

Research Article



Serological Status of Foot and Mouth Disease in Cattle and Buffalo of Andaman and Nicobar Islands of India

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Abstract | Foot and mouth disease (FMD) was first reported from Andaman & Nicobar Islands in 2005 and for the first time serotype “O” was isolated as the causative agent. Since then not any case of FMD has been reported. For routine serosurveillance and monitoring blood samples are being collected and screened for protective antibody titer in the vaccinated animals and antibodies against non-structural proteins (NSPs) for the infected animals. In the present study, samples were collected from the South Andaman district for the period from 2011-2014. The samples were screened for protective antibody titre by indirect liquid phase blocked (LPB) ELISA and for anti NSP protein by DIVA-ELISA. The result of the DIVA-ELISA revealed the prevalence of 9.52%, 7.42% and 9.21% for the year 2011-12, 2012-13 and 2013-14, respectively. The trend of the DIVA positive samples indicated that the animals are still showing DIVA+ve, without showing any clinical symptoms or outbreak since the last outbreak reported in 2005. The result of the LPB-ELISA indicated that, since 2012 there was sharp increase in the trend of pre and post protective antibody titre in the pre and post vaccinated animals. The analysis of the protective antibody titre with respect to herd immunity indicated the protection level of 88% for serotype O, 86% for serotype A and 93 % for serotype Asia -1, respectively. Based on the LPB-ELISA seroprevalence, it is concluded that the level of immunity for the serotype O, A and Asia -1 had increased manifold over the years, however the seroprevalence of NSP +ve cases was high without any infection or outbreak.

Keywords | A&N Islands, Foot and Mouth Disease, Sero-monitoring, DIVA, LPB-ELISA, Immunity

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INTRODUCTION

Foot and mouth disease (FMD) is a highly contagious disease of cloven hoofed animals including cattle, sheep, goat and pigs (Racaniello, 2001). It is one of the most important viral diseases of the livestock causing heavy economic losses to the livestock sector on account of direct losses in the form of productivity and indirect losses in the form of restriction of international trade of livestock and their products including germplasm. In India, direct losses due to FMD has been estimated to be more than 323 million USD (Venkataramanan et al., 2006) while indirect losses could be about 4838 million USD to 5645 million USD annually (Anonymous, 2012). Loss due to milk reduction is approximately 3508 million litres per year (about 8.5% of the total milk production) (Saxena, 1994).

The last report of the clinical FMD in Andaman Islands was 2005 (Sunder et al., 2008; Hemadri et al., 2005) due to serotype O. Since then, no clinical case of FMD has been observed from the A & N Islands. Government of India implemented vaccination based FMD control programme in the A & N Islands in the year 2005 covering entire population of cattle and buffalo of South Andaman. Presently, 17 rounds of vaccinations have been completed. The population of livestock as per the census 2012 is cattle (45,617), Buffalo (7,850), Goat (64,602), Pig (35,401) and poultry (10,80,022) (DAHDF report/Livestock census, 2012). There are no sheep population in the A & N Islands. The movement of the animals from mainland is very less. During the last ten years, no animals (Cattle and buffalo) have been imported from mainland. Animal Husbandry department brought some goats (Malabari and

Black Bengal goats) from animals. After thorough examination at quarantine station they were mixed with the local population. The movement of animals in the islands is also very less, which is mainly from North Andaman to South Andaman. All the islands in the A & N Islands are separated by sea barrier, therefore the transportation and movement of animals is very difficult.

A longitudinal study was conducted from the year 2011-2014 to estimate the sero-prevalence of FMD and to evaluate the herd immunity during this period in the A & N islands. The sero-prevalence of FMD was estimated by r3AB3 Indirect ELISA which can differentiate FMD infected from vaccinated animals (DIVA; Mohapatra et al., 2011). Herd immunity was estimated by quantifying the antibody titers against structural proteins of FMD virus serotypes O, A and Asia-1 by end-point dilution method of liquid phase blocking ELISA provided by the Project Directorate on Foot and Mouth Disease, Mukteswar.

