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Foot-and-mouth disease in elephants in Kerala state of India during 2013

MANORANJAN ROUT[1], NANDAKUMAR S. NAIR[2], BISWAJIT DAS[3], SARAVANAN SUBRAMANIAM[4], JAJATI KESHARI MOHAPATRA[5] and BRAMHADEV PATTNAIK[6]

ICAR-Project Directorate on Foot and Mouth Disease, IVRI Campus, Mukteshwar, Uttarakhand 263 138 India

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ABSTRACT

Foot-and-mouth disease (FMD) is a highly contagious acute vesicular disease of the cloven-hoofed animals including cattle, buffalo, sheep, goats, pigs along with more than 70 wildlife species. During the year 2013, FMD outbreaks were recorded in the southern peninsular India comprising the states of Karnataka, Tamil Nadu, Kerala and Andhra Pradesh. Besides domestic livestock, captive elephants in Kerala were also affected by FMD. The suspected FMD outbreak in six elephants occurred in Neendoor of Kottayam district, Guruvayoor and Thrissur of Thrissur district in Kerala during November - December 2013. The first clinical signs recorded in the elephants were loss of appetite and lameness with mild fever. Frank lesions were grossly evident on the tongue, palate and inner mucous membrane of trunk with exudates from nostrils. There was copious salivation often appeared to be drooling. Severe lameness led to recumbency. Erosive lesions were also noticed in foot-slipper. The foot with blisters turned to open sores making the animals difficult to stand and walk. Clinical samples (foot/oral/tongue/trunk/nasal epithelium) from the FMD-suspected elephants were collected in 50% phosphate buffered saline/glycerol medium (pH-7.5). Supernatants of the homogenized clinical samples were used in a serotype differentiating antigen detection ELISA and samples found negative were further subjected to multiplex PCR. All clinical samples were found positive for FMD virus (FMDV) serotype O in antigen detection ELISA and in mPCR. The VP1 region based phylogenetic analysis indicated the involvement of O/Middle East-South Asia/Ind2001d sub-lineage of FMDV serotype O, which was also responsible for severe disease in domestic livestock in southern states of India during 2013.

Key words: Elephant, Foot-and-mouth disease, India, Kerala

Foot-and-mouth disease (FMD) is a highly contagious acute vesicular disease of the cloven-hoofed animals including cattle, buffalo, sheep, goat, pig along with more than 70 wildlife species and considered as a menace to the livestock industry (Pattnaik *et al.* 2012, Ding *et al.* 2013). FMD in India is enzootic, where three serotypes of the virus such as O, A and Asia 1 are prevalent and about 80% of the outbreaks are attributed to serotype O (Subramaniam *et al.* 2013). The elephant (*Elephas maximus*) has been considered as an integral part of India's history, tradition, myth and culture. During 2013, many FMD outbreaks due to serotype O were recorded in India. Besides domestic animals, outbreaks were also recorded in captive elephants in the state of Kerala. The present study investigates the FMD virus (FMDV) infection in elephants of Kerala with regard to partial characterization and phylogenetic analysis of the viruses involved in the disease outbreak.

MATERIALS AND METHODS

The suspected FMD outbreak in six elephants occurred in Neendoor of Kottayam district, Guruvayoor and Thrissur of Thrissur district in Kerala during November - December 2013. Clinical samples (foot/oral/tongue/trunk/nasal epithelium) from those elephants were collected in 50% phosphate buffered saline/glycerol medium (pH-7.5). Supernatants of the homogenized clinical tissue samples were used in a serotype differentiating antigen detection ELISA as per the method described by Bhattacharya *et al.* (1996) for confirmation of the serotype of the virus involved in the outbreak. Samples found negative in ELISA were further subjected to 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). The serotype differentiating mPCR was performed using Hotstar Kit essentially as described previously (Giridharan *et al.* 2005). The mPCR products were visualized on ethidium bromide stained 2% agarose gel.

PCR amplification of VP1 region was performed using *Pfu* DNA polymerase and the primer combination of ARS4 and NK61 (Knowles and Samuel 1995) following thermal conditions described earlier (Hemadri *et al.* 2002). Cycle

Present address: [1]Scientist (drmrout@gmail.com); [3]Research Associate; [4]Scientist (SS); [5]Senior Scientist; [6]Project Director. [2]Co-PI, FMD Collaborating Centre, Chief Disease Investigation Office, Palode, Thiruvananthapuram.

sequencing reactions of gel purified PCR products were carried out using BigdyeV3.1 terminator kit and sequences were resolved on ABI 3130 genetic analyzer. The nucleotide sequences were aligned using clustal W algorithm (Thompson *et al*. 1994). Phylogenetic analysis was conducted using MEGA 6.06 software (Tamura *et al.* 2013) employing the best fit nucleotide substitution model, TN93+G+I. Phylogenetic tree was reconstructed using Neighbor joining (NJ) method available and the robustness of the tree topology was evaluated with 1000 bootstrap replicates.

