Project Directorate
on
Foot and Mouth Disease

Annual Report 2013-2014

Mukteswar - 263138
Nainital, Uttarakhand, India

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Contents

Contents

1 Executive Summary

Foot-and-mouth Disease (FMD) is a highly contagious viral disease primarily of cattle and buffalo. The disease also affects goats, sheep, pigs, wild ruminant species and elephants. The causative FMD virus (FMDV) is antigenically diverse having seven distinct serotypes (O, A, C, Asia1 and Southern African Territories (SAT) 1-3) and multiple subtypes/genotypes in each serotype. Currently three serotypes (O, A and Asia1) are prevalent in India. Serotype O is the most dominating one followed by serotypes Asia1 and A. India has a FMD susceptible livestock population of 528 million (DAHD&F, GoI, 2007). The economic losses to the livestock industry attributed to FMD are large. There are direct and indirect losses due to this menace. Direct losses to livestock sector are due to significant drop in milk yield (up to 80%), reduction in meat and wool production, abortion in pregnant animals and mortality in calves. Indirect losses to livestock sector are due to permanent loss of productive and reproductive functions, reduced working capacity in bullocks and flare up of opportunistic infections. Most importantly, loss in livestock and their products trade both of national and international levels and massive expenditure by Government on FMD control and cost of treatment lead to huge economic loss.

During the year 2013-14, a total of 472 outbreaks were recorded (Table 1). Almost 50% of the outbreaks were recorded in the southern states of Karnataka, Tamilnadu, Kerala and Andhra Pradesh. Almost four fold increase in outbreak was observed in the southern region. Maximum number of outbreaks was recorded in the states of Karnataka, Kerala and Tamilnadu. There was no incidence of the disease in the states of Punjab and Delhi during 2013-14, and few sporadic cases were recorded in the states of Haryana and Himachal Pradesh. There has been reduction in the incidence of FMD in the western region compared to previous year possibly due to optimal vaccination coverage/infection immunity. The states of Gujarat and Maharashtra, which are covered under FMD-CP, recorded very few outbreaks/case. Further incidence of FMD reduced in north eastern region compared to previous year, possibly due to infection immunity

Table 1 Number of confirmed FMD outbreaks in different geographical regions of the country during the last eight years.

Year	South	North	Central	West	East	North East	Total
2006-07	224	7	23	32	431	64	781
2007-08	445	20	35	33	258	85	876
2008-09	64	18	33	21	66	43	245
2009-10	59	55	20	24	367	74	599
2010-11	51	9	29	17	30	40	176
2011-12	97	20	34	60	71	65	347
2012-13	68	16	21	14	104	108	331
2013-14	228	32	35	27	103	40	472

Serotype O caused maximum numbers of outbreaks (96.2%) and serotype A and Asia1 were restricted to only a few outbreaks/incidences. Compared to previous year, number of outbreaks caused by serotype O has greatly increased (Table 2). Outbreaks due to serotype Asia1 has decreased by 5 fold compared to the last year and occurrence of serotype A decreased by 2 fold. Serotype O was the most prevalent in all the geographical regions. Serotype Asia1 was recorded only in Eastern, Central and Western regions of the country, where this serotype has been isolated regularly. Serotype Asia1 that caused many outbreaks in the Southern region during previous year could not be encountered during 2013-14. All the three serotypes (O, A and Asia1) occurred in the Eastern and Western regions. In Northern region, serotype Asia 1 could not be detected continuously for last four years since 2010-11. This year, serotype A was recorded only in the Eastern, North eastern and Western regions

Table 2 Year wise break-up of outbreaks and FMDV serotypes involved during last eight years

Year	Total	O	A	Asia1
2006-07	781	491	84	206
2007-08	879	753	67	56
2008-09	245	200	21	24
2009-10	600	560	24	15
2010-11	176	150	10	16
2011-12	347	246	16	85
2012-13	331	265	16	52
2013-14	472	454	08	10

This year, increase in number of outbreaks was noticed from June and reached its peak during November, 2013. Several FMD outbreaks were observed between October and December in the Southern region. Several cases of death in cattle and buffalo were due to secondary bacterial infection following FMD. In many of the Southern states, there was no vaccination against Hemorrhagic Septicemia (HS), and post mortem on selected carcasses revealed lesions skin to HS. These states experienced heavy and prolonged monsoon during 2013 that possibly precipitated HS. FMD outbreaks in all the four states were thoroughly investigated by project staff at field level. Delayed vaccination, lack of vaccination in some areas, extended monsoon, inadequate zoo-sanitary measures and frequent animal movement were found to be the major cause of disease spread.

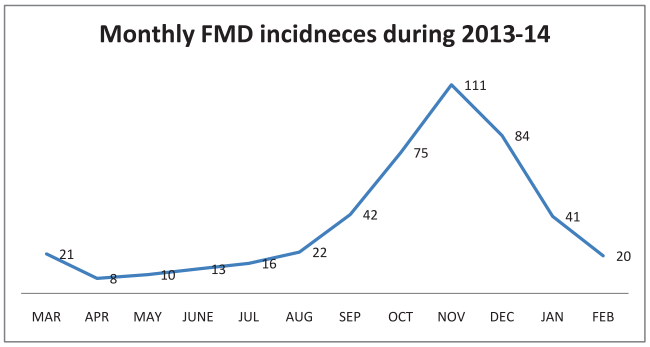
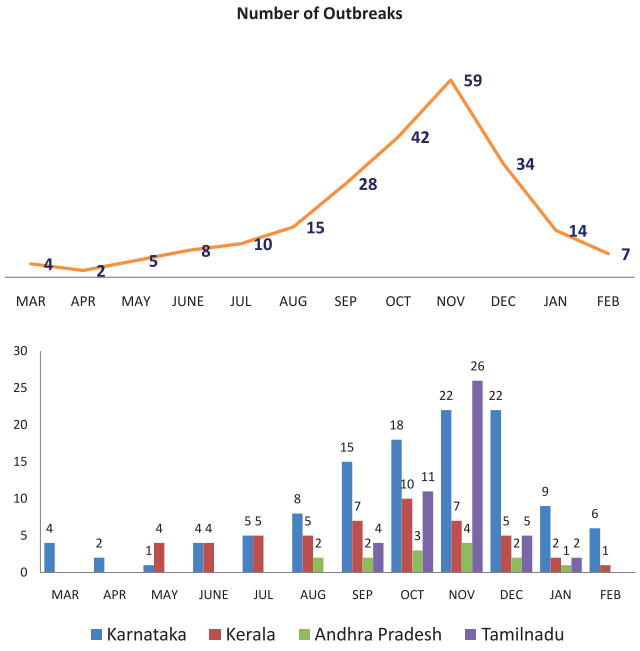


Fig. 1 Month wise occurrence of FMD outbreaks.



