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# Serosurveillance of Foot-and-Mouth Disease in Ruminant Population of Karnataka, India

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## Abstract

Foot-and-Mouth Disease (FMD) is endemic in India and three serotypes viz, O, A, and Asia-1are prevalent in the country. In the current study a total of 7923 serum samples were collected randomly from 4639 cattle, 1363 buffaloe, 1187 sheep and 734 goats from different districts of Karnataka state, India. The samples were screened for antibodies against Non-Structural Proteins (NSPs) and Structural Proteins (SPs) of FMD virus to gather evidence with respect to the FMD virus circulation. The study revealed NSP antibodies in 33% bovines and 16% small ruminants. Higher level of NSP antibodies was observed in cattle (35%), buffaloes (27%), goats (23%) and lower prevalence in sheep (12%).The antibodies against SP was observed in 78% bovines and 18% small ruminants. The study reiterates the importance of strengthening of FMD surveillance in small ruminants as they could pose a potential risk of virus transmission to cattle.

## Introduction

Foot-and-Mouth Disease (FMD) remains a serious threat to the livestock population of India where the disease is endemic. Three (O, A and Asia-1) of the seven serotypes of Foot-and Mouth Disease Virus (FMDV) are prevalent in India, while serotype C has not been detected in the country since 1995 [1]. The occurrence of the disease results in damaging consequences for the livelihoods of local farmers due to impacts upon productivity, food security, and loss of income. India has ~528 million FMD susceptible livestock population (19$^{th}$ Livestock census of India), and the direct economic loss on account of FMD is more than Rs. 20000 crores per annum (~USD 3.2 billion) [2]. Karnataka, the seventh largest state in India has a sizeable proportions of livestock (9.5 million cattle, 3.4 million buffalo, 9.5 million sheep, 4.8 million goats and 0.3 million pigs) susceptible to FMD. FMD outbreaks are regularly recorded in the state of Karnataka and serotype O has been the most prevalent one. Intermittent circulation of FMDV serotypes A and Asia-1 has also been confirmed in the state [3]. Since September 2011, cattle and buffalo (bovines) population of Karnataka state are regularly vaccinated with a trivalent vaccine containing all the three FMDV serotypes circulating in the country, at 6 monthly intervals under the government funded National FMD control programme (FMDCP) [1]. The herd immunity among the vaccinated population against all the three serotypes in the state of Karnataka had increased from 5% to 59% at the end of IV$^{th}$ phase of vaccination leaving 61% population still susceptible to FMD outbreaks [3]. Sheep and goat population are not vaccinated against FMD under the FMD-CP. Although length and size of FMD outbreaks has been significantly reduced due to systematic vaccinations, an abrupt increase in FMD incidences due to serotype O was recorded in the year 2013 [4-6] involving all the species (cattle, buffaloe, sheep, goat, pig) throughout the southern peninsular India (Karnataka, Tamil Nadu, Kerala, Andra Pradesh, Pondicherry).

Earlier, serological survey for antibodies against FMDV was restricted to bovine population only [7] which revealed the prevalence of antibody against both the Nonstructural Proteins (NSP) and Structural Proteins (SP) in 21% and 33% of the animal population in Karnataka state. In this study, all the four susceptible ruminant species (cattle, buffaloe, sheep and goat) with or without the history of recent outbreaks were included to determine the prevalence of antibodies against FMDV SP and NSP. Further, clinical materials collected from FMD suspected animals were tested in antigen differentiating ELISA to identify the prevalent serotypes.