RESULTS AND DISCUSSION

The first clinical signs recorded in the suspected elephants were loss of appetite and lameness with mild fever. Frank lesions were grossly evident on the tongue, palate and inner mucous membrane of trunk with exudates from nostrils. There was copious salivation often appeared to be drooling. Severe lameness led to recumbency. Erosive lesions were also noticed in foot-slipper. The foot with blisters turned to open sores making the animals difficult to stand and walk. The lesions in mouth and feet of elephants can be severe, which may be attributed to the invasiveness of the virus strains and highly susceptible nature of the elephant to the virus, but mechanical stress to which the affected tissues are subjected might also be an important factor contributing to the severity of the disease. In elephants, the excessive stress on the feet due to heavy weight of the animal may result in severity of the foot lesions. Moreover, in Kerala, since elephants are mostly rented out for '*ezhunnellippu*' (parading with statues of deities mounted on the elephant's back) during temple festivals, they need to work continuously for several hours that may aggravate the foot lesions in the affected elephants. However, the affected elephants should not be used for this purpose; otherwise the infectious virus may rapidly spread to other healthy animals depending upon its quantum and virulence. Investigation of an outbreak of FMD in elephants used for ceremonial purposes in Nepal found that the titre of the virus recovered from tongue epithelium was of the order of 10^6 to 10^8 ID_{50} per gram of tissue (Mahy 2005). Hence, due care must be taken to keep the affected animals segregated from the healthy ones.

During 2013–2014, a total of fifty outbreaks of FMD due to serotype O were recorded in Kerala. The incidences were reported in the districts of Thiruvananthapuram (04), Kollam (07), Alappuzha (03), Pathanamthitta (02), Kottayam (01), Idukki (04), Ernakulam (01), Thrissur (05), Palakkad (04), Malappuram (04), Kozhikkode (05), Wayanad (05), Kannur (02) and Kasaragod (03) (Annual Report PDFMD 2013–2014). During the same period several FMD incidences were also recorded in other three southern states of the country. In Kerala, the apparent non-structural protein (NSP) antibody seroprevalence against FMDV in cattle and buffalo population stands at 6.87% during 2014–2015 (Annual Report PDFMD 2014–2015).

All clinical samples collected from the suspected elephants were found positive for FMDV serotype O in antigen detection ELISA and in mPCR. The NJ tree depicting phylogenetic relationships of serotypes O elephant isolate is presented (Fig. 1). The recovered isolate grouped within the lineage Ind2001 of Middle-East South Asia (ME-SA) topotype, precisely in the sub-lineage Ind2001d, which re-emerged in the year 2008 and has been dominating serotype O outbreaks in the country since then (Subramaniam *et al.* 2015). The elephant isolate grouped closely with the contemporary isolates collected from domestic animals in nearby areas, which indicates the probable transmission of FMDV from domestic livestock. Thus, tracing the origin of field outbreak through characterization/sequencing of the virus genome followed by phylogenetic analysis plays a pivotal role in understanding the track of virus movement that has an immense significance in the epizootiology of FMD.

India is enzootic for FMD with a complex disease supporting environment. It must be remembered that most of the reports of FMD in wildlife from Southeast Asia have been derived from India and in almost every case, the evidence of initiation of the outbreak was observed from nearby livestock (Weaver *et al.* 2013). Similarly, in the present case, the intermixing of infected domestic animals with the elephants was suspected to have been the probable source of infection. As mentioned earlier, in Kottayam and Thrissur districts, several outbreaks occurred in the nearby areas in cattle. At the same time, the elephants were also found to have an unrestricted access to the grazing area meant for the local cattle, which might have been a presumable cause of transmission of virus either through aerosol exposure or direct contact. Again, the elephants were looked after by mahouts residing in FMD affected areas, which might have probably facilitated mechanical transmission of the virus causing the disease in the elephants. Moreover, these elephants are not usually vaccinated against FMD, for which the protective titre of antibody remains at poor level. Strict movement restriction pertaining to human and animals is also not followed in any part of the country. Hence, intermingling of affected animals with apparently healthy domestic cattle and other susceptible livestock also play a vital role in transmission of infectious virus. The symptoms of FMD being very mild or inapparent in small ruminants, the disease very often go unnoticed in these species, which also silently play an important role in the epizootiology of the disease (Pay 1988). The virus also spreads through direct contact with infected animals, farm tools, clothes, humans and tends to affect other healthy animals. Sometimes, the same person taking care of infected animals are also involved in the maintenance of healthy stock in the farms, which can also act as a contributing factor behind the spread of infection. It is also noteworthy to mention that in Tanzania, FMD outbreaks from 2001 to 2006 appeared to be the result of uncontrolled human activity (Picado *et al.* 2011). The same factor was also exemplified in Uganda, where human and livestock movements were the predominant reason behind

