Isolation and characterization of foot-and-mouth disease virus from Odisha, India

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Abstract. Foot-and-mouth disease (FMD) is a highly contagious and rapidly transmissible disease of cloven footed animals. Emergence of genetically divergent strains of FMD virus (FMDV) is a major concern globally. FMDV is endemic in India and three serotypes (O, A and Asia 1) prevail. The study was undertaken to characterize the isolates from the state of Odisha, India both genetically and antigenically. FMDV was detected in 7 of the 17 clinical samples collected from FMD affected/suspected animals, in which serotype O and A were found in three and four samples, respectively. Serotype O field isolates clustered in an unnamed group (designated here as Eastern cluster) circulating mostly in the Eastern region of the country and had 10-12.7% divergence from the Ind2001 lineage circulating predominantly throughout the country. The serotype A isolates sequenced in this study was grouped within VP359–deletion group of genotype 18, precisely in clade 18c, having high genetic homology to the virus circulating in the neighboring states, suggesting interstate movement. Both the serotype O and A isolates showed good antigenic relationship value with the respective vaccine strains currently used in the country.

INTRODUCTION

Foot-and-mouth disease virus (FMDV) causes an acute and highly contagious disease of susceptible cloven footed animals, thus encompassing a dramatic influence on livestock trade and production. The virus, a member of the family Picornaviridae belongs to genus Aphthovirus (Racaniello, 2001) occurs as seven genetically and antigenically distinct serotypes (O, A, C, Asia1 and Southern African Territories (SAT) 1-3) with multiple subtypes (Domingo et al., 2003). All the four Euro Asian serotypes (O, A, C and Asia1) have been recorded in India whereas serotype C has not been encountered in the country since 1995 (Subramaniam et al., 2012). One of the significant aspects in the FMDV evolution is high antigenic variations arising due to extreme heterogeneity among viral population driven by mutation of viral genome along with amino acid substitution consequently making control of this economically crippling disease, very difficult (Knowles & Samuel, 2003; Sutmoller et al., 2003). Moreover the lack of cross protection among serotypes adds further to the grueling task of control and management of the disease. India has one of the largest livestock populations in the world with about 528 million FMD susceptible animals and the state of Odisha shares nearly 5% of the total figure, thus exposing it exponentially to FMD associated losses (Mohanty et al., 2015). Regional prevalence of FMD in India suggests maximum proportions (43%) of the total outbreaks occurred in the Eastern
region (States of Bihar, Odisha, West Bengal and Jharkhand) followed by 31.5% in the Southern region which include the States of Tamilnadu, Kerala, Karnataka and Andhra Pradesh (Subramaniam et al., 2012).

Serotype O is the most prevalent of all the serotypes circulating in the state of Odisha, which is also the scenario in other regions of the country. Outbreaks due to serotype A has also been recorded in the state in 2007-08 (April-March) and 2009-10 but serotype Asia1 has not been recorded in the last five years (Annual report PD FMD 2011-12). In order to implement effective control on the disease, it is essential to have information on the pattern of outbreaks and virus strains involved. Despite vigilant screening of antibodies against FMDV in the state the antigenic status still remains hazy. So irrespective of the sero-monitoring and prophylaxis aided by an inactivated trivalent vaccine, it becomes imperative to periodically assess the protection provided by the candidate vaccine strains against the continuously evolving field isolates. Therefore in the current study an attempt has been made to isolate virus from various outbreak areas and characterize them both genetically and antigenically to understand the dynamics of viral incidence.

**MATERIAL AND METHODS**

**Collection of field samples & processing**
Seventeen clinical materials (tongue epithelium) were collected from suspected animals from various regions of Odisha with probable FMD outbreaks. The samples were processed for obtaining virus supernatant which was stored at -20°C for further use.

**Cell line & Reference sera**
BHK-21 clone13 cell line maintained at the ICAR-PD on FMD, Mukteswar was used for virus revival and Two dimensional micro-neutralization test (2D-MNT). Bovine Vaccinate Serum (BVS) against the currently used vaccine virus INDR2/1975 and IND40/2000 for “O” and “A” serotype respectively available at the PD on FMD, Mukteswar was used in 2D-MNT.

**Serotyping**
The processed virus supernatant obtained were tested in sandwich ELISA as per the method described by Bhattacharya et al. (1996). ELISA negative samples were further subjected to multiplex PCR (m-PCR) as described earlier (Giridharan et al., 2005). For this, genomic RNA of field isolates was extracted from sample supernatants by RNeasy mini Kit (Qiagen) and reverse transcribed using FMDV universal primer pNK61 (Knowles and Samuel, 1995). m-PCR was performed with g reverse primer pNK61 and forward primers DHP9, DHP13 and DHP15 (specific for serotypes Asia 1, O and A, respectively). Further, all the samples were passaged 5 times in BHK-21 for the virus revival.

