Tropical Biomedicine 33(4): 753–760 (2016)

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

Mohanty, N.N.[1*], Das, B.[2], Sarangi, L.N.[3], Subramaniam, S.[2], Mohapatra, J.K.[2] and Panda, H.K.[4]
[1]ICAR – Indian Veterinary Research Institute, Mukteswar, Uttarakhand, India
[2]ICAR – Directorate on Foot-and-Mouth Disease, Mukteswar, Nainital, India
[3]NDDB R&D Laboratory, National Dairy Development Board, IIL Campus, Gachibowli, Hyderabad, India
[4]Department of Microbiology, College of Veterinary Science & Animal Husbandry, Odisha University of Agriculture and Technology, Bhubaneswar, Odisha, India
[*]Corresponding author e-mail: just_nihar@rediffmail.com
Received 27 December 2016; received in revised form 10 July 2016; accepted 12 July 2016

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. FMD 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 $VP3^{59}$–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 (Subramanium *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 transcripted using FMDV universal primer pNK61 (Knowles and Samuel, 1995). m-PCR was performed with g reverse primer pNK61 and forward primers DHP9, DHP13and 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 100$TCID_{50}$ (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 VP3[59]–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

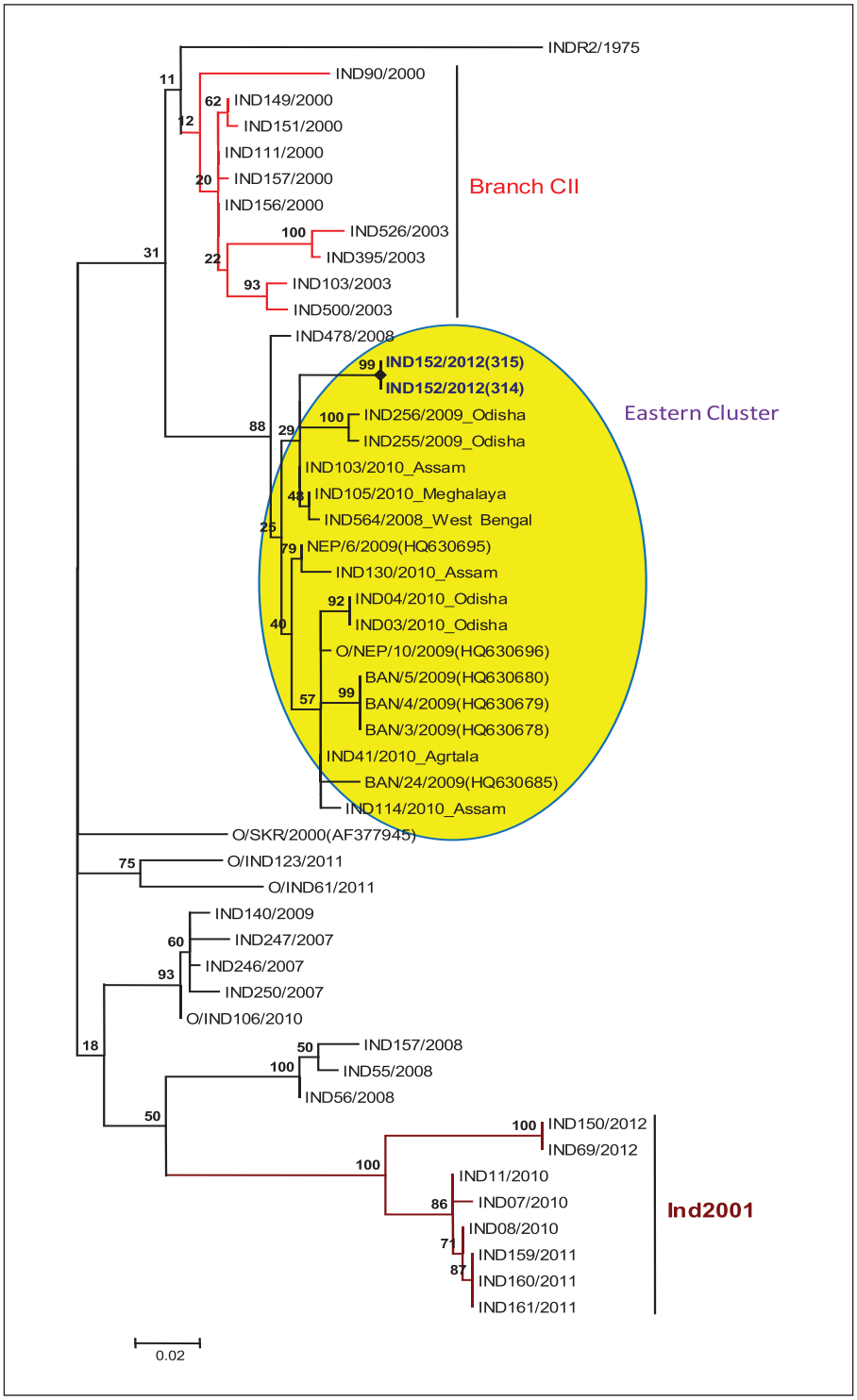

Figure 1. Maximum Likelihood estimated Phylogenetic relationships of FMDV serotype O isolates. Isolates sequenced in the study are highlighted in blue. Both the isolates were collected from same outbreaks and grouped in Eastern cluster which shares ancestry with Branch CII.