Maximum number of outbreaks were recorded in the month of November and increasing trend in the outbreak numbers was recorded since June 2013. Initially outbreaks were reported from Karnataka during March and April, and then both in Karnataka and Kerala through May, June and July. In the beginning, outbreaks in Andhra Pradesh were recorded in the month of August and in Tamilnadu in the month of September. During September 2013 to January 2014,

the disease was reported in all the Sothern states. Four seasons which include winter (December to early April), summer (April to June), monsoon (June to September) and post monsoon (October to December) prevail in the country. It is believed that high relative humidity (RH) and heavy rain during rainy season inhibit aerosol transmission. Incidences of FMD increased from August and peaked in September and maintained until January 2014. Maximum FMD incidences occurred at the end of the monsoon and post monsoon season due to comparatively dry weather and moderate RH which is very much conducive for virus transmission. Outbreaks in summer months were less possibly due to very high ambient temperature.

Phylogenetic analysis based on VP1 (1D) coding region was carried out to assess genetic variations, inter-strain relationships and track movement of the virus. During the year, phylogenetic analysis of serotype O virus shows that Ind2001 strains, which reemerged in late part of the year 2008, nearly out-competed PanAsia lineage in causing outbreaks in the county. The sub-lineage Ind2001d was distributed widely covering many states including Karnataka, Kerala, Andhra Pradesh and Tamilnadu (Southern region); Uttar Pradesh, Uttarakhand and Jammu & Kashmir (Northern region); Gujarat and Maharashtra (Western region); Odisha, West Bengal and Bihar (Eastern region); Madhya Pradesh (Central region) and Assam and Manipur (North Eastern region).

In case of serotype A, all the isolates were found to cluster within the genotype 18 in the maximum likelihood tree, and grouped both in the clade 18c of the VP359-deletion lineage and non-deletion lineage. In case of serotype Asia1, the isolates clustered within the lineage C indicating its exclusive prevalence since 2005.

Vaccine matching exercise was carried out to evaluate antigenic relationship of field isolates with currently used vaccine strains to monitor antigenic variation, if any, occurring in the field, and assessing appropriateness of in-use vaccine strains. Selected virus isolates of all three serotypes were subjected to one-way antigenic relationship analysis (r-value) using Bovine Vaccinate Serum (BVS) against respective vaccine strains. In case of serotype O, the vaccine strain INDR2/1975 covered 79% of the field isolates. Emergence of antigenic variant in an endemic country is a normal phenomenon and the currently used vaccine strain INDR2/1975 still is able to provide near optimal antigenic coverage to the field isolates. Some isolates were found divergent from the vaccine strain and emergence of such antigenic variants in the field is a regular phenomenon and is not alarming at present. In case of serotype A, none of the isolates showed perfect match with the vaccine strain, IND40/2000. Therefore, study has been initiated to evaluate alternate candidate strains for better antigenic coverage with broader match potential. In serotype Asia1, the field isolates (n=2) analyzed had perfect match with the currently used vaccine strain, IND63/1972. Almost 25% of the isolates collected during 2012-13 had less antigenic match with the currently used vaccine strain. An alternate vaccine candidate panel [IND13/2001, IND78/2011 and IND68/2012] has been identified and evaluation is under progress.

National FMD Virus Repository was upgraded with latest/new virus isolates. The virus repository has served the cause of the country by providing isolates for molecular epidemiological studies, evaluation of antigenic relatedness between the field and vaccine strains and selection of new candidate vaccine strains whenever required. A total of 77 virus isolates (32 type O, 19 type A and 26 Asia 1) were added to the repository during the reported period. At present the National FMD virus Repository holds a total of 1851 isolates (O-1180, A-298, C-15 and Asia 1-358).

Under National FMD Serosurveillance, 52,224 bovine serum samples collected at random from various parts of the country were tested in r3AB3 NSP-ELISA for assessing NSP-antibody (NSP-Ab) response, which is an indicator of FMD virus exposure regardless of vaccination status. The test revealed overall seropositivity in ~ 29.2% samples/animals, which is almost equal to the previous year.

During 2013-14, a total of 1,74,076 pre and post vaccinated serum samples were tested under FMD Control Programme (FMDCP) and of which, 42,514 serum samples were from first phase FMDCP districts representing XIII, XIV, XV and XVI phases of vaccinations, and remaining 1,31,564 serum samples were from expanded FMD CP districts of 2010-11 representing Phases II, III, IV V and VI. After phase XVI vaccination, 87.3, 58.4 and 90.3 percent of animals tested were having protective antibody level (log10 1.8 and above) against serotypes O, A and Asia 1, respectively in post-vac serum samples. After phase II vaccination under expanded FMDCP, 74.1, 81.1 and 84.4 percent of animals tested were having protective antibody level against serotypes O, A and Asia 1, respectively in post-vac serum samples. The extended FMDCP areas are likely to yield better result soon.

Six training programmes for the scientific staff of Regional Centers and Collaborating/network units were conducted on use/application of virus serotyping ELISA, LPB-ELISA and DIVA ELISA. Overall performance of the regional centers and network units were monitored periodically and any technical difficulties faced by them were removed instantly through electronic guidance. Requirement of diagnostics kits in the Government sector and vaccine industry was met by the institute. During the period, r3AB3 DIVA Kit for FMD to test 87,850 samples was supplied to the AICRP units and vaccine manufacturing companies. Similarly, virus serotyping Kits for 21,500 tests and LPB-ELISA Kits for 2,36,640 were supplied to FMD Regional centers/network units for sero-surveillance and sero-monitoring of FMD. Diagnostic kits were also supplied to SAARC Countries under FAO programme.

