circulating in Asia and serotype Asia1 is the
27 least prevalent (1, 3, 4).

28 Serotype Asia1 is unique to Asia, and was first detected in India in 1951-52 and in
29 Pakistan in 1954 (3-5). Previously this serotype was classified into lineages (6), while Valarcher
30 et al. (2009) divided these FMDVs into groups based on 5% nucleotide differences (4). FAO
31 World Reference Laboratory for FMD (WRLFMD) has currently established a standard
32 reference list for the nomenclature of FMDV serotypes (7). According to that, the topotype for
33 Asia1 serotypes is ASIA and the lineages are G-I to G-VIII (4, 5, 8). Interestingly, Valarcher et
34 al. (2009) designated previous lineage D as G-III (4) and Subramaniam et al. (2013) designated
35 strains circulating after 2005 under previous lineage C as G-VIII (8), while both lineages were
36 endemic in Indian subcontinent (6). In recent years (2016-17), Sindh-08 (G-VII) is the only
37 lineage of serotype Asia1 that is in continuous circulation in its endemic region (9) whereas
38 outbreaks with other lineage G-VIII were reported sporadically (10, 11).

39 The last report of Asia1 from Bangladesh was in 2013 (11, 12), while all of the previous
40 reported isolates from Bangladesh were within lineage G-VIII (10). After 2013, no Asia1 strains
41 were reported from Bangladesh and India (11, 13). Here we report the emergence of a novel
42 lineage of FMDV serotype Asia1 BD-18 (G-IX) in Bangladesh in 2018.

43 **The Study**

44 On 18th January 2018, an outbreak of FMD occurred in the BGB (Border Guards
45 Bangladesh) Dairy Farm, Pilkhana, Dhaka. Tongue epithelium tissue samples were collected
46 from FMD-suspected cattle, and subsequently virus isolation and generation of VP1-coding
47 sequences were performed using standard protocols of our laboratory (*1*).

48 We reconstructed VP1 phylogeny by maximum likelihood method for the assorted
49 dataset (Technical Appendix (TA) Table 1) in MEGA7 under on general time-reversible model
50 of substitution with gamma distributed rate variation among sites with 1,000 pseudo-replicates
51 and estimated nucleotide divergence (ND) among the established lineages of FMDV serotype
52 Asia1. Using BEAST v2.4.5 (*14*), we accommodated the coalescent constant tree prior using a
53 lognormal distribution in an uncorrelated relaxed molecular clock model across tree branches,
54 and sampled out 1600 trees from 80 million iterations of Markov Chain Monte Carlo (MCMC)
55 chain. Finally, summarized maximum clade credibility tree (MCC) was annotated and depicted
56 in FigTree representing 95% HPD confidence intervals over the nodes to evaluate the reliability
57 (TA).

58 The proposed lineage BD-18 (G-IX) showed at least about >8% nucleotide divergence
59 with all the eight established lineages of Asia1 serotype and highest sequence identity value of
60 its closest genetic lineage G-VIII (Table 1). Moreover, the clades of G-VIII and G-IX separated
61 in the distance tree with an ND of >5% demonstrating the presence of two separate lineages (TA
62 Figure 2). The significant nucleotide variation of BD-18 with other lineages validated the
63 existence of an independently evolving lineage within Bangladesh. The phylogenetic tree clearly
64 represented that all the established lineages and novel lineage BD-18 (G-IX) formed distinct

65 clades signifying the variation within lineages with well-supported bootstrap values of the key
66 nodes (Figure 1, Panel A). Before the emergence of this lineage, lineage G-VIII was
67 sporadically circulating in Bangladesh and its neighboring countries (10, 11, 13). Remarkably,
68 the current vaccine strain (IND63/72) frequently used in Bangladesh is distantly related to newly
69 emerged G-IX in the phylogenetic trees with ND of 16.5-16.7%.

