

Poster Board Number:

SUNDAY-010

Publishing Title:

Molecular Phylogeography of Foot and Mouth Disease Virus in Bangladesh

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Abstract Body:

Foot-and-Mouth Disease Virus (FMDV), etiological agent of FMD, causes an endemic animal disease in Bangladesh. Here we report circulatory FMDV serotypes and its genome-wide analysis to develop strategies of trans-border disease control and containment. RT-PCR was employed to amplify VP1 gene of viral RNA from blister tissue samples of infected animals collected from 24 districts of Bangladesh since 2011 to 2015. Bioinformatics analyses of cultured FMDV isolates' whole genomes and retrieved VP1 sequences was done along with Bayesian MCMC method to predict phylogeography of circulatory FMDV. FMD was predominant in female animals (55.87%) than males (44.13%) with increased susceptibility in aged cattle (51.17%) than the young animals (38.97%) and calves (9.86%). FMDV serotypes O, A and Asia 1 were circulatory in Bangladesh with predominant serotype O (~80%) within the lineage ME-SA/Ind-2001. Within the Ind-2001 clade, the type O complete genome sequences were clustered with those of type O of Uttarkhand, India 2010; Assam, India 2012 and Karnataka, India 2013. FMDV type O from pig formed a sub-clade homologous to 2013 sequences of cattle. Sequences of serotype A retrieved from samples of Chittagong and Gazipur districts, belonged to genotype VII under topotype Asia clade. Serotype Asia 1 re-emerged in 2012 after last outbreaks in 1996 and was confined only in certain areas of Jessore and Gazipur districts with sequences clustered within genetic lineage C. The Phylogenetic analysis confirmed the cross-border movement of FMDV from India to Bangladesh. VP1 sequences of Bangladesh-FMDV detected a notable 8 nucleotide substitutions in the immune-dominant epitope located in GH loop conferring antigenic heterogeneity; most likely the reason behind vaccination failure

using imported vaccine. Unrestricted unidirectional animal movement from India is the main source of intrusion of FMDVs in Bangladesh. Presence of significant positive selection pressure within the VP1 antigenic sites implicates evolution of VP1 under high immune surveillance. Regular monitoring is necessary for checking genotype turnover and emergence of new antigenic types that might lead to new outbreaks.

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Antibiotic Resistance Scenario in Bangladesh; Mechanism and Ecological Impact

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Abstract Body:

Clinical overuse or misuse of antibiotics, inappropriate clinical liquid waste (CLW) management and its discharge to ecological water (EW), and mismanagement of clinical patients create antibiotic resistance situation alarming in developing countries; and this antibiotic resistance markers may spread beyond the boarder. Here we report antibiotics and resistome pollution, mechanism of resistance harborage and its spreading scenario in Bangladesh. Active antibiotics in CLW, admixing point of EW (Buriganga River) and sediment were analyzed using Liquid Chromatography Mass Spectrometry (LCMS/MS) after solid phase extraction. Resistome marker integron class 1 and resistant genes were analyzed using PCR in total DNA isolated from CLW, EW and from phenotypically screened MDR bacteria. CLW and admixing point of Buriganga river (10 m down) contained Ciprofloxacin, Cloxacillin, Amoxicillin and Tetracycline at sub-MIC level. Accumulation of antibiotics at sediment soil (per gm) around admixing point amounted high Ciprofloxacin (4.255 ng), Cloxacillin (4.365 ng) and Tetracycline (5.55 ng). CLW is also loaded with MDR bacteria ($1 \times 10^5 \sim 1.2 \times 10^7$ cfu/ml) and resistant gene pool *qnrS*, *bla*CTX-M and integron class 1. About 47.13% isolates showed MDR properties among which 31.2% were ESBL-positive harboring specific genes (*bla*CTX-M, *bla*TEM and *bla*SHV). On the other hand, 96.7% ESBL isolates showed resistance towards Ciprofloxacin with harborage of *qnrS* gene in plasmid and/or the presence of *acrA*, *acrB* and *tolC* genes encoding AcrAB-TolC efflux pump. Imipenem resistance was screened among 434 clinical isolates of which 16.8% showed Imipenem resistance with MIC_IMP value as high as 256 µg/ml. The carbapenem resistant *bla*VIM-2

gene was detected to be encoded in chromosomally harbored Integron class 1 associated gene cassette in resistant *Pseudomonas stutzeri*. Antibiotics and resistome pollution is increasing alarmingly in Bangladesh and may spread beyond its origin to environments. Selective pressure of antibiotics in EWB and mixing of autochthonous species with allochthonous may influence horizontal gene transfer (HGT) resulting origin of new species of clinical importance.

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Comparative Genomics of Foot and Mouth Disease Virus Type O Circulating in Bangladesh

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Abstract Body:

Background: The causative agent of Foot-and-mouth disease (FMD), FMD virus (FMDV), is a positive sense RNA virus and belongs to the genus *Aphthovirus* within the family *Picornaviridae*. There are seven immunogenically distinct serotypes, i.e. O, A, C, Asia-1 and South African Territories (SAT) 1-3 and vaccine protect against one serotype to another are limited. Here we report isolation and genome wide analysis of FMDV type O from Bangladesh.

Methods: Virus from infected blister tissue was cultured in BHK-21 cell line followed by nucleic acid extraction. Serotype O [BAN/NA/Ha-156/2013] virus was selected for complete genome analysis. **Results:** The genome was found 8131 nt. in length, which includes a 1020 nt. 5' un-translated region (UTR), a 6999 nt. length open reading frame (ORF) encoding a polyprotein of 2332 (excluding stop codon) amino acids or 2304 amino acid residues due to two alternative initiation sites and 91nt 3'-UTR plus 21nt poly(A) tail. 5'-UTR contains a short S-fragment, poly C tract, multiple pseudoknots of unknown function, *cis* acting replication element and internal ribosome entry sites (IRES) which directs the initiation of poly protein synthesis. The genome sequence has been deposited in to the NCBI Genbank database with Accession Number KF985189. Regarding to coding region, the sequence of non-structural proteins is much more conserved than that of the structural proteins in the FMDV genome. Among all the compared regions, 5'-UTR, VP1 and 3A were found lowest conservative. Comparative genome wide analysis with reference sequence (RefSeq) revealed that an 82 nt. deletion in S-fragment and 43 nt. consecutive insertion in 5' untranslated region (UTR) was evident introducing an extra pseudoknot (PK) structure. Comparison of structural protein VP1 indicated variation in B-C loop

(40~60), G-H loop (133~160) and C-terminal linear epitope amino acid segment (200~211) whereas non-structural protein 3A showed that a 10 amino acid insertion (position 92~101) in the 3A protein. **Conclusions:** The genomic structure of serotype O [BAN/NA/Ha-156/2013] differed from Refseq of the virus. The functional implication of this altered genomic structure needed to be further elucidation.

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