I am happy to share that PDFMD is now a member of the Global FAO/OIE Network of FMD Reference Laboratories that constitutes of ten other FMD laboratories in the world. The institute also functions as the FAO-FMD Reference Center and SAARC Regional Leading Diagnostic Laboratory for FMD. The institute is also now a member of GFRA (Global FMD Research Alliance). Construction of International Center for FMD has already been started since March 2014. Creation of this international laboratory with state-of-the-art features of bio-safety and bio-containment (BSL 3Ag) will facilitate Global participation and control of the disease in India and SAARC region. I thank all my fellow scientist colleagues, administrative, accounts and laboratory staff of the institute for their sincere efforts and contribution in accomplishing the tasks assigned to the Institute. We are indebted to the scientific and administrative support of Hon'ble Director General, ICAR and Dy Director General (AS), as well as Asst Director General (AH) and Principal Scientist (AH) for their support.

B.PATTNAIK

2 Vision, Mission, Mandate, objectives and Technical Programme:

Vision:

To make India free from Foot and Mouth Disease.

Mission:

Active epidemiological surveillance through regularly monitoring antigenicity and genomic make up of Foot and Mouth Disease virus strains responsible for disease outbreaks, to provide training in diagnosis and epidemiology, and to develop technologies for making country free from FMD.

Mandate:

Active epidemiological surveillance through regularly monitoring antigenicity and genomic make up of the FMD virus strains responsible for disease outbreaks, and also to provide training in diagnosis and epidemiology.

Objectives:

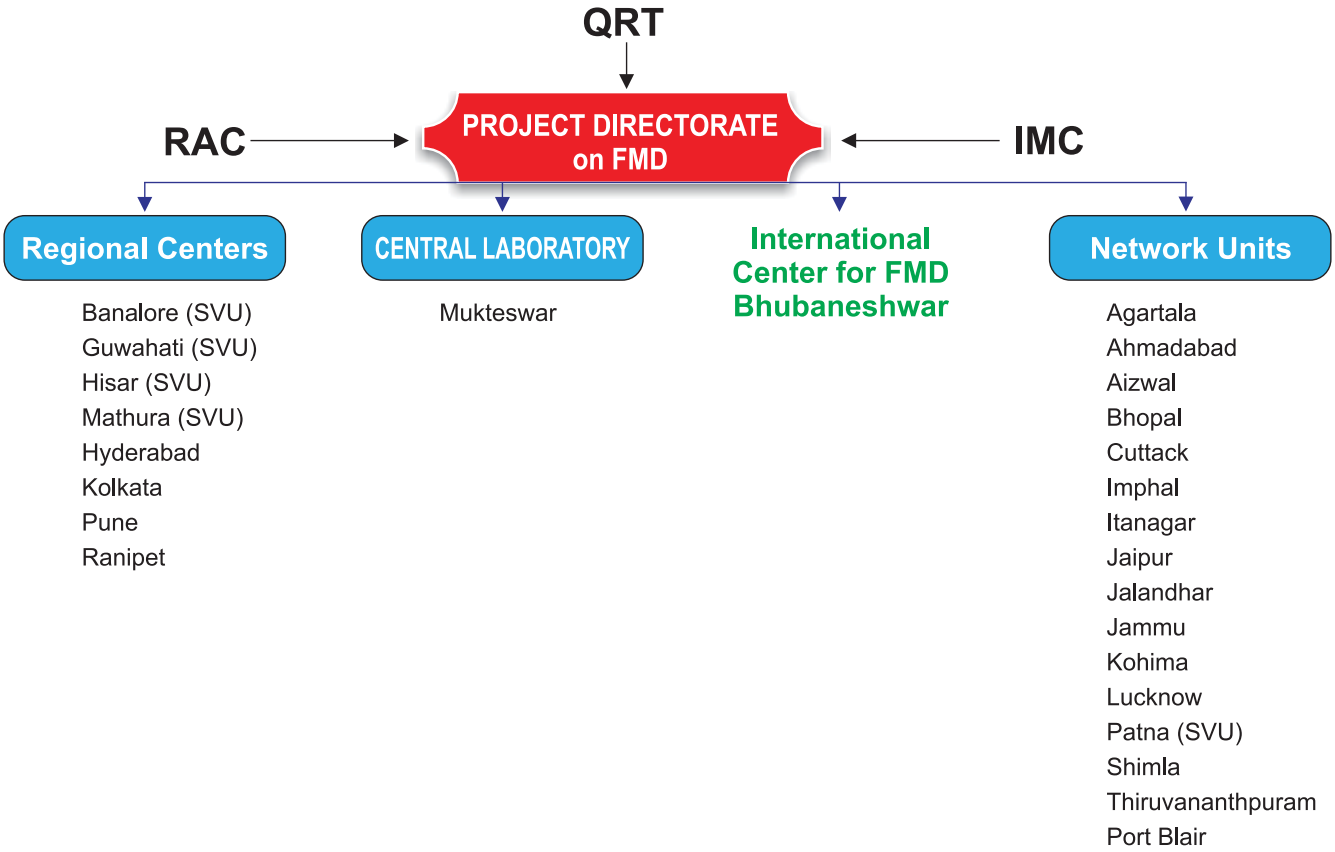
1. To conduct systematic epidemiological and molecular epidemiological studies on Foot- and- Mouth Disease (FMD), and also to study carrier status of the infection and latency of the virus.
2. Antigenic and molecular characterization and cataloguing of FMD virus strains isolated from outbreaks, and monitoring suitability of the vaccine strains in use along with maintenance of National Repository of FMD Virus.
3. Production, standardization and supply of diagnostic reagents for FMD virus serotyping and post-vaccinal sero-conversion. Maintenance and supply of most appropriate vaccine strain to the FMD vaccine manufacturers.
4. Development of newer diagnostic techniques using cutting-edge technologies in molecular biology.
5. Analysis of economic impact of FMD on livestock industry
6. To act as referral laboratory for FMD in South Asia.

