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Genetic and antigenic relationship of foot–and–mouth disease virus serotype O isolates with the vaccine strain O1/BFS

Wanhong Xu [a], Zhidong Zhang [a,b], Charles Nfon [a], Ming Yang [a,*]

[a] National Centre for Foreign Animal Disease, 1015 Arlington Street, Winnipeg, Manitoba R3E 3M4, Canada
[b] State Key Laboratory of Veterinary Etiological Biology, Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Sciences, No 1, Xujiaping, Lanzhou, Gansu 730046, PR China

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ABSTRACT

Foot–and–mouth disease serotype O viruses (FMDV/O) are responsible for the most outbreaks in FMD endemic countries. O1/BFS is one of the recommended FMD/O vaccine strains by World Reference Laboratory for FMD. In the current study, FMDV/O1 BFS vaccine strain and serotype O field isolates (45) were analyzed phylogenetically and antigenically to gain more insight into the genetic and antigenic characteristics of the vaccine strain and field isolates.

O1/BFS showed similarity with 89% of the field isolates using a virus neutralization test (VNT). The P1 region encoding the FMDV capsid was sequenced and analysed for 46 strains of FMDV/O. Phylogenetic analysis showed these viruses originated from five continents and covered eight of 11 reported topotypes. Five isolates that demonstrated low antigenic similarities with O1/BFS were analyzed for their antigenic variation at the known neutralizing antigenic sites. Three of the five isolates demonstrated unique amino acid substitutions at various antigenic sites. No unique amino acid substitutions were observed for the other two unmatched isolates. Positively selected residues were identified on the surface of the FMD virus capsid supporting that it is important to continuously monitor field isolates for their antigenic and phenotypic changes.

In conclusion, the vaccine strain O1/BFS is likely to confer protection against 89% of the 45 FMDV/O isolates based on VNT. Thus O1/BFS vaccine strain is still suitable for use in global FMD serotype O outbreak control. Combining data from phylogenetic, molecular and antigenic analysis can provide improvements in the process of vaccine selection.

1. Introduction

Foot–and–mouth disease (FMD) is highly contagious in cloven–hoofed animals and may cause significant economic losses in the livestock industry. Its presence poses a constant threat to regions free of the disease. The causative agent, FMD virus (FMDV), exists as seven immunologically distinct serotypes: O, A, C, Asia 1, and Southern African Territories (SAT) 1, 2 and 3. FMDV has many antigenically distinct variants within each serotype, due to the low fidelity of RNA polymerase and the ability of the genome to accommodate considerable amounts of mutations. FMDV is a positive–sense ssRNA virus, belonging to the Aphthovirus genus of family *Picornaviridae*. FMDV contains a single copy of RNA encoding a large polyprotein, which is cleaved by viral proteases to form four structural and numerous non–structural proteins and partial cleavage intermediates. The four structural proteins, known as VP1–4, constitute a viral capsid with 60 copies of protomers [1]. VP1, VP2, and VP3 are exposed on the surface of the virus, while VP4 is entirely internal. Thus, the antigenic determinants and cell receptor binding elements are located on the three capsid proteins VP1–3 [2–4]. Five antigenic sites for FMDV/O have been described with critical residues determined by monoclonal antibody escape mutant studies [5–8]. Recently, three novel antibody binding determinants (VP2–74, VP2–191, and VP3–85) for the FMDV/O have been reported using a reverse genetics approach [9]. Serotype O remains the most predominant FMDV serotype globally according to annual FMD reference laboratory network reports by the World Organisation for Animal Health / the Food and Agriculture Organization of the United Nations (OIE/FAO). FMDV/O isolates have been classified into eleven genetically and geographically distinct genotypes (topotypes) on the basis of VP1 sequences [10–13].

For FMD control, ring culling is the economically optimal strategy for sparsely populated livestock areas; ring vaccination is an

* Corresponding author.
E-mail address: Ming.Yang@inspection.gc.ca (M. Yang).

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economically optimal control strategy for densely populated livestock areas [14]. Although current vaccines have a narrow antigenic spectrum, provide only short term immunity and are very fragile [15], in comparison to the culling method, vaccination is relatively cheap and, larger areas can be vaccinated in a relatively short period of time. In many FMD-endemic countries livestock movement restrictions and biosecurity measures are difficult to implement. In this case FMD control becomes heavily dependent on vaccine protection [16]. Currently vaccine selections are mainly based on serological cross–reactivity of the bovine post–vaccinal serum with circulating viruses as well as epidemiological information and phylogeny of the gene sequence for evolutionary analysis [17,18]. The virus neutralization test (VNT) is more relevant for *in vivo* protection than other measures [19]. Thus, VNT is widely used to determine the antigenic relationship between the vaccine strains and field isolates. Sequence analysis of genes encoding capsid in field isolates has added benefits for vaccine selection in that it is capable of tracing the origin of FMD outbreaks and revealing how antigenic determinants have changed over the time [20].

Among the seven FMDV serotypes, serotype O shows moderate levels of strain variation in the field with, occasionally, more extreme variants [18]. FMDV O1/BFS is one of the vaccine strain recommended by the World Reference Laboratory for Foot–and–Mouth Disease (WRLFMD) to be used as high priority virus strains. The aim of this current study is to analyze genetic and antigenic relationship of a vaccine strain, O1/BFS with 45 isolates of FMDV/O representing a wide range of geographical distributions during 1994–2011, to provide evidence that this vaccine could provide protection against isolates from worldwide. In addition, for the first time, a detailed analysis of the molecular basis of the antigenic variations of FMDV serotype O isolates was reported.

