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Serological Evidence of Foot-and-Mouth Disease Virus Nonstructural Protein Antibodies in Indian Elephants *(Elephas maximus indicus)*

M. Rout[1*], P. Deka[2], N.S. Nair[3], M. Karikalan[4], V. Manjunatha[5], N. Sahoo[6], A.K. Sharma[4], J.K. Mohapatra[1] and R.P. Singh[1]

[1]ICAR-National Institute on Foot and Mouth Disease, Bhubaneswar, Odisha, INDIA
[2]FMD Regional Centre, Department of Microbiology, College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati, Assam, INDIA
[3]FMD Collaborating Centre, Pallode, Thiruvananthapuram, Kerala, INDIA
[4]Centre for Wildlife Conservation Management and Disease Surveillance, ICAR-Indian Veterinary Research Institute, Izatnagar, Bareilly, Uttar Pradesh, INDIA
[5]Wild Animal Disease Diagnostic Laboratory, Institute of Animal Health and Veterinary Biologicals, Bannerghatta Biological Park, Bannerghatta, Bengaluru, Karnataka, INDIA
[6]Department of Epidemiology and Preventive Medicine, Centre for Wildlife Health, College of Veterinary Science and Animal Husbandry, Odisha University of Agriculture and Technology, Bhubaneswar, INDIA

**Corresponding author: M Rout; E-mail: drmrout@gmail.com*

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ABSTRACT

Foot-and-mouth disease (FMD) remains one of the most economically devastating menace in livestock due to its highly contagious nature with multispecies involvement. In the present study, a serological survey to detect antibodies against structural and non-structural protein (NSP) of FMD virus (FMDV) in elephants was conducted. A total of 255 elephant serum samples from 5 different states of India were collected with due permission from the competent authorities. A competitive ELISA using commercial kit PrioCHECK® FMDV NS (Prionics AG, Switzerland) was performed to assess antibodies against FMDV 3ABC non-tructural protein. A total of 2.74% (7/255) animals were found positive indicating their previous exposure to FMDV. The serum samples were also subjected to in-house liquid phase blocking ELISA to assess the level of protective antibody against FMDV serotypes O, A and Asia 1, where none of the animals was found to have protective antibody (log_{10} titre of ≥1.8) against all three serotype strains used in the vaccine formulation. In conclusion, the study gathered a low level of serological evidence of virus activity as well as lack of protective antibody against FMDV in the sampled elephants. Further investigations into the dynamics of anti-FMDV antibodies supplemented with virological examination should be carried out to understand the virus ecology and disease epidemiology. In order to establish absolute freedom from infection, oesophageal-pharyngeal fluid collected from the NSP-positive animals could further be examined for the presence of viral genome by polymerase chain reaction or for virus isolation to understand the carrier status in this species.

HIGHLIGHTS

- Total 255 elephant sera from 5 states of India were tested for FMDV NSP antibodies.
- Total 2.74% animals were found positive indicating their previous exposure to FMDV.

Keywords: Foot-and mouth disease virus, Elephants, Nonstructural protein-antibodies

The Indian elephant (*Elephas maximus indicus*), native to mainland Asia, is regarded as one among the three recognized sub-species of the Asian elephant and tagged as ‘endangered’ in the International Union for Conservation

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of Nature (IUCN) Red List since 1986. These largest terrestrial species have been credited as the cultural symbol by the native people of Hindu religion. In 2010, Indian elephant has been declared as 'national heritage' of India and is placed in Schedule I and Part I of Indian Wildlife Protection Act (1972) conferring it the highest level of protection and thereby protected by the Project Elephant Programme run by the Ministry of Environment and Forests, Government of India (GoI). As per the All India Synchronized Elephant Population Estimation Report (2017), Project Elephant Division, Ministry of Environment, Forest and Climate Change, GoI, the total elephant population in India was 27,312. Amongst the total population, the South Indian states of Karnataka, Kerala and Tamil Nadu are home to nearly 44% of the elephants of the country, while the north-eastern Indian states of Assam, Arunachal Pradesh, Mizoram, Meghalaya and Tripura together with West Bengal account for 30% of the elephant population. India holds by far the largest number of wild Asian elephants, estimated at about 26,000 to 28,000 or nearly 60% of the population of the species (Baskaran *et al.,* 2011). Captive elephants have been used for a variety of purposes in India including warfare, logging, cultural and religious ceremonies, recreation in zoos and more recently for wildlife tourism and protection of sanctuaries and national parks. Historically, the significance of the elephant in Indian culture and mythology, as well as its economic and military role in sub-continental armies, has also contributed to a remarkable level of tolerance and support of people towards its survival and conservation (Baskaran *et al.,* 2011).