Technical Programme:

1. Active and passive surveillance of FMD in the country in AICRP mode
2. To carryout antigenic and molecular characterization of field isolates.
3. To study molecular epidemiology of FMD in India.
4. Confirmatory diagnosis and expert advice.
5. To carryout vaccine matching exercise for monitoring of appropriateness of in-use vaccine strains.
6. Maintenance of National Repository of FMD virus strains.
7. Production, standardization and supply of diagnostic kits for FMD virus diagnosis (sandwich ELISA and mPCR kit), sero-monitoring (LPB-ELISA) and serosurveillance (NSP-DIVA ELISA)
8. To develop and standardize advanced laboratory techniques in compliance with the International standards and pass them on to the concerned Centres/Users/Stakeholders with proforma details to facilitate and ensure their uniform application.
9. To organize skill orientation programme for the scientific staff of the project for keeping them abreast with the latest knowledge and expertise from time to time through short-term training courses
10. Participation in FMD Control Programme with vital contribution in monitoring pre and post vaccinal antibody response for assessment of individual and herd immunity level.
11. National FMD Serosurveillance
12. International collaborations in areas of interest.

3 Organizational Setup

The Project Directorate on Foot and Mouth Disease (FMD), the premier Institute for FMD in the country, was established as an All India Coordinated Research Project (AICRP) for FMD in 1968. During more than last four decades of its existence the scope of the project has been expanded progressively and several milestones were achieved to reach the current status of a Project Directorate in 2001 with 23 Regional Centers and Network Units covering all the major regions of the country. The Project Directorate has developed scientific expertise in conventional as well as in cutting edge areas, in the field of FMD diagnosis, epidemiology and research. The mandate of the institute is to carry out research on the epidemiology of FMD in the country and develop technologies to control the disease with ultimate goal of eradication. It is also entrusted with the duty of providing technical support and scientific input/information to the planners and strategy making agencies in planning control of FMD in the country and the SAARC region.



4 Staff Position

S.No.	Name of the staff	Designation	Discipline	Joining in the Current Post
1	Dr. Bramhadev Pattnaik	Project Director	Veterinary Microbiology	December 2006
2	Dr. Aniket Sanyal	Pr. Scientist	Veterinary Microbiology	April 2009 (On deputation)
3	Dr.Bana.B.Dash	Sr. Scientist	Veterinary Microbiology	August 2009
4	Dr. Jajati. K. Mohapatra	Sr. Scientist	Veterinary Microbiology	March 2012
5	Dr. Saravanan Subramaniam	Scientist	Veterinary Microbiology	May 2007
6	Dr.Gaurav.K.Sharma	Scientist	Veterinary Microbiology	December 2009
7	Dr. Manoranjan Rout	Scientist	Veterinary Pathology	March 2010
8	Dr. Rajeev Ranjan	Scientist	Veterinary Pathology	September 2010
9	Dr. Jitendra .K.Biswal	Scientist	Animal Biotechnology	September 2011
10	Dr. Sonalika Mahajan	Scientist	Veterinary Microbiology	April 2013

S.No.	Name of the staff	Designation	Joining in Current Post
1	Shri A.K.Rai	AO	June, 2012 (on leave)
2	Shri P.C.Bhatt	AAO	March, 2013
3	Shri Raja Ram	AF & AO	February, 2012
4	Shri Tara Kumar	Assistant	April, 2013
5	Shri Nayan Sanjeev	T-3 (Lab)	October, 2010
6	Shri D.S.Deolia	T-1 (Lab)	January, 2012
7	Shri S.L.Tamta	T-1 (Lab)	April, 2014
8	Shri J.P.Bhan	S. S. Gr. IV	February, 2008
9	Shri R.N.Sahoo	UDC	May, 2012

5 Epidemiology Report

To assess the regional prevalence of FMDV serotypes, country is divided in to five geographical regions namely; Eastern (States of Bihar, Orissa, West Bengal and Jharkhand), Southern (States of Tamilnadu, Kerala, Karnataka and Andhra Pradesh), North Eastern (States of Assam, Manipur, Meghalaya, Mizoram, Arunachal Pradesh, Sikkim and Tripura), Northern (States of Uttar Pradesh, Punjab, Haryana, Himachal Pradesh, Jammu & Kashmir and Uttarakhand), Western (States of Rajasthan, Gujarat and Maharashtra) and Central (Madhya Pradesh and Chhattisgarh).

Table 5.1 FMD cases/outbreaks recorded and diagnosed during 2013-14 and virus serotype(s) involved

States	Reporting AICRP Centre/Unit	No. of. FMD cases/ outbreaks	No. of. Samples tested	Virus Serotyping Results		
				O	A	Asia1
Southern Region						
Tamil Nadu	Ranipet	48	239	48(158)		
Andhra Pradesh	Hyderabad	14	47	13(27)		
Karnataka	Bangalore	116	1639	116(556)		
Kerala	Thiruvanthapuram	50	387	50(150)		
Total		228	2312	228(891)		
Northern Region						
Jammu & Kashmir	Jammu	05	13	05(07)		
Haryana	Hisar	02	03	02(03)		
Himachal Pradesh	Shimla	04	17	04(17)		
Punjab	Jalandhar			No disease		
Uttar Pradesh	Mathura	05	18	05(14)		
	CADRAD	07	30	07(31)		
Uttarakhand	PDFMD	09	96	09(45)		
Total		32	177	32(90)		
Central Region						
Madhya Pradesh	Bhopal	34	92	34(72)		
Chhattisgarh	Pune	01	01	-		01(01)
Total		35	93	34(72)		01(01)
Western Region						
Gujarat	Ahmadabad	03	11	02(02)	01(05)	
Maharashtra	Pune	18	23	17(22)		01(01)
Rajasthan	Jaipur	04	58	04(08)		
Goa	Pune	02	02		02(02)	
Total		27	94	23(32)	03(07)	01(01)

Eastern Region						
Odisha	Cuttack	25	26	25(18)*		
Bihar	Patna	35	150	35(76)		
West Bengal	Kolkata	42	101	32(53)	02(02)	08(10)
Jharkhand	CADRAD	01	10	-	01(10)	-
Total		103	287	92(147)	03(12)	08(10)
North Eastern Region						
Assam	Guwahati	23	35	23(33)		
Sikkim		01	02	01(02)		
Arunachal	Itanagar	02	02	02 (02)		
Nagaland	Kohima	05	05	05 (05)		
Mizoram	Aizwal	02	09		02(05)	
Manipur	Imphal	03	27	03(05)		
Tripura	Agartala	11	93	11(16)		
Total		47	166	45(63)	02(05)	
	Grand Total	472	3136	454 (1295)	08 (24)	10 (10)

Number of samples collected from FMD suspected outbreaks and diagnosed is given in parenthesis.More than one clinical material was collected from many cases/outbreaks of FMD

*Outbreaks diagnosed retrospect

Processing of field samples and Serotyping

A total of 3136 clinical materials were subjected to FMD virus serotype differentiating sandwich ELISA and Multiplex PCR. Preliminary screening of clinical materials using ELISA was carried out at Regional/ collaborating laboratories. After initial diagnosis, the tissue samples were forwarded to PDFMD, Mukteswar for confirmation and detailed characterization. FMDV serotypes could be identified in 1329 samples. Serotype O virus detected in maximum number of outbreak samples (1295), and serotypes A and Asia1 virus were detected in 24 and 10 samples, respectively. Virus isolation was done in BHK-21 cells, and RNA transfection was also used for virus revival from most difficult samples.