70 From the spatio-temporal dynamics of the lineages of Asia1, we can contend that both
71 contemporaneous and time relapsing emergence of different genetic lineages (G-I to G-VII) was
72 noticeable through chronologically distinct evolutionary phases tracing back to most recent
73 common ancestor (MRCA) states in five countries (TA Table 4). After 15 years since the
74 emergence of tMRCA of G-VIII in India, BD-18 (G-IX) emerged possibly around early 2017,
75 well before the documentation of evolving new strains, possible root of which was located in
76 Bangladesh (Figure 1, Panel B). This lineage might have emerged through a series of key
77 founder evolutionary events with the epidemic spreading of Indian G-VIII viruses, firstly in
78 Bangladesh by about 2004 and then there might be intermingling of G-VIII viruses within this
79 country in subsequent years causing the genesis of G-IX (TA Figure 3). There is also strong
80 support behind the idea that India acted as a crucial hub (posterior probability >85%) for
81 dissemination of viruses into Bangladesh with two independent introduction events (TA Figure
82 3). The sequences of combined clade of G-IX and G-VIII Bangladeshi isolates evolved at rate of
83 5.24×10^{-3} nucleotide/site/year, which is significantly higher, compared to an overall substitution
84 rate of Asia1 3.824×10^{-3} nucleotide/site/year.

85 Lineage specific consensus of the VP1 sequences were compared (TA) which showed
86 frequent amino acid substitutions in B-C (10 substitutions) and G-H (8 substitutions) loops,
87 along with heterogeneity flanking the RGD motif (Figure 2). A unique mutation at position 44 of

88 the G-IX (Glutamic Acid, negative charged) was evident in the groove region of B-C loop, while
89 mostly Arginine (hydrophilic, positive charged) in G-I; Alanine (hydrophobic) in G-II, III and
90 VI; Glutamine (hydrophilic) in G-IV and V; deletion in IND63/72 vaccine strains and hyper-
91 variability in G-VII and VIII were existent. Another interesting mutation was at position 3,
92 where hydrophobic Alanine was present in G-IX compared to hydrophilic Threonine in other
93 lineages. Alanine was also present at positions 58 and 86, which matches only with G-VIII.

94 The VP1 snapshot through reconstructing evolutionary parameters and migration
95 pathways inferred the genetic characteristics and evolutionary history that can help in the
96 understanding of a comprehensive picture of Asia1 lineages and evolutionary process operating
97 in this serotype. In case of the genesis of novel lineage BD-18 (G-IX), we can mention the
98 emergence as another key turning point for Bangladesh after the re-emergence of Asia1 serotype
99 during 2012-2013. Expectedly, the G-VIII isolates of Bangladesh revealed close genetic
100 relationships with Indian isolates reported since 1987 that illustrated umbrella-clustering pattern
101 among sequences of near time-period based on both nucleotide and amino acid identity within
102 the VP1 region (15). This may be due to a steady transmission of viruses through porous trans-
103 border between these countries. From India, the gene flow caused the early landmarks of
104 incursion of G-VIII viruses into Bangladesh, and could be an important factor to the emergence
105 of new lineage, which was not possible to determine in this study due to lack of related
106 sequences for recent viruses circulating in the Indian subcontinent and limitation in nature of
107 genetic dataset.

108 **Conclusions**

109 This study reports the emergence of a novel lineage BD-18 (G-IX) of serotype Asia1 in
110 Bangladesh estimated by at least >5% nucleotide divergence, along with the re-emergence of
111 serotype Asia1 in Bangladesh after 2013. There could be cryptic movements or circulation of
112 viruses which cast a little doubt in the provenance of novel virus strains, however, India might be
113 the crucial hub for independent intrusions of viruses into Bangladesh. Episode of emergence of
114 new virus lineages makes the FMD control program complex in Bangladesh.

