



Investigation of foot-and-mouth disease outbreaks in Chikkaballapur district of Karnataka

M ROUT¹, J K BISWAL², B B DASH³, R HEGDE⁴, S SUBRAMANIAM⁵ and J K MOHAPATRA⁶

Project Directorate on Foot and Mouth Disease, IVRI Campus, Mukteswar, Uttarakhand 263 138 India

Received: 12 July 2013; Accepted: 27 November 2013

ABSTRACT

The present study was undertaken to investigate foot-and-mouth disease (FMD) outbreaks in various villages of Chikkaballapur district in Karnataka. The disease was recorded in both small and large ruminant population. Serum samples (357) were collected from clinically sick, recovered and in-contact animals, and clinical samples (31) were collected from sick or recovering animals. In 3AB non-structural protein (NSP) ELISA, 42.85% cattle, 59.93% sheep and 73.91% goats were tested positive indicating an extensive FMD virus (FMDV) activity. The serum samples were also tested in liquid phase blocking (LPB) ELISA to assess the protective antibody titre, which were 42.85%, 6.72% and 8.69% for cattle, sheep and goats, respectively. Hence, it is presumed that poor herd immunity might have been one of the contributing factors for the spread of the disease. The outbreaks were caused by FMDV serotype O and Asia 1 as confirmed in serotype differentiating antigen detection ELISA and multiplex PCR. The VP1 region based phylogenetic analysis indicated the involvement of both Ind2001 and recently identified Ind2011 lineage of serotype O and lineage C of serotype Asia 1 in the outbreaks. The study revealed the epidemiological complexity of FMD in the field with respect to the spectrum of species of animals affected and the contemporaneous involvement of multiple serotypes and lineages.

Key words: Antigen detection ELISA, Foot-and-mouth disease, Karnataka, LPB ELISA, NSP ELISA, Ruminants

India has the distinction of holding the largest livestock population ranking first in cattle and buffalo, second in goat and third in sheep population in the world. Mixed herds with goats, sheep, cattle and buffaloes are found in this system. Foot-and-mouth disease (FMD) is a highly contagious viral disease of cloven-hoofed animals. The disease is of transboundary importance causing enormous economical losses to livestock production and can spread in the form of highly disruptive large-scale epidemics. The disease severity varies between host species, with cattle developing severe clinical signs, whereas small ruminants with mild clinical disease. Three serotypes of the virus, viz. O, A and Asia 1 are prevalent in South Asia, and about 80% of the outbreaks in India are attributed to serotype O (Subramaniam *et al.* 2012). Timely investigation of field outbreaks aid rapid implementation of appropriate vaccination and zoosanitary measures for effective control of the disease and for restricting the dissemination of the virus. In the present study, FMD outbreaks at various villages in Chikkaballapur district of Karnataka were

investigated.