Regional Scenario

Southern Region

Tamilnadu: During the year under report, 48 FMD outbreaks were recorded in the state. FMDV serotype O was responsible for all the outbreaks. The disease was recorded in several districts of the state including Ariyalur (01), Chennai (01), Coimbatore(01), Cuddalore(02), Dharmapuri (02), Dindigul (03), Erode (03), Kancheepuram (04), Karur (02), Krishnagiri (02), Madurai (02), Nagapattinam (02), Namakkal (01), Perambalur (01), Pudukkottai (01), Ramanathapuram (01), Salem (01), Sivaganga(01), Thanjavur (01), The Nilgiris (01), Theni (01), Thiruvallur (02), Thoothukkudi (01), Tiruchirappalli (01), Tirunelveli (01), Tiruppur (01), Tiruvannamalai (01), Vellore (01) and Viluppuram (01). Besides, an outbreak was also recorded in Pondicherry

Investigation of FMD outbreaks in Tamilnadu (7-8 November 2013)

The investigation of FMD in Tamilnadu revealed that the occurrence of the disease was first noticed in Kidarankondan village of the Nagapattinam District. Extended monsoon in the state predisposed the animals to secondary bacterial infections like HS, causing mortality at few places. There was no incidence of FMD in the organized livestock farms of the state where animals have been regularly vaccinated against

FMD. There was no incidence of FMD in the livestock of the District of Kanyakumari, which has been covered under FMD-CP since 2003-04 and where 14 phases of vaccination against FMD have been completed, that is indicative of protection due to vaccination.

The disease was due to serotype O FMD virus. After visit to the affected area, discussion was held with the Director, AH & VS and he was informed that the situation is under control, and immediate area sanitization/ disinfection is required to prevent spread. The following observations/ suggestions were made after visit to the affected 6 villages (Anaimelnagaram, Arayapuram, Kshetrabalapuram, Villiyanallur, Kuthalam and Therazendur) of the Kuthalam block of Nagapattinam district.

- Occurrence of FMD was first observed sporadically in the Nagapattinam district with low virulence and speedy recovery. Mostly high yielding, cross bred animals were affected with about 50 –75% loss in milk production and there was death of about 200 animals out of 130000 cattle and buffaloes in the Mayiladuthurai Division. The death in animals appeared to be due to HS and bacterial toxemia.
- There was about 20% vaccination coverage against FMD in the affected villages visited by the team with the local Veterinary Officials. Many animals that were left out of vaccination due to pregnancy picked up the infection, as reported by one farmer whose neighbor's vaccinated animals in the village Anaimelnagaram were not affected. Other farmers expressed ignorance about FMD vaccination. There was no door to door vaccination system and only animals gathered at a common place in the village(s) were vaccinated, as reported.
- The affected animals had mild lesions under healing.
- There was no vaccination against HS leading secondary infection after FMD resulting in mortality at few places.
- There was poor herd immunity against FMD virus with higher percentage of DIVA reactors.
- There were about 50 unauthorized quacks operating in the area and played a role in the transmission of the FMD virus to nearby homes and villages.
- People from other agencies (Vaccine manufacturers etc) were also visiting affected households /farmers, without the knowledge of the local veterinary officers and collecting biological samples, and in the process, transmitted/transmitting the virus through men and materials to nearby areas/ households.
- Many cross bred animals tied by the road side in the villages visited were not affected in spite of lack of vaccination. Therefore, in the current scenario, virus transmission within and between villages/ households has been effected by movement of men and materials, and it need to be controlled. The competent authority agreed that all animal husbandry and veterinary service activities should be in the knowledge and with the permission of VAS/AD of the village/ area.
- The local veterinary officers were advised to disinfect the affected animal housing areas with 4% NaOH/ Na2CO3 in water, and spray dry bleaching powder on the roads/ in the affected villages to control the spread of FMD virus.

The state veterinary officials were advised as below

1. All the animals need to be vaccinated against FMD including pregnant animals till third semester of pregnancy and calves above 4 months of age.
2. The animals to be vaccinated against HS, 2-3 months before the onset of monsoon regularly.
3. There should be no vaccination against FMD or HS in the affected herds right now till the current episode is completely subsided. Vaccination need to be started from the unaffected areas.
4. The vaccination programme against FMD in the state need to be intensified to increase the herd immunity to 80% with DIVA reactors below 10%.
5. All the animals of the organized farms/elite

germplasm to be vaccinated at 4 months interval with sero monitoring of each animal followed by revaccination of non / low responders.

6. The animals once affected to be treated with mild antibiotics (Streptopenincilin/ oxytetracycline) and supportive therapy of minerals and vitamins to check secondary infection and to boost immunity. The animal houses to be thoroughly disinfected with 4% Na OH / Na2CO3 for 10 days to prevent virus spread.