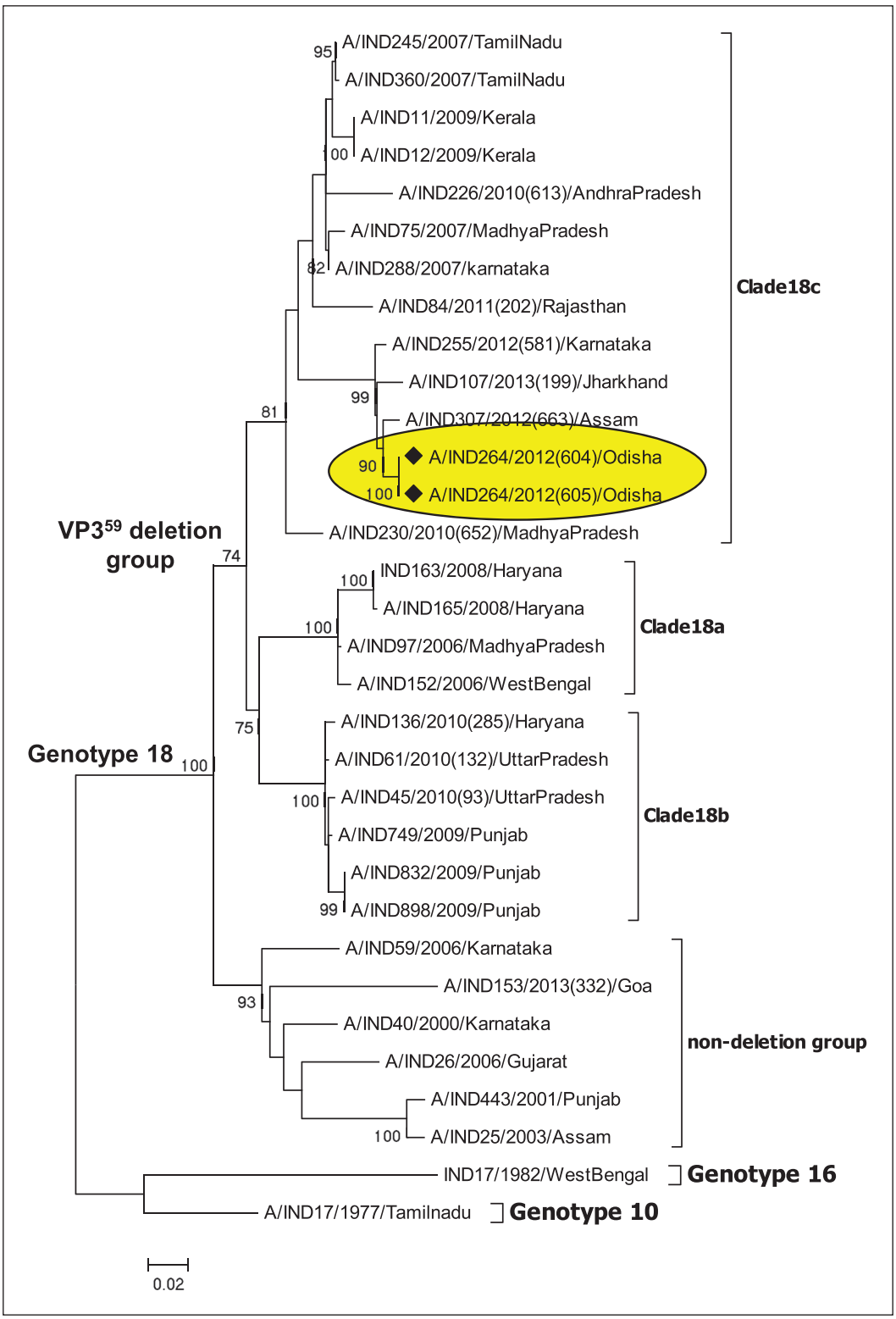

Figure 2. Maximum Likelihood estimated Phylogenetic relationships of FMDV serotype A isolates. Isolates sequenced in the study are encircled. Both the isolates were collected from same outbreaks and grouped in clade 18c.

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 ($VP3^{59}$ 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 $VP3^{59}$– 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 $VP3^{59}$ 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.

Acknowledgement. The authors are thankful to Indian Council of Agricultural Research, Director, Project Directorate on FMD, and College of Veterinary Science & Animal Husbandry, Bhubaneswar for providing necessary facilities to carry out this work. The authors also express their gratitude to Nayan S Singh for the technical support provided during the tests.