2. Materials and methods

2.1. Viruses and cell lines

FMDV/O1 British field strain 67 (GB, BFS) 1860 and all other forty–five FMDV serotype O viruses used in this study were obtained from WRLFMD. Viruses, topotypes and passage numbers are listed in the Supplementary Table. Viral stocks were prepared by growing them in Mengeling–Vaughn Porcine Kidney (MVPK) cell monolayers in Alpha Modification of Eagle's medium (AMEM; WISENT Inc. Canada) supplemented with 2% fetal bovine serum (FBS) and 2 mM l-glutamine, as described previously [21,22].

2.2. Two–dimensional virus neutralization test (2D–VNT)

The O1/BFS 1860 vaccine antiserum collected at 21 days post vaccination (dpv) was obtained from WRLFMD at the Pirbright Institute. The 2D–VNT was performed as previously described by Rweyemamu et al. [23]. Briefly, the post vaccination serum was two–fold diluted vertically in a 96–well plate. Five different virus titers (half log dilution series) were then added horizontally across the plate. Then, the plate was incubated for 1 h at room temperature. MVPK cells were added to the plates and incubated at 37 °C for 2–3 days. Antibody titers were calculated from the regression data as the $\log_{10}$ reciprocal antibody dilution required for 50% neutralization of 100 $TCID_{50}$ of virus ($\log_{10}$ SN_{50}/100 $TCID_{50}$). The antigenic relationship of viruses based on their neutralization by antibodies is given by the following ratio: 'r1' = neutralizing antibody titer against the heterologous virus/neutralizing antibody titer against the homologous virus. Serological relationships between vaccine strain and field isolates were evaluated according to criteria previously described [24,25]. The r1 values $\geq$0.3 are indicative of cross protection, whereas values of <0.3 indicate dissimilar vaccine strains and test isolates.

2.3. Sequencing capsid coding region

RNA extraction, RT–PCR, and DNA sequencing were performed as described previously [21]. Briefly, genomic FMDV RNA was extracted. Full-length cDNA copies of P1 regions were synthesize from genomic RNA (primers: forward, 5′-TTCTGGTGTTTGTCCCGT ACGAT-3′ and reverse, 5′-GTTGACATGTCCTCCTGCATCTG-3′). The cDNAs were amplified by PCR and PCR products were gel purified. DNA sequencing was performed in both directions by use of an ABI Prism BigDye Terminator v3.1 Cycle Sequencing Ready Reaction kit (Applied Biosystems) and an Applied Biosystems Genetic Analyzer DNA Model 3130X. Sequences obtained from both directions were assembled and checked for accuracy with SeqMan®(Lasergene®, Version 12; DNASTAR, Inc.). Pairwise nucleotide sequence alignments were performed using the Martinez–NW method [26] and the Lipman–Pearson method [27] for protein alignments in MegAlign®(Lasergene). The GenBank accession numbers are listed in the Supplementary Table. Phylogenetic trees were constructed using Neighbor–Joining and tested with 1000 bootstrap replicates in MEGA version 7 [28].

2.3.1. 3D structural analyses

Molecular graphics coordinates of the FMDV/O1/BFS1860 crystal structure (PDB #1FOD) [29] were performed using the UCSF Chimera package from the Resource for Biocomputing, Visualization, and Informatics at the University of California, San Francisco [30]. The resulting images were imported into Adobe Photoshop for editing.

2.4. Screening for recombinants

Sequence alignments of 46 FMDV/O P1 regions were screened for recombinant sequences using the programs RDP, GENECONV, MAXCHI, CHIMAERA, 3SEQ, BOOTSCAN and SISCAN as implemented in the RDP4 software package using the default settings. Potential recombinant sequences were identified when two or more methods were in agreement, with p–values <0.001.

2.5. Coalescent analyses

The rate of nucleotide substitution and time of the most recent common ancestor (TMRCA) were estimated for the P1 region using a Bayesian Markov Chain Monte Carlo (BMCMC) method [31] as implemented in the program BEAST, version 2.3.0 [32]. The best fit nucleotide substitution model, TN93+G+I, was used. The relaxed exponential clock and exponential growth population model was chosen for the analysis. Model selection was performed by comparing the model marginal log–likelihood through the Akaikes information criterion [33]. The BEAST analyses were run for a sufficiently long time to ensure that all parameters had an effective sample size (ESS) of >200. The log file was analyzed using Tracer v 1.6 [34], and statistical uncertainties are reflected as the 95% highest posterior density value (HPD).

2.6. Analysis of selection pressure

Site–specific selection pressures for 46 FMDV/O P1 regions were measured as nonsynonymous (dN) –synonymous (dS) nucleotide substitutions per site. The differences were estimated using the single–likelihood ancestor counting (SLAC), fixed–effects likelihood (FEL), internal fixed–effects likelihood (IFEL), and random effects likelihood (REL) methods [35,36] available at the Datamonkey [37,38] online version of the HyPhy package [39]. All analyses