**Sequencing & Genetic analysis**
Nucleotide sequencing of 1D genome region (VP1 coding region) of the cell culture revived isolates was carried out using Bigdye V3.1 terminator kit according to manufacturer recommendation on ABI 3130 genetic analyzer (Applied Biosystems) using primer pNK61. Phylogenetic analysis was performed in MEGA 6 (Tamura et al., 2011). Reference sequences for comparison were taken from Genetic database of PDFMD and NCBI. Maximum Likelihood (ML) method was used for reconstructing phylogenetic tree for serotype O and A. The bootstrap consensus tree inferred from 1000 replicates was taken to represent the evolutionary history of the taxa analyzed.

**Antigenic Analysis**
2D-MNT was performed using BVS against vaccine strain INDR2/1975 and IND40/2000 following two-fold dilutions (1:2 to 1:1024) of mono specific Bovine vaccinate serum (BVS, against each of the type O and A reference virus) as described (Rweyemamu et al., 1978). The highest dilution of serum neutralizing 100TCID₅₀ (calculated according to Reed and Muench, 1938) of the virus was determined graphically. This value was taken as the serum titre. The serological relationship was found out by regression analysis and expressed as ‘r’ value.
RESULTS

Virus Serotyping
Out of 17 clinical materials tested using sandwich ELISA and m-PCR, 7 samples were found positive for FMDV of which serotype O was found in 3 samples and A in 4 samples. Upon serial blind passage in BHK-21 cells, the virus could be isolated from 4 clinical samples (serotype O and A in 2 samples each). The FMDV positive samples belonged to unvaccinated animals, which was reflected from the history taken while sampling.

Genetic characterization
The sequences of VP1 coding region were determined successfully for 4 isolates. The serotype O isolates (designated as PD314/2012 and PD315/2012) clustered in an unnamed group which includes strains circulating mostly in Eastern region (Assam, Meghalaya, Tripura, West Bengal and Odisha) of the country (Fig. 1) and henceforth named here as Eastern cluster. The cluster also contained isolates collected in Nepal and Bangladesh during 2009. The genetic group was divergent from currently used vaccine strain by 10.6 – 12.3% at nucleotide level and had a close phylogenetic relationship with lineage Branch CII described earlier (Hemadri et al., 2002). The eastern cluster had 4 to 8.8% diversity at nucleotide level from Branch CII. Within group genetic diversity (nt) varied from 2.6 to 4.2%. The genetic cluster had 10-12.7% genetic divergence from Ind2001 lineage circulating predominantly in all the regions of the country. Compared to the vaccine strain, the isolates of this study had variations at 13 positions (P4T, K45Q, I48V, K81R, E95V, D138E, S140P, V141A, I144A, A158T, I194V, N197S and V209G). Besides, the isolates had three unique variations compared other field isolates at position 81 (K to R), 194 (I to V) and 209 (V to G). RGDLXXL and antigenically critical positions (43, 44, 144, 148, 149, 154 and 208) were found conserved.

Both the serotype A isolates (designated as PD604/2012 and PD605/2012) sequenced in this study grouped within VP359–deletion group of genotype 18, precisely in clade 18c (Fig. 2). The isolates were 1.1% divergent from an isolate collected from Uttar Pradesh (PD388/2012) and 1.6 to 1.8% divergent from isolates collected in Karnataka (PD584/2012 and PD585/2012).

Antigenic Characterization
2D-MNT was performed using BVS against currently used vaccine strains IND R2/1975 and IND40/2000 for serotype O and A, respectively. All the four revived field strains showed an ‘r’ value greater than 0.3, with a lower limit of 0.38 and the higher limit being 0.58. The values projected protection conferred by the current vaccine strains against the field isolates.