## Materials and Methods

### Study Area and Design

Karnataka, the seventh largest state in India (191.791km$^2$ area, 5.8 % of the country), is situated between 11°40' and 18°27' N latitude and 74°50' and 78°33' E longitude, in the centre of the western peninsular India. The state has a topography ranging from the narrow stretch of coastal plains to the elevated terrains of the Western Ghats sloping gently to the West to the drier plateau regions in the East. The state has 30 revenue districts and can be divided into 10 agro-climatic zones: Northeastern transition, Northeastern dry, Northern dry, central dry, Eastern dry, Southern dry, Southern transition, Northern transition, Hilly, and Coastal. Each district consists of villages (29340 numbers for the state) where the majority of the livestock animal populations are reared by the farmers in small holdings (2-3 animals/household). All 30 revenue districts were included in the study.

Thirty five clinical materials (tongue epithelium and vesicular fluid) were collected from eighteen suspected FMD incidences from the state during 2014. The incidences were not extensive in nature, only 3 to 4 animals were affected in each episode and most of them were unvaccinated. The tissue samples were processed (chloroform extracted 10% PBS suspension) and serotype was confirmed using sandwich ELISA [8] at our laboratory and further by multiplex PCR [9] at the FMD central laboratory, Mukteswar, India.

A total of 7923 serum samples [Table 1] (4639 cattle, 1363 buffalo, 1187 sheep, 734 goats) were collected from the ruminant population between May and August of 2014 by following a multistage convenience sampling. The sample size for the population was estimated [10]. Fifteen villages were selected in each district by using randomization calculator (RANDOM.ORG) and serum samples were collected from these villages following simple random sampling in which each member of the subset had an equal probability of being chosen with an unbiased representation. The serum samples were screened for antibodies against NSP and SP of FMDV.

### Detection of Antibodies against NSP (NSP-Ab) Of FMDV in Bovines

The 3AB3 NSP-ELISA developed by Mohapatra *et al.*, [11], validated and adopted as the primary screening assay for NSP serosurveillance in India, was employed for detecting the NSP-Ab in bovine serum samples. The 3AB3 NSP indirect ELISA was considered to be valid if the mean absorbance (OD) of the positive control was above 0.8, OD of the supplied negative control was less than 0.3 and the background was less than 0.10. The final result of each test serum was expressed as the Percent Positivity (PP) value and the sample was declared positive if PP value was more than 40 percent. The PP value was calculated by dividing the OD of the test serum by that of the positive control serum and then multiplying by 100.

### Detection of Antibodies against NSP (NSP-Ab) Of FMDV in Small Ruminants

A multi-species indirect ELISA for detection of non-structural protein 3ABC specific antibodies to FMDV [12] was used to screen serum samples collected from small ruminants. The serum samples were diluted (1:21) in the serum diluent buffer and assayed using the indirect ELISA format. The absorbance values of the positive control ($OD_{pos}$) and the samples ($OD_{sample}$) were corrected by subtracting the Optical Density (OD) of the negative control ($OD_{neg}$). The final result of each test serum was expressed as the Percent Positivity (PP) value and samples with a PP value of ≥25 relative to the normalized positive sample OD value were considered as positive and those below 25 as negative. Percent Positivity (PP) value for the sample was calculated by using the formula PP value = $(OD_{sample}-OD_{neg})^*100/ (OD_{pos}-OD_{neg})$.

### Single Dilution Liquid Phase Blocking ELISA (SdLPBE) For Quantitative Estimation of Antibody Levels against SP

Antibody titres to SP were quantified by SdLPBE as described by Sharma *et al.*, [13]. The kit has been validated and adopted as the screening assay for seromonitoring of FMD control programme (FMDCP) in India. In brief, test serum samples were diluted to 1:32 and mixed individually with equal volume of pre-titrated inactivated whole virus antigen of the three serotypes making the final dilution of serum to 1: 64. Antigen–antibody mixtures were incubated at 4°C for overnight, and trapped/unblocked antigens were traced by the serotype-specific hyperimmune guinea pig serum. The OD values of the ELISA plates were analyzed using PDFMD ELISA Analyst version 2.0. The titers are presented in this study as $\log_{10}$ value. Antibody titer of $\log_{10}$ 1.8 or more obtained by SdLPBE was adopted as cut off to categorize animals as potentially protected based on the sero-monitoring data generated over the last ten years in the country [13].