Karnataka: During the year, 116 outbreaks were reported in the state. Serotype O caused all the outbreaks. The disease spread through entire state and recorded in the districts of Bagalkot (03), Bangalore Rural (08), Bangalore Urban (07), Belgaum (05), Bellary (01), Bidar (02), Bijapur (02), Chamarajanagar (03), Chikamagalur (05), Chikkaballapur (09), Chitradurga (04), Dakshina Kannada (03), Davanagere (05), Dharwad (03), Gadag (02), Gulbarga (01), Hassan (03), Haveri (02), Kolar (06), Koppal (04), Mandya (06), Mysore (07), Raichur (01), Ramanagara (07), Shimoga (04), Tumakuru (08), Udupi (03), Uttara Kannada (03)

Investigation of FMD outbreaks in Karnataka (14-16 October 2013)

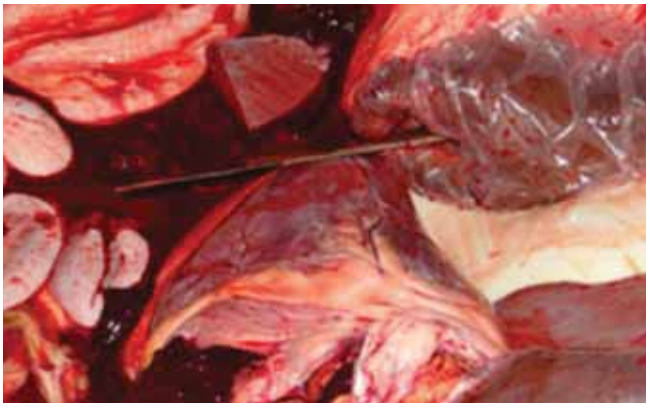
Kolar district

The district of Kolar suffered the most among other districts and complicated by Hemorrhagic Septicemia (HS) leading to mortality of Cattle. The district had very poor herd immunity at 20% in the IV Phase of FMD-CP, and this indicated inadequate vaccination coverage. On the day (14-10-13) of visit to Rajakallalli village, one young cow died and post mortem was conducted. Externally the animal (carcass) had typical FMD lesions in mouth and hoof and also had sub-mandibular edema (as in HS).

The local veterinarians informed that the animal had FMD signs and treated with sulphadimidine from day 1 and subsequently with other antibiotics and also with steroids. The animal recovered and died instantly afterwards.

Post mortem findings

Massive accumulation of peritoneal fluid (>4 liters) and Jelly like fluid under mandibular was found as in microbial toxicity. Hemorrhagic lesions were observed in the base of the heart.. Death of the animal appeared to be due to toxemia caused by Bacillus cereus or due to Hemorrhagic septicemia by Pasteurella multocida following FMD. Over medication also caused the problem, probably arising due to liver dysfunction.



Massive fluid in peritoneal Cavity

Massive fluid in peritoneal Cavity

Meeting with all Deputy Directors of AH department of Karnataka and Dr RN S. Gowda, Chairman of the state Committee on 15/10/13

- Nature of FMD virus and its genetic and antigenic makeup were briefly presented by PDFMD.
- Fourth round of vaccination under FMDCP was carried out in January-February 2013. FMD outbreaks occurred in some pockets during

July-August 2013, during the period the level of protective antibody titre is expected to come down.

- Fifth round vaccination was carried out during September 2013, mostly in the face of outbreaks, which might have resulted in virus spread through mechanical transmission by men and material
- The state received heavy and prolonged rainfall during and after regular monsoon that is very conducive for HS. In many parts of the state, HS vaccination was not conducted.
- FMD affected animals were predisposed to secondary bacterial infections and mortality increased with disease spread. From some of the dead animals, Pasteurella mutocida has been isolates by IAH& B, Bengaluru
- In some districts after HS vaacination, death incidence came down. In many cases though clinical signs of FMD was found to be severe, mortality was not observed as such animals were earlier vaccinated for HS.

Bangalore rural, Taluk: Hoskote

1. **Village:** Muthsandra (Visited on 16-10-13) The village has about 300 cattle and during 5th round of vaccination under FMD-CP >90% of the animals were covered in September 2013. Vaccination in that village was carried out under the supervision of the local veterinarian (Dr. Suresh). Animals in the village were also vaccinated with HS before monsoon.

Six animals (HF cross) of Shri Mahadevayya which were last vaccinated during February 2013, but not vaccinated during September due to unwillingness of the owner developed FMD (both mouth and feet lesions) in the first week of October 2013. No mortality was observed and the animals were recovering under treatment. One 15 days old calf in his herd did not develop FMD, probably due to maternal immunity.

Farmer Shri Mahadevayya

Farmer Shri Ramesh

About 100 feet away from Shri Mahadevayya's herd, sixty HF cross breed cows including 25 milching animals of Shri Ramesh had received FMD vaccination in September 2013 and therefore were found protected from FMD indicating vaccination derived immune protection. With known ability of the FMD virus to cross even continents in a short time frame, it is again proved from the example at the village Muthsandra

that proper and timely vaccination prevents clinical disease even in the presence of diseased animal in close vicinity.

Herd of Shri Mahadevayya (HF cross cows on the left side of the road are suffering from FMD)

Herd of Shri Ramesh (No FMD, across the road)

Village Thathanur (Visited on 16-10-13)

Shri Shantharaj has 18 cattle (HF cross), and about 100 feet away from the piggery of his brother Shri Srinivasayya. The pigs had FMD in early September 2013 and mortality was continuing even on the day of the visit. The pigs were not vaccinated against FMD, and some of the animals were procured from nearby market. Subsequently, the virus spread and eight of the eighteen cattle of Shri Shantharaj developed FMD during late September. Pigs are highly susceptible to FMD and releases huge amount of FMD virus through aerosol, and this exactly happened here. The Hoskotte taluk is having about 1 lakh pigs and rearing of pig and cattle together increased the risk of FMD.

Shri Srinivasayya's Piggery (Dead piglets)

Shri Shantharaj's HF cross cow
(Recovering from FMD) with 80% rop in dairy milk production (from 30L/day to 6L/day)

Village N Hosahalli (Visited on 16-10-13)

FMD occurred during April 2013 and cause of the disease was traced to introduction of new animals (without history of vaccination) which were brought from nearby Chintamani market. FMD occurred in the other side of the village during September 2013. There was death of one calf and a cow.

FMD investigation at Hosahalli

HF cross cow having mouth lesions

Village Muthukatahalli (Visited on 16-10-13)

Poor vaccination coverage (32%) was recorded due to reluctance of the farmers to vaccinate the animals. In the village FMD was recorded only in the unvaccinated animals. Ms Gowdamma had five HF cross cow which were not vaccinated in September and the animals were still struggling to recover from FMD. All the 3 high yielding HF cross cows of Shri Govindappa which were not vaccinated due to pregnancy (objected by owner) were severely down with FMD. Shri Muniraj had 8 HF cross cows, vaccinated during September and he had moved the animals out of the village, and they remained without FMD. Ms Panchamma had 3 HF cross cow; 2 were vaccinated and one was not vaccinated. Only the unvaccinated pregnant animal was severely affected with FMD and the two vaccinated cows were free from the disease in spite of very close proximity. This again showed the usefulness of regular and timely FMD vaccination.