MATERIALS AND METHODS

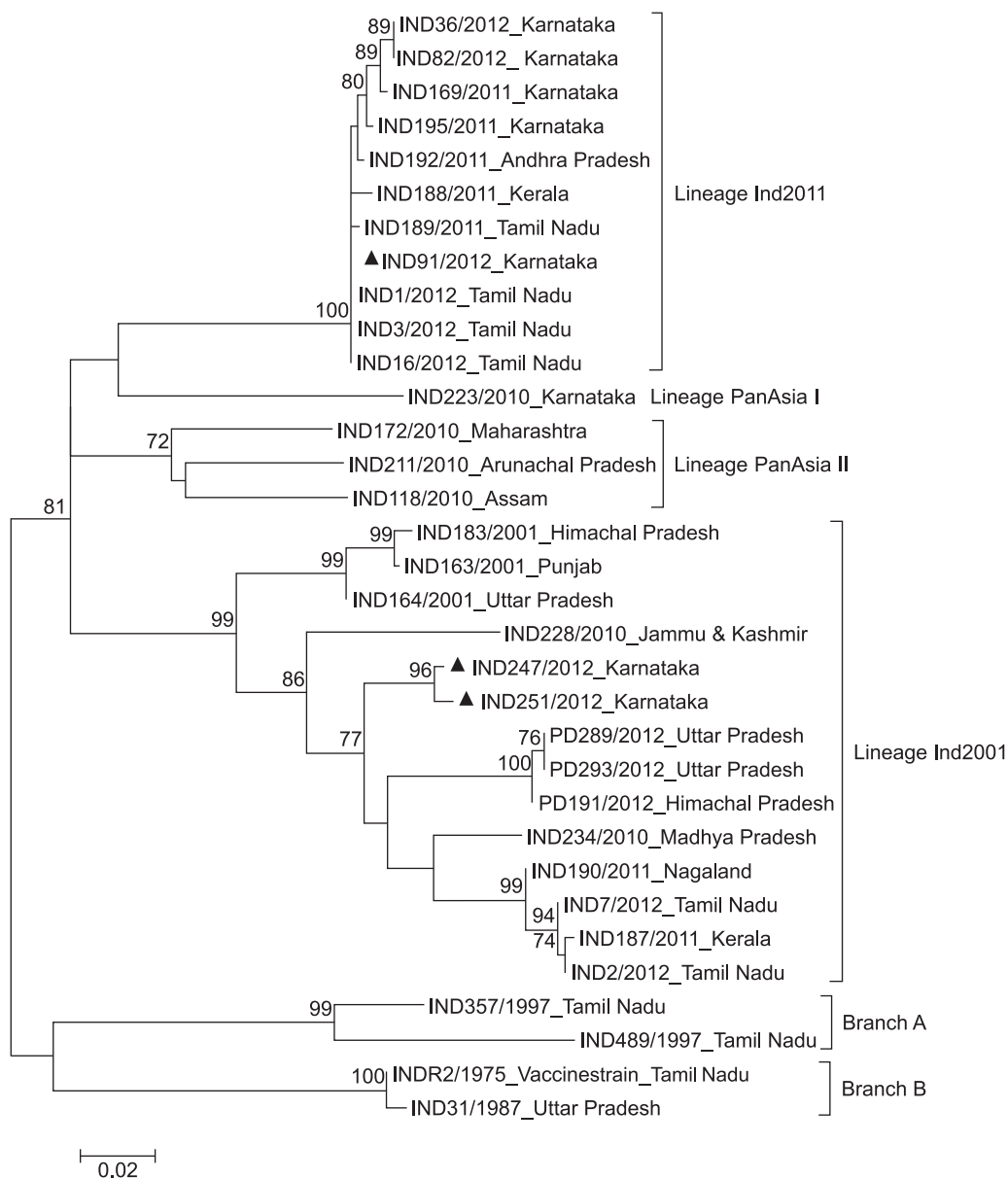
Sample collection: Serum samples (357) from small and large ruminants (cattle 7; sheep 327; goat 23) were collected during April 2012 from different villages within a radius of approximately 30 km in the Chikkaballapur district of Karnataka that had FMD outbreaks. A total of 31 clinical tissue samples (vesicle/tongue epithelium) from 4 sheep (3 from Anur and 1 from Jatawarahosahalli), and 27 cattle (8 from Danamitnahalli, 8 from Kamenahalli, 3 from Devastanahosahalli, 6 from Nanjareddipalli, 2 from Jatawarahosahalli) were collected in 50% phosphate buffered saline/glycerol medium (pH, 7.5).

3AB NSP ELISA for detection of anti-FMDV non-structural protein (NSP) antibodies: An indirect ELISA was performed as per Mohapatra *et al.* (2011) using the in-house r3AB3 NSP ELISA kit to assess antibodies against 3AB NSP of FMDV. Bovine test serum samples were diluted at 1: 20, while sheep and goat samples were diluted at 1: 50 ratio in the diluent buffer. Subsequently, anti-bovine and anti-ovine/caprine horse radish peroxidase conjugated antibodies were dispensed at 1: 2000 and 1: 20000 dilutions, respectively. Serum samples producing corrected optical density value $\geq 40\%$ of that of the positive control were scored positive.

Present address: ^{1-2,5}Scientist (drmROUT@gmail.com, jkubiswal@gmail.com, saranvirol@gmail.com), ^{3,6}Senior Scientist (bbdash08@gmail.com, jajati1@gmail.com). ⁴Epidemiologist and Officer In-charge (honkankeravi@yahoo.com), AICRP Regional Centre on FMD, IAH & VB, Hebbal, Bengaluru 560 024.