### Data Analysis

Statistical analysis of the data generated from the study was performed using **survey toolbox for livestock diseases (Epitoolsepitools**.ausvet.com.au/content.php). The alpha level was set at 0.05 and 95% confidence interval ($CI_{95\%}$) was calculated. Pearson's Chi-square test was used to detect significant differences in the seropositivity between the species and between districts. If the probability value (P value) is less than or equal to set alpha level (0.05) then the result was considered as statistically significant. ANOVA was carried out to know the significant difference in SP level between the species and serotypes and also to know the significant difference in mean titres of NSP between species.

## Results

### Detection of FMDV Serotypes in Clinical Samples

During the year 2014, a total of 10 incidences were confirmed as FMD outbreaks from the processed clinical samples. FMD virus serotype 'O' was recovered from 10 clinical samples. Cattle were the main species affected (n=30) followed by pig (n=25). FMD incidence due to FMDV serotype O was confirmed in seven revenue districts.

**Table1:** Seroprevalence of FMD in livestock of Karnataka as assessed by NSP-ELISA.

| Species | No. Tested | % NSP positive (95%CI) | $\chi^2$ for districts |
|---|---|---|---|
| Cattle | 4639 | 35 (33.5-36) | 444.5*** |
| Buffalo | 1363 | 27 (25-30) | 181*** |
| Sheep | 1187 | 12 (8.5-15) | 63*** |
| Goat | 734 | 23 (19-28) | 57*** |