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119 **Author Bio**

120 Mr. Ali is a research assistant at Microbial Genetics and Bioinformatics Laboratory,
121 Department of Microbiology, University of Dhaka. His research interests include foot-and-mouth
122 disease viruses and their molecular characterization.

123 **References**

- 124 1. Siddique MA, Ali MR, Alam ASMRU, Ullah H, Rahman A, Chakrabarty RP, et al.
125 Emergence of two novel sublineages Ind2001BD1 and Ind2001BD2 of foot-and-mouth disease
126 virus serotype O in Bangladesh. *Transbound Emerg Dis.* 2018 Feb 18.
- 127 2. Nandi SP, Rahman MZ, Momtaz S, Sultana M, Hossain MA. Emergence and distribution
128 of foot-and-mouth disease virus serotype A and O in Bangladesh. *Transbound Emerg Dis.* 2015
129 Jun;62(3):328-31.

- 130 3. Brito BP, Rodriguez LL, Hammond JM, Pinto J, Perez AM. Review of the global
131 distribution of foot-and-mouth disease virus from 2007 to 2014. *Transbound Emerg Dis.* 2017
132 Apr;64(2):316-32.
- 133 4. Valarcher JF, Knowles NJ, Zakharov V, Scherbakov A, Zhang Z, Shang YJ, et al.
134 Multiple origins of foot-and-mouth disease virus serotype Asia 1 outbreaks, 2003-2007. *Emerg*
135 *Infect Dis.* 2009 Jul;15(7):1046-51.
- 136 5. Jamal SM, Ferrari G, Ahmed S, Normann P, Belsham GJ. Molecular characterization of
137 serotype Asia-1 foot-and-mouth disease viruses in Pakistan and Afghanistan; emergence of a
138 new genetic Group and evidence for a novel recombinant virus. *Infect Genet Evol.*
139 2011;11(8):2049-62.
- 140 6. Sanyal A, Subramaniam S, Mohapatra JK, Tamilselvan RP, Singh NK, Hemadri D, et al.
141 Phylogenetic analysis of Indian serotype Asia1 foot-and-mouth-disease virus isolates revealed
142 emergence and reemergence of different genetic lineages. *Vet Microbiol.* 2010;144(1-2):198-
143 202.
- 144 7. WRLFMD. FMDV Prototypes. 2017 [cited 2018 June 21].
145 http://www.wrlfmd.org/fmd_genotyping/prototypes.htm
- 146 8. Subramaniam S, Mohapatra JK, Sharma GK, Das B, Dash BB, Sanyal A, et al.
147 Phylogeny and genetic diversity of foot and mouth disease virus serotype Asia1 in India during
148 1964-2012. *Vet Microbiol.* 2013 Dec 27;167(3-4):280-8.
- 149 9. Ali W, Habib M, Khan RSA, Zia MA, Farooq M, Sajid S, et al. Molecular investigation
150 of foot-and-mouth disease virus circulating in Pakistan during 2014-17. *Arch Virol.* 2018 Mar 7.

- 151 10. WRLFMD. Genotyping report: Myanmar (Batch- WRLFMD/2017/00012). 2017 [cited
152 2018 June 21]. http://www.wrlfmd.org/fmd_genotyping/asia/mya.htm
- 153 11. Ullah H, Siddique MA, Amin MA, Das BC, Sultana M, Hossain MA. Re-emergence of
154 circulatory foot-and-mouth disease virus serotypes Asia1 in Bangladesh and VP1 protein
155 heterogeneity with vaccine strain IND 63/72. *Lett Appl Microbiol.* 2015;60(2):168-73.
- 156 12. Ali MR, Alam ASMRU, Amin MA, Ullah H, Siddique MA, Momtaz S, et al. Complete
157 genome sequence of the circulatory foot-and-mouth disease virus serotype Asia1 in Bangladesh.
158 *Genome Announc.* 2017 Oct 26;5(43).
- 159 13. Subramaniam S, Mohapatra JK, Das B, Sharma GK, Biswal JK, Mahajan S, et al. Capsid
160 coding region diversity of re-emerging lineage C foot-and-mouth disease virus serotype Asia1
161 from India. *Arch Virol.* 2015 Jul;160(7):1751-9.
- 162 14. Drummond AJ, Rambaut A. BEAST: Bayesian evolutionary analysis by sampling trees.
163 *BMC Evol Biol.* 2007 Nov 08;7:214.
- 164 15. Gurumurthy C, Sanyal A, Venkataramanan R, Tosh C, George M, Hemadri D. Genetic
165 diversity in the VP1 gene of foot-and-mouth disease virus serotype Asia 1. *Arch Virol.*
166 2002;147(1):85-102.