MATERIALS AND METHODS

STUDY AREA

The study area was divided into two zones. In the first zone, the districts of South Andaman were selected because >32% of the total livestock population of cattle, goat, pig and buffalo are in these districts and currently animals of the South Andaman districts are being vaccinated bi-annually under government endorsed FMD control programme. In addition, only animals of these districts were clinically affected during the last FMD outbreak during the year 2005. In the second zone, districts of North and Middle Andaman were sampled with no available history of FMD outbreaks.

SERUM SAMPLES

A total of 5501 serum samples were collected from cattle and goat population of which 2943 serum samples were collected on 0 DPV and 2558 on 21-28 DPV during 2011-12 to 2014-15. Sera samples were collected from vaccinated and non-vaccinated cattle and goat population. The population of cattle represented mainly the non-descript type with 10% population of (16117) HF and Jersey cross. Samples were collected from vaccinated as well non vaccinated animals including the calf less than 6 month of age.

SEROLOGICAL ASSAYS

DIFFERENTIATION OF INFECTED FROM VACCINATED ANIMALS (DIVA)

A panel of four non-structural protein based indirect enzyme linked immunosorbent assays (ELISA), which could detect the antibodies against NSPs of FMDV in the FMD infected animals, were used for detection of FMD infec-

tion (Sharma et al., 2014). If the animal were not exposed to FMD virus infection but vaccinated with inactivated purified polyvalent FMD vaccine, no anti-NSP immune response is elicited in host's body. The test was carried out as per the protocol developed by PD-FMD, Mukteswar. The sample producing OD value more than the fixed cut off ratio $\{(test\ serum\ sample\ mean\ OD/positive\ control\ serum\ mean\ OD) \times 100\}$ i.e. percent positivity value or PP value $\geq 40\%$ is qualitatively diagnosed as positive for FMD infection.

LIQUID PHASE BLOCKING -ELISA (LPB-ELISA)

Serotype specific antibody titres were quantitatively measured by LPBE to assess the overall herd immunity status against FMD virus serotypes O, A and Asia1. The assay was carried out using the end-point dilution LPBE kit developed by PD-FMD, Mukteswar. Briefly, series of 2 fold dilution of test serum were mixed with an equal volume of a fixed amount of virus in a low binding plate and allowed to react overnight at 4°C. Next day the free antigen (not blocked by the antibodies in the test serum) were trapped to the wells of the ELISA plate by the pre-coated type specific rabbit antibodies. Subsequently, the presence of antigen was traced by adding pre-titrated serotype specific guinea pig serum and anti guinea pig HRPO conjugated antibodies. Colour reaction was developed by addition of substrate solution and OD was measured at 492 nm with background at 620nm. Antigen control (OD_{max}) was used for comparison with the test samples. Background was kept to find out if there is any non-specific rabbit (coating) and guinea pig (tracing) antibody binding in the test. Mean OD of background wells is subtracted from OD of each well for calculation. Percent reactivity against each serum dilution is calculated as follows:

$$\% \text{ reactivity} = \frac{\text{Mean OD of test wells}}{\text{Mean OD of antigen control wells}} \times 100$$

Titre of the serum samples is expressed as the reciprocal of the serum dilution giving 50% OD as compared to the antigen control. The samples showing log₁₀ titre of ≥ 1.8 against all the serotype such as O, A & A-1 in the vaccine were considered as protective antibody titre.

RESULTS AND DISCUSSION

SEROPREVALENCE OF FMD

The result of the DIVA tests indicated that out of 2632 sera samples, 210 samples (7.97%) showed positive to 3rAB3 NSP. The percentage of +ve DIVA samples varied from 5.3 % to 9.52% during different year of survey (Table 1). The trend of the positive samples shows that the DIVA positivity is declining during the last four years. However, the villages such as Rangachang, Wiberlygunj, Junglight, Port Mort, Garacharma and Manglutan of South An-

damam district showed very high DIVA positivity up to 34.5%. The history of FMD outbreak due to serotype O was reported in the year 2005 from these villages (Figure 1). However, the samples of North & Middle Andaman district did not show positive NSP to DIVA ELISA.