Shri Govindappa's herd

Ms Gowdamma's herd

Summary and conclusions:

- Four meetings were conducted and five villages were visited in Bengaluru Rural and Kolar districts with the help of local officers between 14 and16 October 2013.
- FMD had affected un-vaccinated cattle (mostly HF cross cows and heifers) and Pigs (those which were not vaccinated in FMDCP Phase 5). A few cases in buffaloe were also noticed.
- Vaccination driven protection was clearly observed in HF cross breed cows in close vicinity of clinically sick animals.
- Laboratory diagnosis has revealed that the current of FMD in Karnataka is due to serotype O virus of lineage Ind2001, and all the outbreak strains characterized are homogeneous among them and are strongly related to the current serotype O vaccine virus at all the critical antigenic sites.
- There is no divergent serotype O virus strain in the current episode of FMD. The strain associated with disease outbreaks in Karnataka presently has been in circulation in India since 2001
- HS vaccination was not conducted in many districts before the onset of monsoon. Current prolonged monsoon flared up HS subsequent to FMD. FMD predisposes to HS as the animals were vaccinated against the later.
- Absence of HS vaccination before monsoon has aggravated the situation resulting in mortality of

animals due to HS following FMD. The causative agent, Pateurella multocida has been isolated from dead animals. Post mortem lesions also suggested microbial toxemia (Bacillus cereus).

- Vaccination (both FMD and HS) during the face of the outbreaks lead to quick spread of the virus by men and material.
- In spite of occurrence of FMD in the villages of Bengaluru Rural districts, the organized Central and state government farms in Hasserghatta having elite cows, bulls and pigs have remained free from the disease. This establishment has been following routine bi-annual FMD vaccination and seromonitoring (supported by FMD regional center, Bengaluru) since last occurrence of FMD there in 2008. This is an example that needs to be repeated elsewhere.
- On field visit to some affected villages, it was once again assured/confirmed that timely and regular vaccination with FMD can only give protection from FMD. The details mentioned later

Meeting with Director and Deputy Directors of CFSP & TI, Pig breeding center, Central Cattle Breeding Farm, Nandini Sperm Station and State Semen Bank, Hasserghatta

The CFSP&TI farm of Hasserghatta had an FMD outbreak due to serotype A in the year 2008. Since then no FMD has been recorded there. The farm animals in the elite herds receive two doses of FMD vaccine at six-month interval regularly and remain insulated/protected from FMD even in the currently ongoing wide spread FMD outbreaks in surrounding villages of Bengaluru rural.

Following points were advised.

- Strict biosecurity measures need to be followed as the disease is in surrounding villages.
- Bio-risk officer need to be appointed to monitor movement (animal and human) activities in the area and the farms.
- Profile of the employees and animal handlers working in the farms to be maintained.
- Changeover of employees coming from nearby villages before entering in to the farm.
- Large/wider disinfection pit for vehicle to pass through and decontaminate the wheels.
- Quarterly vaccination of animals as they are very valuable
- Periodic (every 3 months) DIVA testing of animals in the herd with the help of regional FMD center, Bengaluru.

Andhra Pradesh: With the launch of FMD CP, the state remained relatively free of FMD. During 2012-13, only a single case of FMD was recorded due to serotype O. This year a total of fourteen outbreaks were reported in the state. The outbreaks were recorded in the districts of Chittoor (02), Karimnagar (01), Hyderabad (01), Rangareddy (01), Kurnool district (01), Krishna District (01), Vizianagaram (01), Kurnool (02), Ananthapur (01), Tirupathi (01), Kadapa (01) and Srikakulam (01). All the outbreaks were caused by serotype O FMDV.

Investigation of FMD outbreaks in Andhra Pradesh (29-30 October 2013)

The investigation of FMD in Andhra Pradesh revealed that the occurrence of FMD was first observed in the wild life of S. V. Zoological Park, Tirupati in the last week of August, then in the bulls of FSBS, Karimnagar and Nilgais of Nehru Zoological Park, Hyderabad. Subsequently the disease was noticed in the other parts of the state involving domestic livestock.

The following observations were made after visit to the affected zoo in Hyderabad, FSBS, Karimnagar, Bollaram village, Malkagiri Mandal in Rangareddy District. The occurrence of FMD was observed sporadically in 6 out of 23 districts with low virulence and speedy recovery. Mostly high yielding, cross bred animals were affected with about 50 % – 75% loss in milk production without mortality, but it affected the livelihood of the affected farmers.

Outbreak investigation in Nehru Zoological Park, Hyderabad:

- The zoological park of Hyderabad is situated on the side of the National highway covering 380 acres of area.
- The enclosures of the antelopes are near the main gate entrance adjacent to the high way. It was noticed that unauthorized and temporary livestock markets are running adjacent to the compound wall of the park near the entrance along the national highway which pose risk of infection for the antelopes kept near the vicinity.
- At present the disease has been subsided with all animals apparently healthy. The following advisory was suggested to the officials of the zoo for better management and control of FMD in susceptible animals.

Advisory

1. One Bio-risk officer with veterinary science background may be in place in the zoo to implement and monitor the biosecurity measures strictly for control of infectious and contagious diseases.
2. FMD susceptible animal (like Antelopes, Elephant and Giraffe etc.) if found sick, should be segregated/ quarantined immediately irrespective of diagnosis in an enclosure far from healthy susceptible animals. These animals should be seromonitored against FMD.
3. The newly introduced FMD susceptible animals should be vaccinated against FMD during quarantine and serum samples to be collected before and 30 days after vaccination for investigation.
4. Necessary information should be provided well in advance before introduction of any FMD susceptible animal from abroad (especially Africa and Middle-East) to the AH department of Govt. of AP and PDFMD, Mukteswar to eliminate the threat of introduction of exotic strains of FMD virus serotype (Southern African Territories (SAT) 1, 2 and 3).
5. The premises having susceptible animals should be thoroughly disinfected with 4% sodium hydroxide/sodium carbonate; the fodders to be sprayed with 2% sodium carbonate if outsourced from villages around, drinking water should be treated with 2% sodium bicarbonate (once in 15 days as a matter of routine).
6. Dip tank with 4% sodium hydroxide sufficient to dip the wheel of tractors should be in place at the entrance to the zoo area (with minimum 15 ft).
7. Separate animal handlers/attendants should be engaged for each species of FMD susceptible animals. The attendants may be provided with separate uniform, footwear, gloves, face mask and caps, before they enter animal enclosures. The profile of each animal attendant should be recorded and they should be advised not to maintain FMD susceptible livestock and should be discouraged to visit any livestock market and premises. Residents inside the zoo should be debarred from rearing any FMD susceptible livestock inside the zoo premises.
8. Wherever possible, animal (may be elephants) should be vaccinated against FMD twice in a year and seromonitored to keep them free from FMD.
9. Administrative measures may be initiated to ban the temporary and unauthorized livestock market on the road side adjacent to the park to reduce the risk of transmission of FMD to captive animals of the zoological park.