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168 Address for correspondence: M. Anwar Hossain, Department of Microbiology, University of
169 Dhaka, Dhaka-1000, Bangladesh; email: hossaina@du.ac.bd

170 Table 1. Percent nucleotide identity/divergence in VP1 encoding gene of FMDV Asia1
171 established groups with newly proposed group IX.

Genetic lineages	Intergroup (G-IX)	
	Nucleotide identity (%)	Nucleotide divergence (%)
G-I	83.9-85.2	17-18.7
G-II	86.6-88.3	13-15.2
G-III	87-88.6	12.6-14.6
G-IV	84-86.6	15-20.4
G-V	83.6-85.6	16.3-18.9
G-VI	87.8-89.4	11.4-13.7
G-VII	82.3-84.7	17.5-20.6
G-VIII	90-91.8*	8.8-10.9

172 *The least identity/divergence has been observed with G-VIII. From the perspective of our
173 dataset, two strains (IND227/07 & IND12/07) of G-VIII from different states of India showed
174 the highest identity (~92%) to the novel G-IX lineage. Both of the virus strains had tracked back
175 to an Indian origin in about 2005.

176

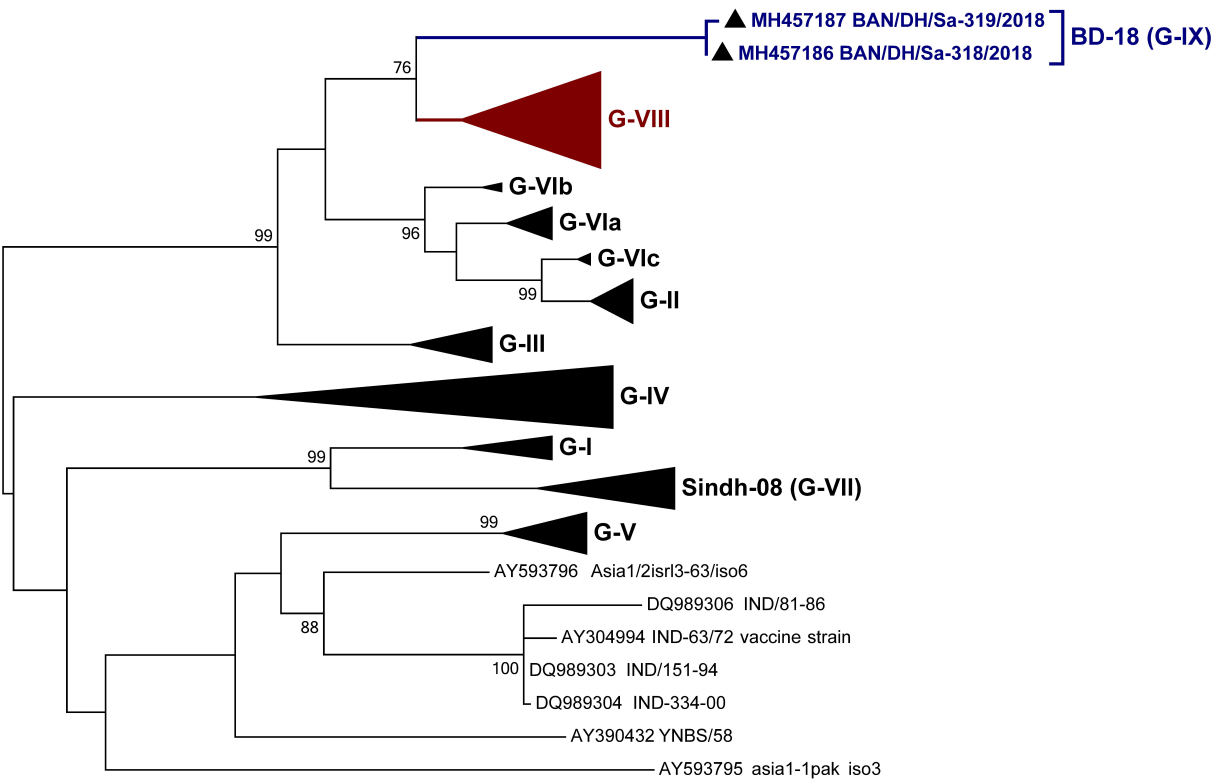
177 Figure 1. Phylogenetic analyses of VP1 coding region of serotype Asia1 covering all the
178 lineages. A) Phylogenetic relationship among the established lineages of FMDV Asia1 serotype
179 and novel lineage G-IX. Scale bar indicates nucleotide substitution per site. The sequences
180 generated for this study are shown in bold letter and marked with (filled triangle) symbol.
181 Lineage BD-18 (G-IX) formed a completely different clade seemingly originating from G-VIII,
182 which was in circulation in Bangladesh during 2012-2013 and never found in India and/or
183 Bangladesh after 2013. B) Maximum clade credibility (MCC) tree summary of spatio-temporal
184 reconstruction based on VP1 sequences of FMDV serotype Asia1. The time-tree (tips
185 corresponding to year of sampling) was generated using TreeAnnotator in BEAST2 package and
186 annotated in FigTree with discrete location (country) based depiction. Some terminal nodes were

187 collapsed for clarity and represented the established genetic lineages. Internal node colors reflect