Table 1. Seropositivity of samples collected from outbreak areas for FMD virus SP and NSP antibodies

Species	Total samples	3AB NSP-Ab positive	Number of animals showing log ₁₀ titre of ≥ 1.8 against all 3 serotypes	Spike of SP-Ab and NSP-Ab positivity	Type O	Type A	Type Asia1
Cattle	7	3 (42.85%)	3 (42.85%)	SP-Ab 4 fold spike Both SP-Ab 4 fold spike and NSP-Ab positive	0 (0%) 0 (0%)	0 (0%) 0 (0%)	0 (0%) 0 (0%)
Goat	23	17 (73.91%)	2 (8.69%)	SP-Ab 4 fold spike Both SP-Ab 4 fold spike and NSP-Ab positive	6 (26.08%) 6 (26.08%)	0 (0%) 0 (0%)	1 (4.34%) 1 (4.34%)
Sheep	327	196 (59.93%)	22 (6.72%)	SP-Ab 4 fold spike Both SP-Ab 4 fold spike and NSP-Ab positive	125 (38.22%) 95 (29.05%)	6 (1.83%) 5 (1.52%)	2 (0.61%) 1 (0.30%)



Continued...

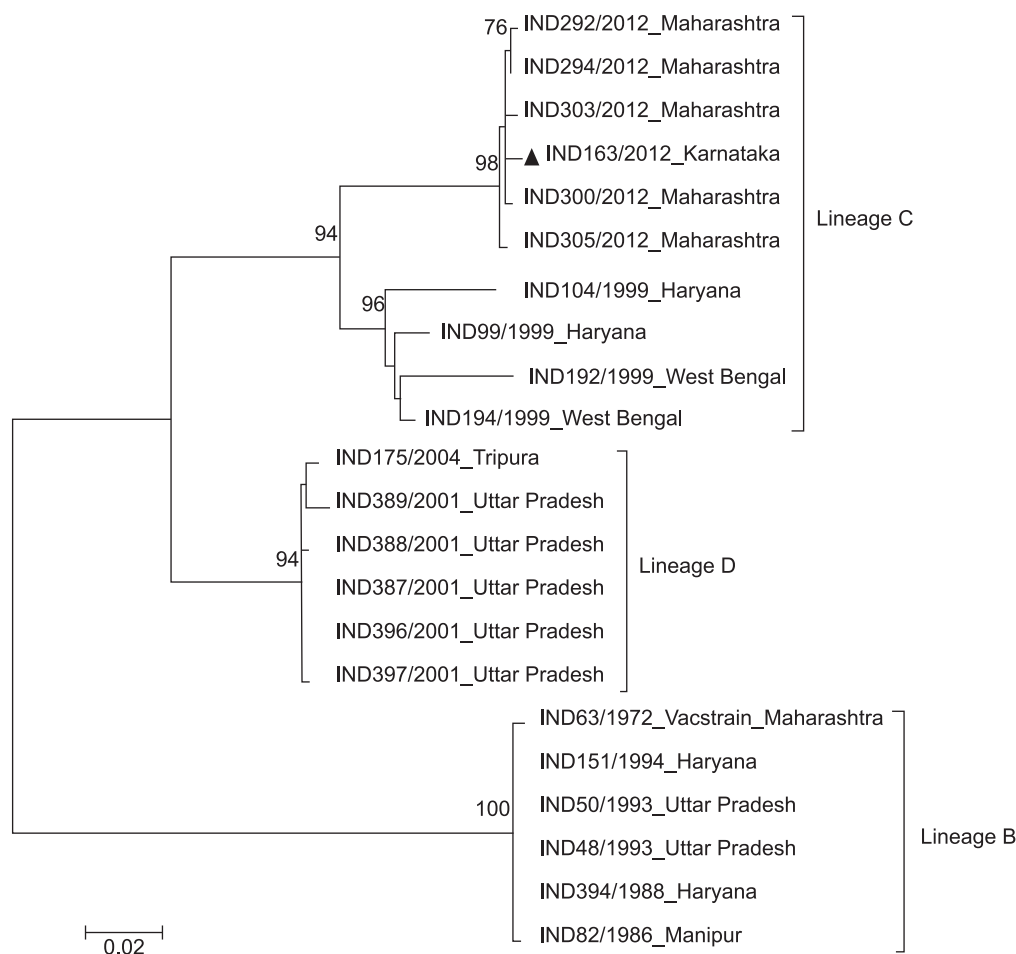


Fig. 1. Neighbor-joining tree at 1D region showing the phylogenetic relationship of FMDV serotype O (1a) and Asia 1 (1b) isolates. The isolate sampled in the outbreaks are marked with solid triangle. Bootstrap support values above 70% are shown near the major nodes