**F= 10.42*** between the Species, ***significant at< 0.0001**

**Table 2:** District level seroprevalence of foot and mouth disease in ruminant population in Karnataka state, India.

| District | Livestock Population | Animal Sampled | % NSP positive (95%CI) | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Cattle | Buffalo | Bovine (Cattle& Buffalo) | Goat | Sheep | Small ruminants (Goat &Sheep) |
| Bagalkote | 1611199 | 300 | 33 | 30 | **31.5 (21.5-38)** | 20.3 | 22 | **21 (11-31)** |
| Bengaluru Urban | 266794 | 248 | 43 | 75 | **44 (17-29)** | 0 | 7 | **4.2 (3-8)** |
| Bengaluru Rural | 376564 | 249 | 22 | 0 | **22 (33.5-53)** | 24 | 7 | **12 (10-15)** |
| Belagavi | 2718252 | 297 | 43 | 37 | **40 (11-23)** | 21.5 | 22 | **22 (10.5-33)** |
| Ballari | 1417107 | 316 | 16 | 2 | **13 (37-56)** | 33 | 23 | **24 (10-38)** |
| Bidar | 621789 | 250 | 46 | 54 | **50 (43-57)** | 5 | 10 | **6 (10-12)** |
| Chamarajanagar | 512312 | 200 | 56 | 10 | **49.5 (43-56)** | - | - | **-** |
| Chikkamagalur | 1575522 | 297 | 7 | 7 | **7 (4-11)** | 27 | 3 | **12 (1-26)** |
| Chikkaballapur | 833322 | 268 | 32 | 7 | **26.5 (21-33)** | 64 | 14 | **22 (12-32)** |
| Chitradurga | 546118 | 301 | 52 | 25 | **47.5 (41-54)** | 0 | 1 | **1 (0.4-2)** |
| Dakshina Kannada | 290594 | 253 | 27.5 | - | **27.5 (22-34)** | 21 | 0 | **21 (9-32.5)** |
| Davangere | 924207 | 266 | 50.5 | 32 | **48 (41-55)** | 0 | 3 | **3 (1-7)** |
| Dharwad | 413793 | 300 | 42 | 34 | **40 (33-47)** | 19 | 28.5 | **21 (13-29)** |
| Gadag | 573822 | 246 | 45 | 51 | **47.5 (41-54)** | 25 | 11.5 | **17 (6-29)** |
| Hassan | 1011052 | 250 | 39 | 25 | **35 (29-42)** | 26 | 9 | **18 (4-32)** |
| Haveri | 768964 | 300 | 12.5 | 16 | **13.5 (9-19)** | 21 | 9 | **14 (8-20)** |
| Kalaburgi | 1008178 | 240 | 23 | 28 | **25 (19.5-31)** | 32.5 | 0 | **32.5 (11-54)** |
| Kodagu | 118885 | 200 | 30 | 31.5 | **30.5 (2.5-37)** | - | - | **-** |
| Kolar | 790330 | 234 | 38 | 17 | **37 (31-44)** | 0 | 3 | **3 (0.3-6)** |
| Koppal | 1023493 | 350 | 14 | 14 | **14 (10-19)** | 0 | 11 | **11 (3-19)** |
| Mandya | 1098738 | 254 | 42 | - | **42 (35-49)** | 0 | 4 | **4 (1.5-9)** |
| Mysuru | 962065 | 253 | 30 | 0 | **30 (24-37)** | 0 | 8 | **7.5 (0.3-15)** |
| Raichur | 1385051 | 301 | 11 | 4 | **9 (6-14)** | 22 | 20 | **21 (3.5-38)** |
| Ramanagara | 542113 | 295 | 32 | 50 | **36.5 (30-43)** | 24 | 22 | **23 (7-39)** |
| Shivamogga | 778723 | 300 | 38 | 21 | **35 (29-42)** | 28.5 | 12 | **18 (3-33)** |
| Tumakuru | 1971345 | 250 | 26 | 17 | **24.5 (20-31)** | 5.5 | 0 | **2 (0.3-4)** |
| Udupi | 240445 | 227 | 69.5 | - | **69.5 (63-75)** | 15 | 0 | **15 (4-34)** |
| Uttara Kannada | 431181 | 235 | 52.5 | 52 | **52.5 (46-59)** | 53 | 35 | **4 (11-69)** |
| Vijapura | 1095245 | 233 | 36 | 28 | **33 (27-40)** | 53 | 12.5 | **33 (14-52.5)** |
| Yadgiri | 1113142 | 210 | 19 | 13 | **15.5 (33.5-36)** | 60 | 0 | **60 (50-78)** |
| **Total for the State** | **27020345** | **7923** | **35 (33.5-36)** | **27 (25-30)** | **33.2 (32-34)** | **23 (19-28)** | **12 (8.5-15)** | **16 (14-18)** |

### Prevalence of Antibodies against FMDV NSP

A total of 33% bovines and 16% small ruminants were seroreactors in NSP ELISA. Overall, 35% of cattle, 27% of buffaloe, 12% of sheep and 23% of goats were seroreactors in NSP-ELISA [Table 2]. The prevalence of anti-NSP antibodies varied significantly between the ruminant species ($\chi^2$ = 264.8 df =3 P<.0001) and between the districts [Table 2]. Comparison of mean titre of NSP revealed significant difference between the species (F=10.42, P<0.0001). Seroprevalence in cattle was higher than in buffalo ($\chi^2$ = 26.91, df = 1, p < 0.0001), sheep ($\chi^2$ =240.87, df =1 p < 0.0001) and goats ($\chi^2$ = 38.1, df = 1, p = 0.574). The seroprevalence varied significantly between large and small ruminants ($\chi^2$ = 205, df = 1, p < 0.0001) and also between small ruminants ($\chi^2$ = 44.14, df = 1, p < 0.0001).