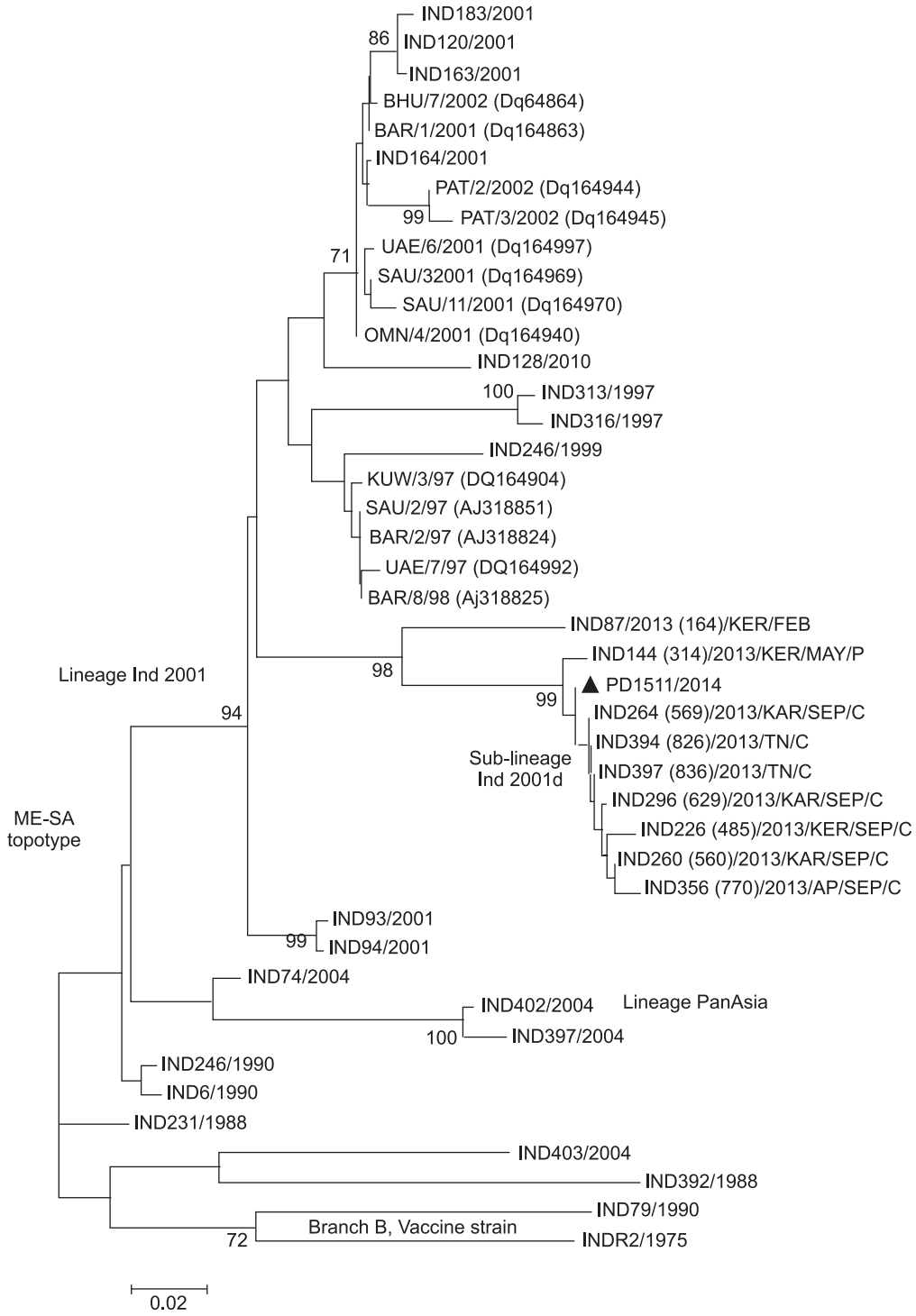

Fig. 1. Neighbour-joining phylogenetic tree estimated at VP1 coding region (639 bp) depicting relationships of FMDV serotype O isolates collected from elephants with contemporary isolates from domestic animals. Bootstrap values (>70%, out of 1000 replicates) is shown near the nodes. The elephant isolate sequenced in this study is indicated by filled triangle.