188 inferred locations for the clades, while tip and branch colors represent the sampling locations for
189 tip branches. Diameters of internal node circles represent posterior location probability values.
190 The sequences generated by our lab for this study were shown in navy blue color and other
191 sequences reported from Bangladesh but not from this study are marked with dark pink color.
192 Blue lines on the node indicate 95% high posterior density of the time of the most recent
193 common ancestor (tMRCA). The tree clearly represents the evolutionary changes throughout the
194 time span (1921-2018) and G-IX emerged as the last group probably from G-VIII.

195

196 Figure 2. Alignment of the respective VP1 amino acid consensus of eight groups under the
197 umbrella of FMDV Asia 1 with newly proposed group IX (contains the most recent report of
198 FMDV Asia1 in the Indian sub-continent so far). Group specific consensus was generated based
199 on the VP1 amino acid multiple sequence alignment of the representative isolates under the
200 flagship of a group using MView tool (EMBL-EBI) where 80% identity was taken into
201 consideration. Amino acids that were found conserved among all the isolates of a particular
202 group (current dataset) were represented as uppercase letter, whereas most abundant (not
203 conserved) amino acid call at 80% identity was denoted as a lowercase letter. In contrary, the dot
204 present in the consensus implies the amino acid variability at those positions at 80% identity
205 ceiling. Considering the alignment of the group specific consensus with reference to newly
206 proposed group IX, frequent amino acid substitution was found restricted at B-C loop (position
207 40-60 (6)) and G-H loop (position 140-160 (6)) of the VP1. This is noteworthy that, a unique
208 mutation at 44 position (most probably within groove region of B-C loop) of the group IX

209 (presence of E- Glutamic Acid, a highly charged amino acid) is evident which is indicated here
210 as down head arrow colored in red.



0.05

