DETECTION OF FOOT-AND-MOUTH DISEASE VIRUS ANTIBODIES IN CAPTIVE ASIAN ELEPHANTS (*Elephas maximus indicus*) IN KERALA

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Abstract

Foot-and-mouth disease (FMD) is the most contagious viral disease of mammals and it is endemic in India.Apart from ungulates, more than 70 wildlife species including elephants are susceptible to FMD.Serum samples from eighteenunvaccinated captive elephants in Kerala were screened for the presence of antibodies against FMD virus serotypes O, A and Asia-1 using Liquid Phase Blocking (LPB) ELISA.Antibody titres varying from 0.61 to 0.76 log 10 against all the three serotypes were detected in all the sera samples of elephants under study, suggesting previous exposure to the pathogen.

Keywords: *captive elephants, FMD, LPB ELISA*

Foot and Mouth Disease is the most contagious disease of mammals (OIE, 2012). FMD virus (FMDV) belongs to the genus *Aphthovirus* of the family *Picornaviridae*. Seven immunologically distinct serotypes of FMDV viz; O, A, C, SAT 1, SAT 2, SAT 3 and Asia 1 exist with no cross protection between serotypes (Ding *et al*., 2013). FMD is endemic in India, with three serotypes viz; O, A and Asia-1 being prevalent. Majority of the outbreaks were due to serotype O, followed by Asia-1 and A (PDFMD, 2014).

The main reservoir for FMD viruses are cattle. Pigs, sheep, goats and water buffalo are also susceptible to FMD among the domesticated species (OIE, 2012). In endemic areas, because of the close contact, the wild and captive animals like Mithun (Hegde*et al*., 2011), Yaks (Prasad *et al*., 1978), Deers (Moniwa*et al*., 2012), Nilgai (Sujatha and Srilatha, 2007), and Elephants (Barman *et al*., 1999) can get exposure to the virus from domestic animals, leading to transmission of the disease (Chakraborty *et al*., 2014). More than 70 wildlife species including African buffalo, giraffes, elephants, mithun, deer etc. contract FMD (Michel and Bengis, 2012) and all seven

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serotypes of FMDV have been demonstrated in wild animals (Weave r*et al.*,2013).Kerala had recurrent outbreaks of FMD among domestic cattle inspite of regular vaccination. This paper reports seroconversion of captive elephants of Kerala against FMD due to exposure to the pathogen from the field.

Materials and methods

Eighteen captive elephants under the custodianship of Kerala Forest department, Temples and private individuals which were having no history of vaccination against FMD were included in the study. The blood samples were collected from ear vein during the period between August 2015 and January 2016.Sera were separated and stored at -20□C until the time of testing.

The serum samples were then subjected to Liquid Phase Blocking (LPB) ELISA as described by Hamblin *et al*. (1986). LPB ELISA was done in collaboration with the laboratory of IndianImmunologicals Ltd. (IIL), Hyderabad. All the facilities, reagents and control sera were provided by IIL.

For each serotype, three flat bottomed ELISA plates were coated with 50 µl of the reconstituted serotype specific immune rabbit serum (IRS) against O IND R2/75, A IND40/2000 and Asia1 IND 63/1972. Plates were then incubated at 4°C overnight. Test sera and positive control (serotype specific Bovine Vaccinated Serum [BVS]) were serially diluted in PBS from 1:4 to 1:512. Fifty µl of diluted antigens were then added to all wells except the wells of 12th column, of the specific plates for each serotypes. Tenth column was kept as positive control, 11thcolumn as antigen control and 12th column as blank. These plates were then incubated at 4°C overnight. Contents of the flat bottomed ELISA plates were discarded and the plates were washed with 200 µl of PBSTfor five times. Fifty µl of the incubated Ag-Ab mixture was transferred from the 'U' bottomed plates to the respective wells of the flat bottomed ELISA plates. Plates were incubated at 37°C for one hour and washed. Fifty µl of reconstituted serotype specific tracing immune guinea pig serum (IGPS) was added to each type-specific plate and incubated at 37°C for one hour and the plates were washed. Fifty µl of reconstituted anti-guinea pig-HRPO conjugate (1 in 2000 in blocking buffer) was added to each well and incubated at 37°C for one hour. Plates were washed and 50 µl substrate solutions (OPD activated with 30-33% Hydrogen peroxide solution) was added to each well in a dark room. All the plates were incubated at 25°C for 15 minutes. The colour reaction was stopped by adding 50 µl of stopping solution (1.25*M* Sulphuric acid). The optical densities of the wells were read at 492 nm using ELISA plate reader with help of the software Magellan. Data were transferred into MS Excel format and results were interpreted using forecasting. The titer of the serum sample was calculated as the reciprocal of the highest dilution showing 50 percent inhibition of OD value as compared to the antigen control wells.

Results and discussion

Antibodies to FMDV serotypes O, A and Asia-1 were found in all the eighteen animals (Table 1). The mean antibody titres were 0.65 log10, 0.68 log10 and 0.70 log10 against O, A and Asia-1 respectively. Statistical comparison was done by one-way ANOVA (Snedecor and Cochran, 1991) but no significant difference was observed in the antibody titres developed against the three serotypes.

The result agrees with the findings of others who reported serotypes being identified from infected captive elephants in India were types O (Pyakural *et al*., 1976; Barman *et al*., 1999), A (Gomes*et al*., 2010) and Asia-1 (Rahman *et al*., 1988). Since none of these eighteen elephants manifested any clinical signs of FMD, the presence of serum antibody titre indicates previous exposure to the virus. Similar findings have been made by Gomes*et al*. (2010) who reported seropositivity against FMDV serotype Aby LPB ELISA, in captive Asian elephants without any history of clinical disease.Yadav *et al.* (2012) reported an overall seroprevalenceof 11.36 per cent for FMD in Asian elephants from North-Eastern

Table 1. Antibody titres developed against FMDV serotypes in elephants

Sl. No.	Animal No.	Antibody Titres against FMDV Serotypes (Log 10)		
		O	A	Asia-1
1	1	0.61	0.76	0.76
2	2	0.61	0.61	0.76
3	3	0.76	0.76	0.61
4	4	0.61	0.61	0.76
5	5	0.61	0.76	0.76
6	6	0.76	0.61	0.61
7	7	0.76	0.76	0.61
8	9	0.61	0.61	0.76
9	10	0.61	0.61	0.61
10	17	0.76	0.61	0.76
11	18	0.61	0.76	0.61
12	19	0.61	0.61	0.76
13	20	0.76	0.76	0.76
14	21	0.61	0.61	0.61
15	22	0.61	0.61	0.76
16	23	0.61	0.76	0.76
17	24	0.61	0.61	0.76
18	25	0.61	0.76	0.61
Mean titers		**0.65**	**0.68**	**0.70**

India using LPB ELISA, which include multiple seropositivity against Types O, A and Asia1.

The source of exposure of elephants to FMD might be the presence of infected domestic animals, as suggested by Barman*et al*. (1999). According to Weaver *et al*. (2013), passive infection of wildlife was reported when there were outbreaks among domestic animals. Outbreaks of FMD in captive elephants from Tamil Nadu (Biswal *et al*., 2015) and Kerala (Rout *et al*., 2016) have been reported during the height of FMD outbreak among domestic animals in 2013 and the studies showed O/ Middle East-South Asia/Ind2001d sub-lineage of FMDV serotype O was responsible for the outbreak in both domestic animals and elephants suggesting probable transmission from cattle to elephants.

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