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Selection of vaccine strains for serotype O foot-and-mouth disease viruses (2007–2012) circulating in Southeast Asia, East Asia and Far East

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ABSTRACT

Foot-and-mouth disease (FMD) is endemic in Southeast Asia (SEA) and East Asia with circulation of multiple serotypes and multiple genotypes within each serotype of the virus. Although countries like Japan and South Korea in the Far East were free of FMD, in 2010 FMD serotype O (O/Mya-98) outbreaks were recorded and since then South Korea has experienced several FMD outbreaks despite regular vaccination. In this study a total of 85 serotype O FMD viruses (FMDV) isolated from 2007 to 2012 from SEA, East Asia and Far East were characterized by virus neutralisation tests using antisera to four existing (O/HKN/6/83, O/IND/R2/75, O/SKR/2010 and O/PanAsia-2) and one putative (O/MYA/2009) vaccine strains, and by full capsid sequencing. Serological studies revealed broad cross-reactivity with the vaccine strains; O/PanAsia-2 exhibited a good match with 95.3%, O/HKN/6/83 with 91.8%, O/IND/R2/75 with 80%, and the putative strain O/MYA/2009 with 89.4% isolates employed in this study. Similarly O/PanAsia-2 and O/IND/R2/75 vaccines showed a good match with all eight viruses belonging to O-Ind-2001d sublineage whereas the vaccines of O/Mya-98 lineage, O/MYA/2009 and O/SKR/2010 exhibited the lowest match indicating their unsuitability to protect infections from O-Ind-2001d viruses. A Bayesian analysis of the capsid sequence data indicated these circulating viruses (n = 85) to be of either SEA or Middle East-South Asian (ME-SA) topotype. The ME-SA topotype viruses were mainly detected in Lao PDR, Vietnam, Myanmar and Thailand reflecting the trade links with the Indian subcontinent, and also within the SEA countries. Implications of these results in the context of FMD control in SEA and East Asian countries are discussed.

1. Introduction

Foot-and-mouth disease (FMD) is a highly contagious disease of domestic and wild cloven hooved animals across the world. It is endemic in Africa, Middle-East and Asia. The causative agent, FMD virus (FMDV) is a single stranded positive sense RNA virus (genus *Aphthovirus*, family *Picornaviridae)* and exists as seven immunologically distinct serotypes, O, A, C, Asia 1, SAT (Southern African Territory) 1, 2 and 3, each with a wide spectrum of antigenically distinct subtypes [1,2]. Serotype C was last reported in Brazil and Kenya in 2004 [1,3] and is probably now extinct. The RNA genome is enclosed in a capsid and encodes for four structural proteins (VP1-4) and 8 non-structural proteins. Sixty copies of the four structural proteins (VP1-4) form the capsid; VP1-3 are exposed on the surface that contain neutralising epitopes whilst the VP4 is internal.

Southeast Asia (SEA), East Asia and Far East have a large population of FMDV susceptible livestock, mainly cattle, pigs and water buffaloes. Regular outbreaks have been reported in countries in SEA: Cambodia, Lao PDR, Malaysia, Myanmar, Thailand, Vietnam, and in East Asia: Mongolia, Hong Kong and China [4]. Indonesia, Singapore and Brunei have remained FMD-free without vaccination. Similarly the Philippines has not reported an outbreak since 2005, and was declared FMD-free without vaccination by OIE in 2011. FMD free countries like Japan and South Korea in the Far East experienced FMD outbreaks in 2010 and since then South Korea has experienced several outbreaks, almost every year. More than 60% of FMD outbreaks in SEA and East Asia are caused by serotype O [1]. Historically the main serotype O topotypes found in SEA and East Asia are Cathay and SEA. In addition another topotype from

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the Middle East, ME-SA (PanAsia strain) caused extensive outbreaks in Japan, South Korea, China, Taiwan and Russia during 1999–2002 [5–8] and continues to circulate in the SEA countries.

The predominant SEA topotype strain, O/SEA/Mya-98 was mainly restricted to SEA countries until 2010 (Table 2). In 2010–2011 this strain caused devastating outbreaks in FMD free countries, Japan and South Korea [3,9–13] resulting in high economic losses. O Manisa vaccine was used to control the disease, however full clinical protection was not observed in South Korea. In spite of a compulsory vaccination campaign, clinical disease has been reported in South Korea every year since 2014 [15, WRLFMD reports; http://www.wrlfmd.org/] which raises questions about the effectiveness of vaccination in pigs. Amongst many possible reasons, a non-matching vaccine could be important [15]. Indeed vaccine matching work carried out at World Reference Laboratory (WRL), Pirbright using South Korean viruses from the years 2010 and 2011 indicated that only 60% (three out of five) of the isolates matched with O Manisa vaccine in-vitro [WRLFMD report; http://www.wrlfmd.org/]. Recently another strain of ME-SA topotype, O-Ind-2001d originating from the Indian subcontinent has spread to new areas and caused outbreaks thereby complicating the epidemiological situation. Initially these O-Ind-2001d viruses were restricted to the Indian subcontinent [16], however they have been detected in the Middle East and North Africa since 2013 [17,18]. These O-Ind-2001d viruses has also been detected in Lao PDR since 2015 and have now spread to other neighbouring countries like Vietnam, Myanmar, Thailand and South Korea (Table 1), possibly by trade links with Indian subcontinent, and animal movement between SEA countries [19,20].They have also been detected in Russia in 2016 and China in 2017 emphasizing the importance of a matching vaccine for use in FMD control programmes. To our knowledge there is no published report on the FMD vaccine strain selection for SEA, East Asia and Far East. Therefore this study was designed to carry out a systematic study to select an appropriate serotype O vaccine strain for use in the SEA and East Asian countries for FMD control.