REFERENCE

Bhattacharya, S., Pattnaik, B. and Venkataramanan, R. (1996). Development and application of a sandwich enzyme linked immunosorbent assay (ELISA) for type identification of foot-and mouth disease (FMD) in virus in direct field materials. *Indian Journal of Animal Science*, **66**: 1201-09.

Domingo, E., Escarmýìs, C., Baranowski, E., Ruiz-Jarabo, C.M., Carrillo, E., Núñez, J.I. and Sobrino, F. (2003). Evolution of foot-and-mouth disease virus. *Virus Research*, **91**(1): 47-63.

Giridharan, P., Hemadri, D., Tosh, C., Sanyal, A. and Bandyopadhyay, S.K. (2005). Development and evaluation of a multiplex PCR for differentiation of foot-and-mouth disease virus strains native to India. *Journal of Virological Methods*, **126**(1): 1-11.

Hemadri, D., Tosh, C., Sanyal, A. and Venkataramanan, R. (2002). Emergence of a new strain of type O foot-and-mouth disease virus: its phylogenetic and evolutionary relationship with the PanAsia pandemic strain. *Virus Genes*, **25**(1): 23-34.

Hemadri, D., Tosh, C., Venkataramanan, R., Sanyal, A., Samuel, A.R., Knowles, N.J. and Kitching, R.P. (2000). Genetic analysis of foot-and-mouth disease virus type O isolates responsible for field outbreaks in India between 1993 and 1999. *Epidemiology and Infection*, **125**(3): 729-736.

Jangra, R.K., Tosh, C., Sanyal, A., Hemadri, D. and Bandyopadhyay, S.K. (2005). Antigenic and genetic analyses of foot-and-mouth disease virus type A isolates for selection of candidate vaccine strain reveals emergence of a variant virus that is responsible for most recent outbreaks in India. *Virus Research*, **112**(1): 52-59.

Knowles, N.J. and Samuel, A.R. (2003). Molecular epidemiology of foot-and-mouth disease virus. *Virus Research*, **91**(1): 65-80.

Knowles, N.J. and Samuel, A.R. (1995). Polymerase chain reaction amplification and cycle sequencing of the 1D (VP1) gene of foot-and-mouth disease viruses. Report of the Session of the Standing Technical Committee of the European Commission for the Control of Foot-and-Mouth disease held at Vienna, Austria, September 19–22, 1994. FAO, Rome, pp. 45–53.

Mohanty, N.N., Subramaniam, S., Rout, M., Sarangi, L.N., Bist, P., Pandey, L.K., Mohapatra, J.K. and Panda, H.K. (2015). Serosurveillance of foot-and-mouth disease in ruminant population of Coastal Odisha, India. *Beni-Suef University Journal of Basic and Applied Sciences*, **4**(4): 279-283.

Mohapatra, J.K., Subramaniam, S., Pandey, L.K., Pawar, S.S., De, A., Das, B., Sanyal, A. and Pattnaik, B. (2011). Phylogenetic structure of serotype A foot-and-mouth disease virus: global diversity and the Indian perspective. *Journal of General Virology*, **92**: 873-879.

Pattnaik, B. (2013). PD-FMD Annual Report, 2011–12, pp.16. ICAR – Project Directorate on Foot and mouth disease, Mukteswar.

Pattnaik, B., Venkataramanan, R., Tosh, C., Sanyal, A., Hemadri, D., Samuel, A.R., Knowles, N.J. and Kitching, R.P. (1998). Genetic heterogeneity of Indian field isolates of foot-and-mouth disease virus serotype O as revealed by partial sequencing of 1D gene. *Virus Research*, **55**(2): 115-127.

Racaniello, V.R. (2001). Picornaviridae: the viruses and their replication. In: Knipe, D.M., Howley, P.M. (Eds.), Fields Virology, fourth ed., vol. 1. Lippincott/The Williams & Wilkins Co, Philadelphia, PA;pp 685–722.

Reed, L.J. and Muench, H. (1938). A simple method of estimating fifty per cent endpoints. *American Journal of Epidemiology*, **27**(3): 493-497.

Rweyemamu, M.M., Booth, J.C., Head, M. and Pay, T.W.F. (1978). Microneutralization tests for serological typing and subtyping of foot-and-mouth disease virus strains. *Journal of Hygiene*, **81**(1): 107-123.

Samuel, A.R. and Knowles, N.J. (2001). Foot-and-mouth disease type O viruses exhibit genetically and geographically distinct evolutionary lineages (topotypes). *Journal of General Virology*, **82**(3): 609-621.

Subramaniam, S., Pattnaik, B., Sanyal, A., Mohapatra, J.K., Pawar, S.S., Sharma, G.K., Das, B. and Dash, B.B. (2012). Status of Footandmouth Disease in India. *Transboundary and Emerging Diseases* **60**(3): 197-203.

Sutmoller, P., Barteling, S.S., Olascoaga, R.C. and Sumption, K.J. (2003). Control and eradication of foot-and-mouth disease. *Virus Research*, **91**(1): 101-144.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. and Kumar, S. (2011). MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution*, **28**(10): 2731-2739.