Foot-and-mouth disease virus (FMDV), a member of the *Aphthovirus* genus within the *Picornaviridae* family, is the causative agent of FMD, one of the world's most important infectious diseases of cloven-hoofed domestic animals, responsible for huge global losses of livestock production (Subramaniam *et al.,* 2022). The virus exists as an important livestock pathogen since more than 120 years after its identification with annual costs from production losses and vaccination estimated at US$6.5 - US$21 billion in FMD-endemic areas (Poonsuk *et al.,* 2018). Though primarily a devastating disease of domestic livestock, it also threatens wildlife species. More than 70 wild animal species have been demonstrated to be susceptible to FMDV either by natural infection or by experimental challenge (Weaver *et al.,* 2013). Certainly, in addition to domestic species, there are a number of 'non-domestic species' or 'non-classical species' that are also susceptible to FMD. Though a vast majority of captive elephants in Asia are used for conservation, logging, ceremonies and tourism, to date, neither such captive elephants in Asian range countries have been systematically evaluated for FMD nor their real importance in FMD epidemiology has clearly been addressed. Hence, a preliminary study aiming for serological assessment of FMD was targeted to unravel FMDV activity in Indian elephants targeting FMDV nonstructural and structural protein-antibodies (NSP- and SP-Ab). This seems to be the first report on serological approach in captive Indian elephants in order to gather preliminary evidence for presence of FMDV antibodies in the said species in India. The investigations are likely to add value to the ongoing FMD control and elimination efforts in the country through mass vaccination campaign and associated approaches.

MATERIALS AND METHODS

Study population and sample collection

A total of 255 Indian elephants from 5 different states representing different geographies e.g., Kerala (southern India), Assam (north-eastern India), Odisha (eastern India), Karnataka (southern India), Madhya Pradesh (central India) were sampled with due permission granted from the competent authorities (MoEF and wildlife authority of the concerned state). The reported age of the elephants ranged between 2 to 30 years and they were physically examined correlating with their history for FMD symptoms. Approximately 6 ml of blood was collected aseptically from caudal auricular vein of elephants using sterile 19 gauge winged IV infusion sets and adapters to facilitate collection directly into 10 ml vacutainer tubes that were kept in slanting position away from light for clotting. Then, clear straw coloured serum was separated after centrifuging tubes at 4000 rpm for 10 minutes at 4°C and transferred to 5 ml polypropylene vials with proper labeling and stored at -20° C prior to transport to the laboratory. All serum samples were carried in cold chain to the Central FMD laboratory at ICAR-Directorate of Foot and Mouth Disease, Mukteswar, Uttarakhand, India and were stored at -80° C till testing.

Laboratory tests

Competitive ELISA for FMDV infection-specific antibodies

A competitive ELISA using commercial kit, PrioCHECK® FMDV NS, (Prionics AG, Schlieren-Zurich, Switzerland) was performed for post-incursion surveillance to assess antibodies specific to the highly conserved nonstructural protein (NSP) 3ABC of FMDV due to its reported high sensitivity (97.2%) and specificity (98.1%) (Brocchi *et al.,* 2006; Paton *et al.,* 2006; Engel *et al.,* 2008). Therefore, seroconversion of susceptible animals to 3ABC nonstructural protein is indicative of virus exposure/infection. The test detects antibodies elicited following natural viral replication, irrespective of the virus serotype, but not vaccinal antibodies (Bhatt *et al.,* 2018). Additionally, being a blocking or competitive ELISA, it can be applicable for all species without any need for a species-specific conjugate (Chung *et al.,* 2018). The test was used as per the manufacturer's protocol previously reported by various workers. After the test procedures, the plates were read at a wavelength of 450 nm to measure the optical density (OD). The OD values of all samples including the controls (negative, weak positive and positive) were calculated and expressed as the percent inhibition (PI) using the following formula:

$$PI = 100 - [(OD \text{ of test sample}) / (\text{mean OD of negative controls})] \times 100.$$

A PI value of < 50% was considered negative, whereas that ≥ 50% was categorized as positive. More specifically, PI value of ≥ 50%, but < 70% was considered a weak positive result, while a value of ≥ 70% was considered as strong positive.

Liquid phase blocking (LPB) ELISA for assessment of protective structural protein antibody

In order to correlate the history of 'no FMD vaccination' status of the captive elephants (as informed by the wildlife veterinarians), the samples were tested in LPB ELISA. Two-fold dilution (from 1:16 to 1:128) of serum samples were tested for determining the serotype-specific FMDV SP-Ab titre to assess the overall status of vaccinal immunity/protective antibody against all three serotypes O, A and Asia 1 in the vaccine using the in-house LPB ELISA kit (ICAR-DFMD, Mukteswar) as per the procedure described earlier (Ranabijuli *et al.,* 2010). The results were expressed as percentage reactivity for each serum dilution as follows:

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

The antibody titres were expressed as logarithm of reciprocal of serum dilutions giving 50% of the absorbance recorded in the antigen control wells. The samples demonstrating $\log_{10}$ titre of ≥1.8 were graded to have sufficient protective antibody (Sharma *et al.,* 2015).