2. Materials and methods

2.1. Cells, viruses and bovine post-vaccinal sera (BVS)

Eighty-five serotype O viruses from SEA, East Asia and Far East submitted to the WRL FMD at Pirbright and two vaccine strains from Middle East and India were used in this study (Supplementary Table 1). Three are the vaccine strains O/HKN/6/83, O/PanAsia-2 (O/TUR/5/2009) and O/IND/R2/75 that were originally isolated from Hong Kong, Turkey and India in 1983, 2009 and 1975, respectively. The other 84 viruses were isolated over a six-year period between 2007 and 2012. These originated from eleven countries; Cambodia (n = 7), Democratic Republic of Korea (n = 1), Hong Kong (n = 4), Japan (n = 1), Lao PDR (n = 9), Malaysia (n = 8), Mongolia (n = 1), Myanmar (n = 7), South Korea (n = 6), Thailand (n = 16) and Vietnam (n = 25). In addition, eight serotype O viruses (isolated over a four year period between 2009 and 2012 from the Indian subcontinent) belonging to O-Ind-2001d lineage were also included in this study making a total of 95 viruses (Supplementary Table 1). The samples from SEA countries were derived from either cattle (n = 47), buffalo (n = 9) or pig (n = 27) epithelial tissues except two viruses from Cambodia whose host species is not known (Supplementary Table 1). All eight isolates from the Indian subcontinent were of cattle origin. All the samples were initially grown in primary bovine thyroid cells (BTY) with subsequent two to three passages in IB-RS2 (pig kidney) cells. Stocks of virus were prepared by infecting IB-RS2 cell monolayers and were stored as clarified tissue culture harvest material at −70 °C until required.

Five bovine anti-FMDV post-vaccination sera (BVS) were used in the study. Two of these, namely O/IND/R2/75 and O/PanAsia-2 have been described in detail previously [21,22]; one antisera, O/SKR/2010 (existing vaccine of O/SEA/Mya-98 lineage) was procured from MSD Animal Health, Germany whereas the other two were generated in this study. The antisera were raised against the Cathay topotype vaccine strain, O/HKN/6/83 and the putative strain, O/MYA/2009 of O/SEA/Mya-98 lineage, in cattle at Pirbright as described previously [23] by administering inactivated, purified 146S FMDV particles in ISA-206 adjuvant. The animals were boosted on 21-day post-vaccination and bled one week later. For each antigen, a pool of sera from five animals was used in the serological tests. The homologous neutralising antibody titres of each pooled serum were in the range of $\log_{10}$ 2.58–3.27 (data not shown).

2.2. Antigenic characterization by two-dimensional virus-neutralisation test (2D-VNT)

The 2D-VNT test was carried out using the pooled BVS according to Rweyemamu et al. [24]. Antibody titres were calculated from regression data as the $\log_{10}$ reciprocal antibody dilution required for 50% neutralisation of 100 tissue culture infective units of virus ($\log_{10}SN_{50}$/100 $TCID_{50}$). The neutralising antigenic relationship of viruses is given by the ratio: 'r_1' = neutralising antibody titre against the heterologous virus/neutralising antibody titre against the homologous virus. The serological relationship between two viruses in the range 'r_1' = 0.3–1.0 are indicative of a reasonable level of cross protection whereas values less than 0.3 indicate dissimilar strains and the need to acquire, or develop, a new vaccine strain [25]. All the tests were carried out in duplicates, and repeated at least twice, and average values from at least two tests were used for subsequent analysis.

2.3. RNA extraction, RT-PCR, nucleotide (nt) sequencing and analysis of the sequence data

For generating the nt sequences of the capsid coding region (P1) of the viruses, RNA extraction, reverse transcription (RT), polymerase chain reaction (PCR), sequencing, sequence analysis and assembling, and alignment were performed as described previously [26]. Nt sequences of the viruses were aligned using the CLUSTAL X multiple sequence alignment program [27] and the predicted amino acid (aa) sequences were translated using BioEdit 7.0.1 [28]. The alignments were used to construct distance matrices using the Kimura 2-parameter nucleotide substitution model [29] as implemented in the program MEGA 6.0 [30].

Using jModelTest [31] and MEGA [30], General time reversal (GTR) model with combination of gamma distribution and proportion of invariant sites (GTR+G+I) was determined to be the most suitable nucleotide substitution model for the complete P1 nucleotide sequences of the SEA type O viruses. Bayesian analysis was performed using the BEAST software package v1.8.4 [32]. In BEAUti v1.8.4, the ages of the viruses were defined by the date of sample collection and the analysis used GTR+G+I model to describe rate heterogeneity among sites. Variations in substitution rate among branches were evaluated by comparing four different clocks in BEAST. The maximum clade credibility (MCC) phylogenetic tree was inferred using the Bayesian Markov Chain Monte Carlo (MCMC) method followed by a Bayes factor analysis in TRACER version 1.6 [33] to determine the best-fit model resulting in the selection of an uncorrelated exponential relaxed molecular clock. Tree Annotator program in BEAST was used to obtain the evolutionary tree and FigTree program 1.4.2 was used to view the trees.