### Prevalence of Antibodies against FMDV SP

Antibodies to SP deemed to be protective (titer of $\log_{10}$ 1.8 or more) (SP-Ab) were observed in 78% of bovines (cattle & buffaloes together) and 18% of the small ruminant samples against all three serotypes O, A and Asia-1. Species wise, 79% of cattle, 74% of buffalo, 17% of sheep and 18% of goat samples showed SP-Ab against all the three FMDV serotypes O, A and Asia-1. The SP-Ab was observed in 84%, 86% and 93% of the large ruminants (cattle/buffaloes) and 38%, 32% and 35% of the small ruminants (sheep/goats) against serotype O, A and Asia-1, respectively. The prevalence of SP-Ab varied significantly between bovines and small ruminants (F=55, p<0.0001), against all the three FMDV serotypes and also between individual serotypes O, A and Asia-1(F=53, P<0.0001). The samples negative for NSP antibodies in small ruminants (n=1611) revealed SP-Ab in 36% of the samples against serotype O, 31.6% against A, 35.8% against Asia-1 and 18.2% against all three serotypes. The samples positive for NSP antibodies in small ruminants (n=310) revealed SP-Ab in 49% of the samples against serotype O, 37% against A, 44.8% against Asia-1 and 21.3% against all three serotypes. Both NSP-Ab positivity and ≥4 fold spike of SP-Ab titre (0.6 $\log_{10}$titre increase) was observed in 18% of the samples against any one of the three serotypes in small ruminants.

## Discussion

FMD is endemic and known for its wider distribution in Karnataka state, India. The retrospective study on the epidemiology of Karnataka had revealed the dominance of FMDV serotype O over the other serotypes A and Asia-1 in causing the outbreaks in the livestock [3]. The confirmed outbreaks in the recent past revealed the circulation of all the three serotypes in the state, though outbreaks due to A and Asia-1 were intermittently detected [3]. FMDV serotype O is the predominant serotype in India and responsible for around 80% of the outbreaks in the country. The Southern region of the country, including the state of Karnataka had experienced severe outbreaks during 2013 due to sub-lineage O/ME-SA/ Ind2001d (5). During 2014, all the ten confirmed FMD incidencesin Karnataka were due to serotype O. The phylogenetic analysis based on VP1 (1D) coding region of serotype O virus collected during 2014-15 revealed extended dominance of Ind2001 strains and limited circulation of lineage PanAsia which had been identified to cause two sporadic incidence in the state [14]. Re-emergence of Pan- Asia lineage which had caused many outbreaks in 2006-07 in southern region of the country including Karnataka was considered as an epidemiologically significant event recorded during 2014. In spite of genetic heterogeneity, the serotype O viruses recovered from the Karnataka state were found to be antigenically homologous to current serotype O vaccine strain INDR2/1975 [14].

A systematic vaccination programme is ongoing in Karnataka state to control FMD. Under the programme, all the cattle, buffalo and pig populations are being bi-annually vaccinated with a trivalent vaccine since 2011. Earlier to the implementation of FMD-CP, there was no preventive vaccination against FMD in Karnataka, and vaccination was limited mostly to post-outbreak situations. With the implementation of FMD-CP, the vaccination coverage increased gradually from 58% in the first phase to 80% at the end of the fourth phase. The seromonitoring results indicated that the protective SP-Ab against all the three serotypes increased gradually from 4.5% to 59% at the end of the fourth phase of vaccination, leaving 61% of the population susceptible to FMDV attack with anyone of the three serotypes, circulation of which has been incidentally confirmed [3]. Although clinical disease has been reduced in the state because of the systematic vaccinations, an abrupt increase in the number of FMD cases due to FMDV serotype O involving all the species was recorded in 2013 after four rounds of mass vaccination. Post-outbreak of the disease in 2013, the vaccination campaign was intensified and the vaccination coverage was more than 95 percent. The present study was carried out subsequent to two rounds of mass vaccination after the outbreaks in 2013.