DISCUSSION
Foot-and-mouth disease (FMD) is endemic in India with circulation of three serotypes (O, A and Asia1), serotype O being dominant followed by A and Asia1. Various lineages/genetic groups co-circulate, making the vaccination based control programme a challenging task. The same scenario hold true for the state of Odisha which falls in the eastern coastal region of the country and is a significant contributor to the livestock population of India with approximately 24.02 million FMD susceptible heads (Mohanty et al., 2015). An inactivated trivalent vaccine is in use as prophylaxis under the state level FMD control programme but however is limited to large ruminants like cattle and buffalo. The incidence of clinical disease among cattle prompted the current study by sampling small outbreak pockets of coastal Odisha. During last five years (2007-08 to 2011-12), a total of 59 FMD outbreaks were recorded in the state and of which 54 were due to serotype O and 5 outbreaks owing to serotype A were recorded in 2007. In this study, outbreak due to serotype A and O was recorded in Jagatpur and Athpur block of Cuttack district (one of the coastal districts) during 2011 (Annual report PD FMD 2011-12). The study revealed continuous presence of serotype O, with outbreaks being reported every year coupled with intermittent circulation of serotype A in the state of Odisha. Even, evidence of FMDV structural
Figure 1. Maximum Likelihood estimated Phylogenetic relationships of FMDV serotype O isolates. Isolates sequenced in the study are highlighted in blue. Both the isolates were collected from same outbreaks and grouped in Eastern cluster which shares ancestry with Branch CII.
Figure 2. Maximum Likelihood estimated Phylogenetic relationships of FMDV serotype A isolates. Isolates sequenced in the study are encircled. Both the isolates were collected from same outbreaks and grouped in clade 18c.
and non-structural antibodies among small and large ruminant population of coastal Odisha ascertaining virus circulation has also been reported (Mohanty et al., 2015).

It has been previously reported (Pattnaik et al., 1998; Hemadri et al., 2000) that type O FMD virus population circulating in India and causing disease outbreaks are genetically much heterogeneous. According to Samuel and Knowles, (2001) all the isolates from India belonged to Middle East- South Asia (ME-SA) topotype. Seven sub-lineages of the virus, namely Branch A, B, C-I, C-II, PanAsia I & II and ‘Ind2001’ have been described in India. Currently, majority of the outbreaks are caused by Ind2001 followed by PanAsia I and II with sporadic cases of CII. (Subramaniam et al., 2012). The finding of the current study falls in line with the report of the above authors as, it was evident from the phylogenetic tree that there has been circulation of prominent lineages including Branch CII, PanAsia and Ind2001 in Odisha. Except for the two isolates of this current study, Ind2001 lineage is appeared to be predominantly circulating in Odisha. It was also found that the isolates in eastern cluster, which included strains mostly circulating in the Eastern region (Assam, Meghalaya, Tripura, West Bengal and Odisha) also, contained isolates from Nepal and Bangladesh during 2009. The eastern states share border with both Nepal and Bangladesh and it is possible that the virus might have traversed either way. Within group genetic diversity (nt) was less than 4.2% and this group of viruses has been circulating in the state of Odisha since 2009 or may be longer. However it was interesting to note that this group that could not spread outside eastern region irrespective of animal movement across state borders.

Serotype A FMD virus that is genetically and antigenically most diverse could be grouped into 26 global genotypes based on 1D sequence analysis with >15% nucleotide divergence (Mohapatra et al., 2011). Of these, four genotypes (2, 10, 16 and 18) have been identified in India with endemic co-circulation of genotypes 16 and 18 between 1990 and 2001. Within genotype 18, a divergent and unique lineage emerged during the later part of 2002, having an amino acid (aa) deletion at the 59th position of VP3 (VP359 deletion group) and dominated the field outbreak scenario in 2002–2003 (Jangra et al., 2005). With time the deletion group has diversified leading to three clades (a, b and c). Last outbreak owing to serotype A in Odisha was recorded in Kalahandi and Jagatsinghpur during 2009-10. The isolates were found belonged to non-deletion group of genotype 18. However the Serotype A isolates of this study belonged to VP359– deletion group of genotype 18, precisely clade 18c. The clade 18c was first reported in Southern parts of India with a gradual migration toward the Western, Central and Northern regions. But the recent outbreak in Jagatpur with the particular group was the first report from the state of Odisha. Among the eastern regions, the emergence of deletion group has also been observed in the state of Assam and West Bengal in the year 2003 and 2006 respectively. The serotypes had less than 2% genetic divergence from the isolate of UP and Karnataka at VP1 region, clearly suggestive of close epidemiological linkage between the isolates, which could have been due to the direct introduction of the virus among the livestock population of the outbreak area by animal migration. The above statement was well backed up by facts of constant transboundary animal movement through the outbreak area. Moreover an outbreak in Karnataka with serotype A deletion group clade18c in June coincided with an outbreak in Jagatpur in the same month.

Foot-and-mouth disease is endemic in India from a very long time with occurrences in many parts of the country. The progressive evolution of virus poses an eminent threat towards control and management of the disease. The current study highlights the emergence of virus belonging to the VP359 deletion group for the first time in the state of Odisha and circulation of specific strains of serotype O in eastern region including Odisha. Even though the current vaccine strains were capable of providing adequate antigenic coverage against the recent outbreaks, but the continuous emergence of genetic and antigenic variants in the field viruses,
emphasizes on the need to study the outbreaks and characterizing the isolates to monitor the dynamics of the virus. Moreover, while devising control strategies for FMD, evaluation of various genetic clusters and their antigenic characteristics might provide the right direction in this regards.

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REFERENCE