Liquid phase blocking (LPB) ELISA for titration of anti-FMDV structural protein (SP) antibodies: Two-fold dilution of serum samples were tested for serotype-specific FMDV SP-Ab titre using the in-house LPB ELISA kit as per Ranabijuli *et al.* (2010) to assess the protective antibody titre (cutoff for protective antibody being $\log_{10}$ titre of ≥ 1.8 against all 3 component serotypes such as O, A and Asia 1 in the vaccine). The results were expressed as percentage reactivity for each serum dilution as follows:

$$\text{Percentage reactivity} = (\text{OD mean of each test serum dilution} / \text{OD mean of antigen control}) \times 100$$

The antibody titres were expressed as logarithm of reciprocal of serum dilutions giving 50% of the absorbance recorded in the antigen control wells.

Serotype differentiating antigen detection ELISA: Supernatants of the homogenized clinical tissue materials were used in a serotype differentiating antigen detection ELISA as per Bhattacharya *et al.* (1996) for confirmation of serotype of the virus involved in the outbreaks.

Serotype differentiating multiplex polymerase chain reaction (mPCR): Total RNA was extracted from the tissue

samples using RNeasy mini kit. Reverse transcription was performed using M-MLV reverse transcriptase and reverse primer NK61 (Knowles and Samuel 1995). A serotype differentiating mPCR was performed using a commercial kit (Giridharan *et al.* 2005). The mPCR products were visualized on ethidium bromide stained 2% agarose gel.

Nucleotide sequencing: PCR amplification of VP1 region was performed using *Pfu* polymerase. For serotype O, the primer combination of ARS4 and NK61 (Knowles and Samuel 1995), while for serotype Asia 1, the primer combination of 1C505 and NK61 (Knowles and Samuel 1995) were used. The details of sequencing primers and thermal conditions applied were essentially as described earlier (Hemadri *et al.* 2002, Sanyal *et al.* 2010). Cycle sequencing reactions of gel purified PCR products were carried out using a kit and sequences were resolved on ABI 3130 genetic analyzer. Phylogenetic comparisons were made using maximum likelihood (ML) method available in MEGA 5.05 software package (Tamura *et al.* 2011). Indian field outbreak strains were obtained from the institute database and sequences were aligned using clustal W algorithm (Thomson *et al.* 1994). For serotype O, Tamura

3-parameter model with rate variation following a gamma distribution and for serotype Asia 1 Hasegawa-Kishino-Yano nucleotide substitution model were applied for phylogenetic reconstruction. The robustness of the tree topology was evaluated with 1,000 bootstrap replicates.

RESULTS AND DISCUSSION

India is endemic for FMD with a complex disease supporting environment prevailing in the country. Tracing the origin of the outbreak and route of virus movement play a key role in understanding the epidemiology of FMD, which in turn helps in formulation of control policies. The ongoing FMD Control Programme in India covers only bovines with regard to vaccination, overlooking caprine and ovine species. Serotypes, O, A and Asia 1, causing FMD outbreaks were documented. Although a mixed farming and communal grazing system is practiced in the country, only in a few outbreaks, the disease in small ruminants is reported and diagnosed. During the present investigation, sheep and goat populations were targeted along with cattle during an outbreak in the state of Karnataka.