The SP-Ab was observed in 79% of the large ruminants (cattle/buffaloes) against all three serotypes. Post outbreak of the disease in 2013, due to increase in vaccine coverage, the SP-Ab increased from 59% to 79% against all three serotypes. These figures could be inclusive of both vaccine and infection induced protective antibodies against SP. The increase in SP-Ab and reduced disease incidence in the state may be due to infection (outbreak) immunity combined with extensive vaccination [15]. The increase in anti-NSP antibodies in bovines from 21% in 2013 to 33% in 2014 also indicates the circulation of the virus and exposure to infection. In India, only cattle, buffalo and pigs are vaccinated under FMDCP and small ruminants are not vaccinated. SP-Ab (titer of $\log_{10}$ 1.8 or more) was observed in 18% of the small ruminant samples against all three serotypes O, A and Asia-1. As small ruminants are not covered 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 [16]. Since the circulation of all the three serotypes has been confirmed in the state of Karnataka, the presence of SP-Ab in small ruminants indicates that these animals were frequently exposed to all the three serotypes. In total, 18 percentage of the samples demonstrated ≥4 fold relative spike in SP-Ab titre against either of the serotypes further confirming the circulation of FMDV serotypes in small ruminants. Earlier published reports also revealed the circulation of FMDV serotypes in small ruminants [17-19]. Clear 4 fold spike in SP-Ab was not apparent in bovines, 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 [20]. From the above findings, it could be clearly inferred that the small ruminant populations are frequently exposed to FMDV infection by different serotypes of the virus and remain as sub-clinical host. It was observed in the present

study that the samples negative for NSP antibodies in small ruminants revealed SP-Ab in 18.2% samples against all three serotypes. The samples positive for NSP antibodies in small ruminants revealed SP-Ab in 21.3% of samples against all three serotypes. The presence of NSP antibodies in the animals negative for SP antibodies might have been due to residual antibodies arising from the past infection, as earlier study has shown that antibodies against NSP can persist for a longer duration than SP after infection [11]. The NSP-Ab positive but SP-Ab negative category could have been either due to a faster rate of decline of SP-Ab over time subsequent to an infection or due to a weaker SP-Ab response. Though it has been shown that following an infection, animals can remain seropositive to structural antibodies of FMDV for several years [21], the rate of decline of SP-Ab in small ruminants has been shown to be faster than that in cattle [22-23].

Overall, 35% of cattle, 27% buffaloe, 11.6% of sheep and 23.2% of goats were seroreactors in NSP-ELISA. The NSP ELISA results are indicative of the past exposure or ongoing virus activity in susceptible animals. Among small ruminants, prevalence was sig- nificantly higher in goats than in sheep. Lower seroprevalence of FMD in sheep may be associated with low frequency of exposure to the disease. In most of the field outbreaks, we have seen that only the bovine species show clinical manifestations and the small ruminants remain asymptomatic even though cohoused with the large ruminants. The level of virus replication is relatively more luxuriant in bovines than in the small ruminants. Hence, the NSP-Ab response elicited in the infected bovines is expected to be comparatively high than the generally asymptomatic small ruminants. Besides, in ruminants, FMDV is capable of causing a persistent infection with a significant higher duration for cattle (about 3.5 years), than sheep (9 months) and goat (4 months) [24]. These factors related to variability in the degree of virus replication and persistence might have contributed to the observed difference in the apparent prevalence of NSP-Ab between large and small ruminant population. The small ruminants especially goats can be used as tracers when bovine population is routinely vaccinated under FMD-CP.

The study demonstrated that FMD is endemic in the state. The present study gathered the serological evidence of the virus activity in the small ruminant population of Karnataka state. The animal husbandry practice in the state reflects cattle, sheep and goats being reared in close proximity and at many places even co-housed in a single shed. Communal grazing is practiced in many areas where both small and large ruminants are allowed to use the same pasture land and water sources. These unrecognized, sub clinically infected small ruminants may act as a source of infection through their secretions and excretions and could pose a potential risk of virus dissemination to cattle and other animals. The preliminary findings of the present study suggest the need for strengthening the surveillance activities in small ruminants alongside large ruminant population.

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