Table 1: Trend of DIVA (r3AB3) positive samples

Year	No. of samples tested for DIVA (Cattle)	No. of positive samples	% of positive samples
2011	147	14	9.52%
2012	896	67	7.47%
2013	1139	105	9.21 %
2014	450	24	5.3 %

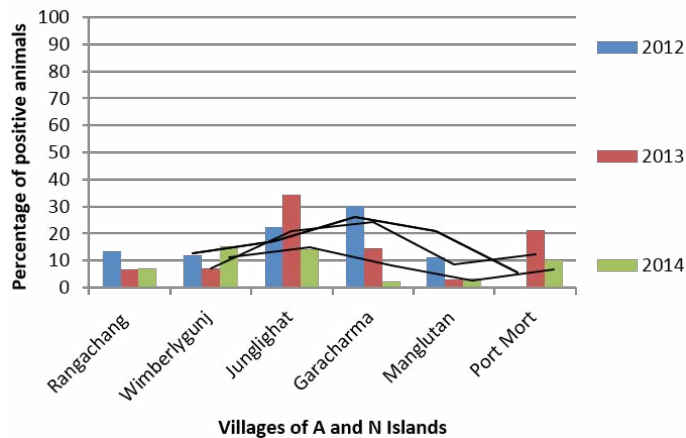


Figure 1: Villages of South Andaman showing high DIVA positive samples

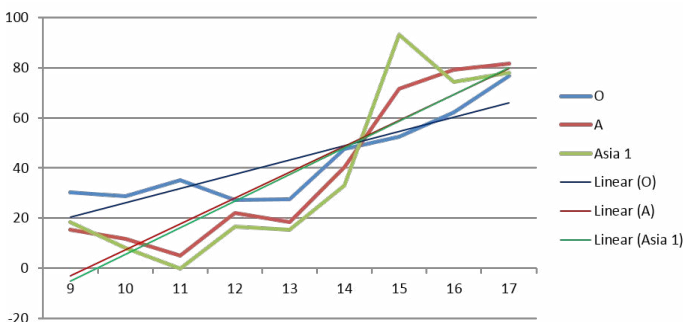


Figure 2: Trend of FMD vaccinal antibody response in FMDCP in cattle and buffalo

The sera samples which showed positive in the DIVA test were further screened by the multiple NSPs indirect ELISAs for detection of antibodies against 2Ct, 3B, 3AB3, 3ABC NSPs. A total of 396 sera samples were screened by the four I-ELISAs, of which, none of the sample showed positive in the all four assay. However, 54 samples showed positive in any one of the four assays. Only 8 samples were found positive in 3 assays (except 3B). The result of the multiple DIVA assay indicated that no specific geographical pattern could be noticed, however, samples collected from Manglutan, Junglighat, Port Mort were relatively

more positive than others.

The sero-prevalence pattern of FMD indicated that about 8% of the samples were positive to DIVA ELISA since last outbreak due to FMD was reported in 2005. Not a single sample was found positive in all the four NSPs which is indicative of absence of clinical FMD in this region of the country. Although, low but some samples were found positive in three of the four assays, which could be attributed to the repeated vaccination during the 17 phases of vaccination programme by partially purified vaccines (Mohapatra et al., 2011). It has been reported that FMDV may persist in some of the infected cattle for about 3 years (Soren et al., 2002) and percent positivity of the DIVA was also found high in the population where the FMD was reported in the year 2005. However, it is very difficult to explain the persistence of infection in the animals for about 10 years without exhibiting any clinical disease in the geographically isolated area. Detailed examination of virus persistence is required in this part of the country by examining oro-pharyngeal fluids for the presence of the virus to confirm the virus persistence.

SERO-MONITORING OF FMD

So far 17th round of vaccination has been carried out in South Andaman with target population of cattle (15,286), buffalo (985) goat (23,794) and pig (906). The results of LPBE indicated that out of 2943 and 2558 pre and post vaccinated sera sample of the last six round of FMD vaccinated animals showed antibody titer greater log₁₀ 1.8 27.62%, 30.82%, 33.98% in pre and 47.54%, 56.61%, 58.48% in post type O, A and A-1 respectively (Table 2). The trend of antibody titre was found to be increasing since 2011 (Figure 2). The post protective antibody titre for type O, A and A-1 for the 17th round of vaccinated animals was found to be 76.78%, 81.56% and 77.87% respectively.

