Foot-and-mouth disease in wildlife population of India


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ABSTRACT
A total of 41 clinical samples (vesicle/tongue/foot/nasal epithelium) from Indian gaur, deer, spotted deer, nilgai, chowsinga, bison, black buck, elephant, sambar deer were collected in 50% phosphate buffered saline/glycerol medium (pH-7.5) during suspected FMD outbreaks. Supernatants of homogenized clinical samples were used in a serotype differentiating antigen detection ELISA and samples found negative were further subjected to multiplex PCR (mPCR). A total of 3/11 (27.2%) samples from Indian gaur, 2/7 (28.5%) chital deer, 5/5 (100%) nilgai, 2/2 (100%) black buck were found positive for serotype O in antigen detection ELISA. A total of 3 ELISA-negative samples from spotted deer, 2 from bison and 2 from sambar deer were found positive for serotype O in mPCR. The VP1 region-based phylogenetic analysis indicated the involvement of both O/ME-SA/Ind2001 and PanAsia lineage of serotype O in the outbreaks. The wildlife species infected with FMD may pose further threat to the surrounding domestic livestock.

Key words: Bison, Black buck, Chowsinga, Deer, Elephant, Foot-and-mouth disease, Gaur, Nilgai, Wildlife.

INTRODUCTION
For many years wildlife species were thought to be the carriers of pathogens of a number of diseases of domestic animals. Slaughter of thousands of wild ruminants was done with an effort to protect domestic ruminant populations, even though their role in the epidemiology of diseases of domestic ruminants was not clearly understood. Wildlife has an essential role to play in the future socioeconomic plans for developing countries. However, consideration must be given to the fact that some species of wildlife can act as reservoir hosts of pathogens of man and domestic animals. Such dangers must be recognized and clearly defined.

Foot-and-mouth disease (FMD) was one of the diseases that had received the most attention, partly because of its high prevalence in domestic stock and partly because its existence acted as an important constraint to livestock exports. In our country, much emphasis is not given on research in the role of wild animals as reservoirs and transmitters of diseases of importance to livestock production and human health. Among the diseases listed in the OIE list A, FMD constitutes a significant constraint to international trade of live animals and animal products. The disease is reported in two of the OIE member states (Vosloo et al., 2002). FMD is the single most important trade-sensitive disease influencing global livestock trade. The role of wildlife species in FMD was extensively reviewed (Thomson et al., 2003). The objective of the present study was to determine the prevalence of FMD in wildlife species of India, because in order for India to establish effective control strategies, it is crucial that the epidemiology of the disease in these species is fully understood.

MATERIALS AND METHODS
Sample collection: A total of 41 clinical samples (in the form of either vesicular epithelium/tongue epithelium/foot epithelium/nasal epithelium as per the availability and convenience) from Indian gaur (2 from Tamil Nadu, 8 from Karnataka, 1 from Kerala), deer (5 from Karnataka, 3 from Kerala), spotted deer (7 from Karnataka), nilgai (2 from Karnataka, 1 from Maharashtra), chowsinga (1 from Andhra Pradesh), bison (3 from Kerala, 1 from Karnataka), black buck (2 from Karnataka), elephant (1 from Tamil Nadu), sambar (2 from Madhya Pradesh) were collected in 50% phosphate buffered saline/glycerol medium (pH-7.5) during suspected FMD outbreaks between 2008 and 2013 (Table 1).

Serotype differentiating antigen detection ELISA: Supernatants of the homogenized clinical tissue materials were used in a serotype differentiating antigen detection ELISA as per the methods described earlier by Bhattacharya et al. (1996) for confirmation of the 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 (Qiagen, Germany). Reverse
transcription was performed using M-MLV reverse transcriptase (Promega, USA) and reverse primer NK61 (Knowles and Samuel, 1995). A serotype differentiating mPCR was performed using Hotstar Kit (Qiagen, Germany) essentially as described previously (Giridharan et al., 2005). The mPCR products were visualized on ethidium bromide stained 2% agarose gel. Only ELISA-negative samples were further subjected to mPCR.