During the survey, clinical FMD in the form of frank vesicular/erosive lesions on tongue and feet of cattle was evident, whereas it subsided in sheep and goats leaving some scar marks on the dental pad and lameness. SP-Abs are present in infected as well as vaccinated animals, while the detection of antibodies against NSPs is accepted as an indication of previous infection (Laddomada 2003). Besides, spiking of SP-Abs against a serotype is also indicative of infection particularly in a non-vaccination scenario. As small ruminants are largely ignored in the vaccination programme adopted in the country, any evidence of SP-Ab in the flock should be considered as an outcome of FMDV infection, and serotype-specific spike in these tracer animals should be suggestive enough of the circulating serotype of the virus (Balinda *et al.* 2009). A total of 42.85% cattle, 59.93% sheep and 73.91% goats were detected to be positive reactors in 3AB NSP ELISA suggesting an exposure of these animals to FMD virus (Table 1). LPB ELISA indicated the overall protective antibody titre ($\log_{10}$ titre of ≥ 1.8 against all 3 prevalent FMDV serotypes as cut-off) at 42.85%, 6.72% and 8.69% for cattle, sheep and goats, respectively (Table 1). Hence, it is presumed that poor herd immunity might be one of the contributing factors leading to outbreaks (Parida 2009). When clear spike in SP-Ab response against any serotype, by which we mean ≥ 4 fold increase in the titre (0.6 $\log_{10}$ titre increase) of any one compared to each of the other 2 serotypes in the vaccine was taken into account, only serotype O specific rise in titre was observed in majority of sheep (38.22%) and goats (26.08%) indicating an extensive circulation of serotype O virus in the locality. The proportion of samples showing both NSP-Ab positivity and ≤ 4 fold spike of SP-Ab titre against serotype O in goat and sheep were 26.08% and 29.05%, respectively. A few samples (4.34% goats and 0.61% sheep) also showed clear spike in SP-Ab response against serotype Asia 1. Although cattle

revealed clinical symptoms, clear 4 fold spike in SP-Ab was not apparent, which could be because of a masking effect of vaccinal antibodies or post-infection due to serotypes other than that involved in the present infection (Mwiine *et al.* 2010). It was also observed that following infection, animals could remain seropositive to structural antibodies of FMDV for several years (Doel 2005).

In serotype differentiating antigen detection ELISA, 2 sheep samples were typed as serotype O. Out of 27 cattle samples, 9 were typed as serotype O, while 4 as serotype Asia 1. Only ELISA negative samples were subjected to mPCR. In mPCR, 1 sheep and 3 cattle samples were positive for serotype O, while 2 cattle samples for serotype Asia 1. In the same village Kamenahalli, concomitant circulation of both serotype O and Asia 1 was evident. Contemporary prevalence of multiple serotypes in the same village was thought to be due to procurement of diseased animals from various neighbouring parts of the state along with long distance trafficking of small ruminants that sub-clinically carry the virus (Kitching 1998).

In VPI region based phylogenetic analysis, the serotype O isolates clustered in 2 different lineages. Isolates (PD564/2012 and PD573/2012) from village Nanjareddipalli clustered within Ind2001 lineage, while 1 isolate (PD184/2012) from village Jatawarahosahalli grouped with Ind2011 lineage (Fig. 1). The advent of Ind2001 lineage was documented in the year 2001 (Hemadri *et al.* 2002). The Ind2001 lineage after causing sporadic cases during 2003–05, re-surged in 2008 and has been dominating the outbreak scenario in the country since 2009. Within Ind2001 lineage, high level of genetic diversity was detected among the strains isolated from the state of Karnataka with circulation of many sub-clusters (Subramaniam *et al.* 2013). The Ind2011 lineage was first identified in 2011 and was restricted to the states of Karnataka, Andhra Pradesh, Tamil Nadu and Kerala in the southern region (Subramaniam *et al.* 2013). The Ind2011 lineage showed a mean nucleotide divergence of 11% from rest of the lineages circulating in the country. The serotype Asia 1 isolate (PD333/2012) from village Kamenahalli clustered with lineage C (Fig. 1), which has been exclusively circulating in India since 2005 (Sanyal *et al.* 2010). Serotype Asia 1 was re-introduced into the state of Karnataka during 2012–13 after a gap of 5 years. The virus isolates of Karnataka were closely related to the isolates collected from Gujarat and Maharashtra (less than 2% nucleotide divergence) suggesting an epidemiological link among these strains. Unrestricted trade of animals in Chikballapur district of Karnataka might be one of the probable causes of circulation of multiple serotypes and lineages of FMDV.

From the present investigation of FMD outbreaks in the field, involvement of multiple serotypes in multispecies was confirmed. Regular surveillance of FMD is thus very important to keep track on the circulating serotypes and strains of the virus. Each and every outbreak in an endemic region warrants rapid diagnosis and detailed scientific investigation to understand the dynamics of FMDV

circulation and to evolve effective control strategies.