RESULTS AND DISCUSSION

Serological testing is undoubtedly a suitable and convenient tool for FMD surveillance done in various countries (OIE, 2023). FMDV exposed/infected animals produce antibodies to both structural and nonstructural proteins of the virus, whereas those vaccinated against FMD using purified vaccines can produce antibodies to viral structural proteins only (Atuman *et al.,* 2020). Thus, a diagnostic approach detecting antibodies against viral NSPs that in principle are elicited only during infection and not upon vaccination, is a clear indicator of infection or virus circulation (OIE, 2023). During the present investigation, a low rate of seropositivity of 2.74% (7/255) elephants for NSP-Abs provides serological evidence of virus activity confirming their previous exposure to FMDV. In India, indirect 3AB3 NSP ELISA with species-specific HRPO conjugate is routinely used for FMD serosurveillance in bovines (Mohapatra *et al.,* 2011). The diagnostic specificity of the PrioCHECK FMDV NS kit for bovine sera was reported as 98.1% by Brocchi *et al.* (2006), while its diagnostic sensitivity in nonvaccinated, experimentally infected bovines approached 100%. FMDV NS kit being a blocking ELISA, is species independent and as such was considered a good choice for testing wildlife sera from a range of species. The same kit has earlier been used by different researchers for detection of NSP antibodies in a wide range of wild animals. Moreover, anti-NSP antibodies can persist for a long period (Paton *et al.,* 2009) and do not necessarily indicate recent FMDV exposure/infection. LPB ELISA could detect no animals with protective antibody ($\log_{10}$ titre of ≥ 1.8) against

all three serotype strains incorporated in the vaccine signifying a 'no vaccination' status in these species in the country correlating with the history taken during sampling. Thus, with the finding of serological evidence of FMDV activity as well as lack of protective antibody against FMD in Indian elephants, further field studies collecting more number of clinical samples from affected elephants can be targeted in order to enable the genome characterization of FMDV lineages circulating within such species. Subsequent investigations into the dynamics of anti-FMDV antibodies in these animals supplemented with virological examination might help better understand the virus ecology and the disease epidemiology in such an important FMD-susceptible species. In order to establish absolute freedom from infection, oesophageal-pharyngeal fluid collected from the NSP-Ab-positive animals may be examined for the presence of viral genome by polymerase chain reaction or for virus isolation, which may show if these animals can act as virus carriers.

Natural FMD cases have been reported in captive African elephants (*Loxodonta africana*) and Asian elephants (*Elephas maximus*) during 1970s and 1980s by several researchers. African elephants were found susceptible to needle inoculation with FMDV, but did not become infected when exposed to artificially infected cohorts or cattle. Furthermore, there was no serological evidence for infection in elephants culled in Kruger National Park (KNP) of South Africa over a period of 30 years. In India, Rout *et al.* (2016) reported clinical FMD with clear lesions in elephants in Kerala during 2013 confirmed by serotype differentiating antigen detection ELISA and multiplex RT-PCR. The VP1 region based phylogenetic analysis has given an indication of 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. The authors mentioned that in Kerala, several FMD outbreaks occurred in cattle during 2013, where at the same time, elephants were also found to have an unrestricted access to the grazing areas used by the local cattle, which might have acted as a possible source 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 virus exposure in the elephants. These facts elucidate the probable sources of virus exposure of elephants in the present study that might have led to seroconversion as detected by the competitive ELISA. It is also common in many Asian elephants and captive Indian elephants to intermingle with domestic cattle during grazing, other social and ceremonial activities to have contact with wild elephants and other wildlife species during various human-driven activities. In India, elephant-back safaris permit tourists to view wildlife and elephants. In addition, captive elephants are used routinely by national park managers to patrol and participate in wildlife capture activities. There are no self-sustaining captive elephant populations anywhere in the world, and many range countries depend on the breeding of captive cows by wild bulls, where the probable source of infection cannot be ruled out. FMD is a threat to Asian elephants because their care in captivity puts them in close proximity to humans and other susceptible wild ruminants. Wild elephants may also be at risk in areas where they intermingle with captive elephants.

CONCLUSION

The present serological study though on a pilot scale, could gather an indication of FMD virus activity in Indian elephants. Further, as the disease has been reported in elephants on several occasions in the country, certain zoosanitary measures can be followed to prevent the disease in these animals. Nevertheless, it cannot be considered out of place to plan for strict biosecurity measures including stringent vaccination schedule at elephant interface during ongoing disease outbreaks in domesticated livestock that could help protect these endangered species on the earth in future. Moreover, such investigations on opportunistic serum samples need to be carried out intermittently so as to assess FMD virus activity correlating with the ongoing disease control interventions.

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