FMD outbreaks (Ayebazibwe *et al.* 2010). The facts and circumstances set forth above indicate that human and livestock movements should be judiciously controlled especially during the face of an outbreak. Thus, the controlled movements of people and livestock as well as proper vaccination before and during outbreaks can be considered to be the effective means of handling FMD outbreaks (Thomson 2011).

FMD in Indian elephant was previously reported by Hedger and Brooksby (1976). Pyakural *et al.* (1976) also reported and isolated FMDV serotype O from a natural outbreak in Indian elephant. Strains of FMDV isolated from elephants so far in India are type O, A, A_{22} and Asia 1 (Pattnaik and Venkataramanan 1989). Serotype Asia 1 was

earlier recorded from Indian elephant by Rahman *et al.* (1988) and the probable source of infection could be traced back to an outbreak of FMD due to serotype Asia 1 in cattle and buffaloes. When FMD-affected cattle enter a reserve forest or any wildlife premises, the chances of the disease spreading among susceptible wildlife species including elephants were reported to be very high. Mahouts are sometimes also responsible for transmission of the virus from the affected cattle in their house or locality as mentioned in this case.

A vaccination-based FMD Control Programme (FMDCP) was launched by the Department of Animal Husbandry, Dairying and Fisheries (DAHD&F), Government of India since August 2003–2004 covering 54 specified districts in the country, which involved biannual vaccination of cattle and buffaloes with trivalent (O, A and Asia 1) FMD vaccine. Under the same programme in 2010, additional 167 districts were further included. So, currently, this programme includes 221 districts of the country covering all the states of southern peninsula including Kerala. Three districts of Kerala namely, Trivandrum, Kollam and Pathanamthitta were covered under FMDCP during 2003–2004 and later in 2010–2011, eleven districts were further added including the districts of Kottayam and Thrissur, from where the clinical samples from FMDV infected elephants were collected. In Kottayam and Thrissur districts, biannual vaccination is being followed in bovine population as in other districts under FMDCP. Mostly, cattle and buffaloes are included under the vaccination programme ignoring most of the small ruminants, pigs, wild and captive animals. Thus, the elephants from the sampled districts were also refrained from FMD vaccination drive. Moreover, there are no commercially available FMD vaccines currently approved for use in susceptible wild/captive animals (Weaver *et al.* 2013). Still, increasing the vaccination frequency to every 4 months with an altered dose may be imperative with the presently available vaccines, as the in-use vaccines are labile and hence must be kept at specified temperatures from the time of manufacture to the point of use through the maintenance of an unbroken cold chain with proper handling that is exceptionally critical (Weaver *et al.* 2013). Concomitant post-vaccination monitoring is also essential to determine if the current vaccination-based control programmes are effective (OIE 2012).

There is no resident population of elephants reared in the reported area. Elephants are usually brought to the area for temple rituals and are taken out once the festival is over. During this temporary period, routine vaccination and other preventive measures were not adopted in these animals. However, the elephant keepers in the nearby areas of sampling were suggested to routinely follow FMD vaccination along with other biosecurity measures in order to prevent FMD in elephants. After the incidence of FMD, biosecurity measures were strengthened during the elephant camp held at Guruvayoor. Along with this, the state animal husbandry department should also take initiatives to bring these species under the umbrella of routine vaccination campaign along with domestic cattle and buffaloes. The pre- and post-vaccinated serum samples should be collected and routinely tested in liquid phase blocking ELISA, which will help to keep vigil over the kinetics of protective antibody titre in the vaccinated elephants. To the best of our knowledge, this appears to be the first confirmed report of FMD in elephants based on partial characterization and phylogenetic analysis of the virus genome. However, after this incidence of FMD in 2013, the disease has not been reported in these species in the particular region.

Hegde *et al.* (2010) having screened 37 elephant serum samples from various zoological gardens and National Parks in Karnataka for FMDV antibodies, suggested the necessity of vaccination of these animals to maintain immunity against the disease. Few reports state that free-ranging elephants are rarely affected when outbreaks occur in ruminants in their territory (Howell *et al.* 1973, Kalanidhi *et al.* 1992); hence, clinical FMD has been supposed to be a disease of elephants in captivity (Chakraborty and Majumder 1990). Clinical cases of FMD are numerous for Indian elephants, which may be linked to their higher susceptibility to infection (Pyakural *et al.* 1976, Hedger and Brooksby 1976). Still the possibility of virus transmission between elephants cannot be underemphasized. Regular surveillance thus becomes the prime need of the hour to keep track on FMD incidences along with the circulating serotypes and evolving strains of the virus. Each and every outbreak in any susceptible species in an enzootic region should assure rapid diagnosis followed by detailed scientific investigation, so that the virus dynamics could be understood paving the way for formulation of effective control strategies.

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