ACKNOWLEDGEMENTS

The authors are highly thankful to the Indian Council of Agricultural Research for granting funds and facilities for carrying out the work. We are also thankful to the farmers who were co-operative enough to allow the collection of clinical and serum samples from their affected animals. The help extended by Mr Nagraj during sample collection is also duly acknowledged.

REFERENCES

- Balinda S N, Tjornehoj K, Muwanika V B, Sangula A K, Mwiine F N, Ayebazibwe C, Masembe C, Siegismund H R and Alexandersen S. 2009. Prevalence estimates of antibodies towards foot-and-mouth disease virus in small ruminants in Uganda. *Transboundary and Emerging Diseases* **56**: 362–71.
- Bhattacharya S, Pattnaik B and Venkataramanan R. 1996. Development and application of sandwich enzyme-linked immunosorbent assay (ELISA) for type identification of foot-and-mouth disease (FMD) virus in direct field materials. *Indian Journal of Animal Sciences* **66**: 1–9.
- Doel T R. 2005. Natural and vaccine induced immunity to FMD. *Current Topics in Microbiology and Immunology* **288**: 103–31.
- 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**: 1–11.
- Hemadri D, Tosh C, Sanyal A and Venkataramanan R. 2002. Emergences of a new strain of type O foot-and-mouth disease virus: its phylogenetic and evolutionary relationship with PanAsia pandemic strain. *Virus Genes* **25**: 23–34.
- Kitching R P. 1998. A recent history of foot-and-mouth disease. *Journal of Comparative Pathology* **118**: 89–108.
- 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 Research Group of the Standing Technical Committee of European Community for Control of FMD*. FAO, Vienna Austria September 1994, 45–53.
- Laddomada A. 2003. Control and eradication of O.I.E. list A diseases the approach of the European Union to the use of vaccines. *Developmental Biology, Basel, Karger* **114**: 269–80.
- Mohapatra J K, Pandey L K, Sanyal A and Pattnaik B. 2011. Recombinant non-structural polyprotein 3AB-based serodiagnostic strategy for FMD surveillance in bovines irrespective of vaccination. *Journal of Virological Methods* **177**: 184–92.
- Mwiine, F N, Ayebazibwe C, Alexandersen S, Olaho-Mukani, W, Okurut Ademun A R and Tjornehoj K. 2010. Seroepidemiological investigation of foot-and-mouth disease virus serotypes in cattle around Lake Mburo National Park in South-Western Uganda. *Journal of Veterinary Medicine and Animal Health* **2**: 46–54.
- Parida S. 2009. Vaccination against foot-and-mouth disease virus: strategies and effectiveness. *Expert Review Vaccines* **8**: 347–65.
- Ranabijuli S., Mohapatra, J K, Pandey L K, Rout M, Sanyal A, Dash B B, Sarangi L N, Panda H K and Pattnaik B. 2010. Serological evidence of foot and mouth disease infection in randomly surveyed goat population of Orissa, India. *Transboundary and Emerging Diseases* **57**: 448–54.
- Sanyal A, Subramaniam S, Mohapatra J K, Tamilselvan R P, Singh N K, Hemadri D and Pattnaik B. 2010. Phylogenetic analysis of Indian serotype Asia1 foot-and-mouth-disease virus isolates revealed emergence and reemergence of different genetic lineages. *Veterinary Microbiology* **144**: 198–202.
- Subramaniam S, Pattnaik B, Sanyal A, Mohapatra J K, Pawar S S, Sharma G K, Das B and Dash B B. 2012. Status of foot-and-mouth disease in India. *Transboundary and Emerging Diseases* **60**: 197–203.
- Subramaniam S, Sanyal A, Mohapatra J K, Sharma G K, Biswal J K, Ranjan R, Rout M, Das B, Bisht P, Mathapati B S, Dash B B and Pattnaik B. 2013. Emergence of a novel lineage genetically divergent from the predominant Ind2001 lineage of serotype O foot-and-mouth disease virus in India. *Infection, Genetics and Evolution* **3**: 18C: 1–7.
- 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**: 2731–9.
- Thompson J D, Higgins D G and Gibson T J. 1994. CLUSTAL W improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weightmatrix choice. *Nucleic Acids Research* **22**: 4673–80.