**Nucleotide sequencing and phylogenetic comparisons:**
PCR amplification of VP1 region was performed using *Pfu* polymerase (Fermentas, Germany). For serotype O, the primer combination of ARS4 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). Cycle sequencing reactions of gel purified PCR products were carried out using BigdyeV3.1 terminator kit and sequences were resolved on ABI 3130 genetic analyzer (Applied Biosystems, USA). Sequences were aligned using clustal W algorithm (Thomson et al., 1994). Phylogenetic analysis was conducted using MEGA 5.05 software (Tamura et al., 2011) employing the best fit nucleotide substitution model, TN93+G+I. Phylogenetic tree was reconstructed using Unweighted Pair Group Method with Arithmetic Mean (UPGMA) method available and the robustness of the tree topology was evaluated with 1000 bootstrap replicates (Figure 1).

**RESULTS AND DISCUSSION**
Phylogenetic analysis of the target gene of the virus involved in the outbreaks always help tracing the origin and route of virus movement, which in turn helps in understanding the disease epidemiology. Present FMD Control Programme in India covers only bovines with regard to vaccination leaving the entire wildlife species even in the sanctuaries, zoos and National Parks. Hence, these species are affected with FMD by natural infection. This calls for evaluation of the potential role of wildlife species in the epidemiology of the disease in the country. During the period of study, clinical FMD in the form of frank vesicular/erosive lesions on tongue/feet/nasal epithelium of various wildlife species was observed. A total of 3/11 (27.2%) samples from Indian gaur (2 from Karnataka and 1 from Tamil Nadu), 2/7 (28.5%) spotted deer (from Karnataka), 5/5 (100%) nilgai (2 from Andhra Pradesh, 2 from Karnataka, 1 from Maharashtra), 2/2 (100%) black buck (from Karnataka) were found positive for serotype O in serotype differentiating antigen detection ELISA. A total of 3 ELISA-negative samples from spotted deer (from Karnataka), 2 from bison (from Kerala) and 2 from sambar (from Madhya Pradesh) were found positive for serotype O in mPCR.

During January 2008, FMD was recorded in Bannerghatta National Park, Karnataka. FMD virus (FMDV) serotype O was identified in necropsied sample (Heart) collected from a wild gaur. Phylogenetic analysis revealed
clustering of the isolate in O/ME-SA/PanAsia lineage. Severe outbreaks of FMD in domestic animals were recorded in the southern region of India during 2007-2008 and the causative lineage was identified to be O/ME-SA/PanAsia lineage (Subramaniam et al., 2012). In another instance, FMDV was isolated from nilgai in Shivarama Wildlife Sanctuary, Andhra Pradesh in March 2011 and the lineage was identified to be O/ME-SA/Ind2001. Recently, during 2013-2014, FMD was recorded in chital deer and black buck in Bannerghatta Biological Park, Karnataka, and in sambar deer in Van Vihar National Park, Madhya Pradesh. In all the cases FMDV serotype O was confirmed and the isolates were found to group within O/ME-SA/Ind2001, which has been widely prevalent in India since 2008.

It is noteworthy that FMDV serotype O is predominant in India involving different species of animals (Annual Report, PDFMD, 2013-2014). From the present investigation, FMD in various wildlife species was confirmed. The virus might have been transmitted from domestic livestock to wildlife species or vice versa, could not be pointed out. Still regular focus on surveillance is very important in these animals to keep track on the circulating serotypes and strains/lineages of the virus.

CONCLUSION

This study confirms the involvement of both O/ME-SA/Ind2001 and PanAsia lineage of serotype O FMD virus in the disease outbreaks of various wildlife species in India. The wildlife species infected with FMD may pose further threat to surrounding domestic livestock. Hence, these wildlife species ought to be brought under the sphere of ongoing surveillance and control measures including prophylactic vaccination coupled with zoosanitary measures. Further research needs to be carried out to collect and test the oesophageal-pharyngeal fluid from various susceptible or infected wildlife species to detect FMD virus genome by PCR or virus by isolation methods.

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REFERENCES