The quantum sero-epidemiology of the animals screened for LPB-ELISA assay indicated that the herd immunity for type O, A and Asia1 was found to be 88%, 86% and 93%, respectively. The proportion of susceptible population was found to be only 12%, 14% and 7% respectively for type O, A and A-1, respectively. The latest trends of the antibody protective titre in the vaccinated animals were found to be high compared to the previous round of vaccination.

The only outbreak of FMD due to serotype was reported in the year 2005 (Sunder et al., 2008; Hemadri et al., 2005). Since then the islands is free from FMD, as no clinical case of FMD has been reported. The initiation of FMD control programme by Government of India has created a major impact due to judicious vaccination programme, the outbreak could be controlled and since then not a single case of FMD was reported. The sero-monitoring and continuous surveillance of the animals, sera samples from

Table 2: FMDCP phase wise pre and post vaccination status (LPB-ELISA) of protected animals

Number and date/month of phase of vaccination	Month of vaccination	No. of serum sample tested (pre-vac)	No. of serum sample tested (post-vac)	No. & % of sample with pre-vaccinated animals protected			No. & % of sample with post-vaccinated animals protected			No & % of animals protected against 3 serotypes of FMDV
				O	A	Asia1	O	A	Asia1	
Phase-XI	Feb 2011	20	20	1 (5%)	0	0	7 (35%)	1 (5%)	0	
Phase- XII	Sep 2011	180	180	36 (20%)	19 (10.5%)	11 (6.1%)	49 (27.2%)	40 (22.2%)	30 (16.6%)	25 (13.8%)
Phase-XIII	Jun 2012	283	283	26 (9.1%)	12 (4.2%)	15 (5.3%)	78 (27.5%)	52 (18.3%)	44 (15.5%)	29 (10.2%)
Phase-XIV	Feb 2013	758	596	141 (18.6%)	98 (12.9%)	81 (10.7%)	145 (47.5%)	211 (40.3%)	189 (32.9%)	180 (30.2%)
Phase-XV	Aug 2013	651	516	164 (25.1%)	233 (35.7%)	356 (54.6%)	271 (52.5%)	370 (71.7%)	480 (93.0%)	257 (49.8%)
Phase-XVI	Feb 2014	530	502	220 (41.5%)	243 (45.8%)	251 (50%)	312 (62.2%)	398 (79.3%)	394 (74.3%)	294 (58.56%)
Phase -XVII	Aug 2014	521	461	225 (43.1%)	302 (57.9%)	286 (54.9%)	354 (76.8%)	376 (81.6%)	359 (77.8%)	293 (63.5%)

cattle, buffalo and goat resulted in the status of the vaccinal antibody response as well as the status of the population showing positive in DIVA assay. The trend of the vaccinal antibody response in the cattle indicated that there has been considerable increase in the overall protection with all the three serotype viz. O, A and Asia-1 from 13.8% to 63.5%.

The trend of DIVA positive cases also declined over the last four years of screening. The positive sample has been found to be clustered in the villages where the history of FMD was reported in 2005. However, no clinical case of FMD has been reported from the DIVA positive zones. The trend of DIVA positive showed that in the year 2014 only 5.3% of the samples showed DIVA positive, which is very low, compared to the national average of 27% (Annual Report of FMD 2013-14). However, the case of DIVA positive without any clinical cases of FMD is alarming. Andaman & Nicobar Islands is geographically isolated from mainland with a sea barrier of almost 1200 km and there is almost negligible movement of animals from mainland. So there is very little chance of attracting the entry of pathogen from mainland. To make the island free from FMD it is advisable to follow the strict vaccination programme under FMDCP and routine sero-monitoring of the livestock. Both the tests viz. LPB-ELISA and DIVA have been found to be very effective in continuous sero-monitoring of the large number of samples.

AUTHORS CONTRIBUTION

All the authors contributed in various phases of this experiments viz. conduction experiment, guidance to conduct the research, scientific discussion and preparation of manuscript.

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CONFLICT OF INTEREST

The authors have no conflict of interest.

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