Outbreak investigation in Rangareddy district:

Taluk: Malkagiri, Village: Bollaram

One farmer had 25 HF and Jersey cross bred animals. The animals were vaccinated against FMD in April 2013. The animals are located near the main road with free movement of animals for trade. One pregnant animal without any history of vaccination against FMD was introduced from the local market. FMD sings was noticed in 8 animals on 3rd September 2013. FMD was confirmed and was due to serotype O. The animals in the farm were vaccinated against FMD on 21st September 2013 to prevent further spread. Overall the milk yield was reduced from 80 liters per

day before the disease to 20 liters resulting in the huge economic loss to the farmer. One lactating animal became completely dry.

Outbreak investigation in Frozen Semen Bull Station (FSBS), Karimnagar:

The FSBS maintain 57 Bulls, and 11 Bulls (8 Murrah and 3 Cross bred Jersey) were down with FMD on 25 August 2013 and the disease lasted till 2 September 2013. On visit, all animals had recovered. The animals were vaccinated against FMD on 6 July 2013. But the seroconversion for protective antibody was not satisfactory with low herd immunity. The source of FMD virus for FSBS was possibly being the adjacent private dairy where the animals had FMD prior to disease in FSBS. This private dairy is also engaged in procuring and selling animals routinely without any routine vaccination against FMD. The contractual animal workers of FSBS were from the nearby villages having FMD. FMDV was transmitted to FSBS probably through men and materials.

Following advisory was suggested to the farm management

1. The profile of animal handlers/attendants needs to be maintained in all the organized bull farms and they should be discouraged to rear FMD susceptible animals in their houses.
2. In order to maintain FMD free status in future, biosecurity in the station has to be strengthened along with vaccination against FMD. The workers may be provided with working uniforms, shoes, masks and caps. They should wash their hands with soap and nostrils before entering the sheds.
3. The animals are to be vaccinated at 4 months interval against FMD with regular seromonitoring by the Hyderabad FMD center. The low responders have to be revaccinated with double the dose of the vaccine.
4. The animals are to be regularly sero monitored (before and 21 days after vaccination) for antibodies against structural and non structural proteins of FMD virus.
5. Semen of affected and in contact apparently healthly bulls to be tested for virus shedding every month till the last bull is FMD free. Semen free from FMD virus can be used. Semen samples may be sent to PDFMD, Mukteswar for testing.

Discussion with the Director, Dept. of AH, Govt. of AP

The meeting was held to take stock of the FMD incidences in Andhra Pradesh, and the following points were discussed.

1. There were sporadic incidences of FMD involving organized farms and livestock of 6 districts of Andhra Pradesh though there was no outbreak of FMD in Andhra Pradesh since last four years.
2. The FMD in the state livestock was of low morbidity and mortality due to the effect of previous vaccination under FMDCP.
3. The strain associated with disease outbreaks presently has been in circulation in India since 2001
4. The animals were not vaccinated against HS
5. Some farmers did not allow their animals to be vaccinated due to one or the other reason. Pregnant animals and calves were not vaccinated which pick up the infection very fast.
6. There were incidences of FMD in the neighboring states of Karnataka and Tamilnadu with frequent inflow of animals to Andhra Pradesh which might have triggered the disease incidence in the state. Heavy torrential rain/prolonged monsoon might also have aggravated the incidences of FMD.
7. The economic losses due to FMD are high in high input and high output livestock herds.

Following advisory was issued.

1. Low seroconversion for protective antibodies after vaccination with higher percentage of DIVA reactors (30-55%) in the affected districts need to be addressed with intensification of vaccination and regular seromonitoring.

2. All the cattle and buffaloes above four months of age need to be vaccinated 2 times in a year (once in six month) against FMD including pregnant animals till third semester of pregnancy and calves above 4 months of age, using single needle for each animal.
3. Vaccination against HS needs to be carried out in the state 2 months before the onset of monsoon.
4. The extension programme may be strengthened to apprise the livestock owners of the benefit of vaccination and control of FMD.
5. The vaccination against FMD in the state to be intensified with regular seromonitoring with an emphasis to increase the herd immunity above 80% with DIVA reactors below 10%.
6. All the animals of the government organized farms/elite herds/bull stations to be vaccinated at 4 months interval with sero-monitoring of each animal followed by revaccination of non responders. Biosecurity measures need to be strengthened at these farms.
7. The animals once found sick/affected to be segregated immediately and treated with mild antibiotics (Streptopenincilin/ oxytetracycline) along with supportive therapy of minerals, calcium, glucose infusion and multi vitamins to check secondary infection and to boost rapid recovery. Boroglycerin (5%) need to be applied in the oral lesions and/or Ragi flour mixed with honey as a bolus in the affected animals. The hoof / feet lesions to be washed/cleaned with soap water/ KMnO4/ Dettol dried with cotton and applied with antibiotic ointments/Himax/ Neem Oil etc as fly repellants.
8. The animal houses, drains, dunk pits etc to be thoroughly disinfected with 4% NaOH, fodders and drinking water to be treated with 2% Na2 CO3. Bleaching powder to be sprinkled around the animal houses and at the entrance. Strict biosecurity measures to be maintained.
9. Sick animals to be fed with the rice, wheat and ragi MIX (1:1:1) broken to small pieces and boiled in water and supplemented with mineral mixture, vitamins, concentrate, molasses/ honey twice a day to maintain the nutrient requirement of the animal and fast recovery.
10. The affected/ infected animals can shed virus in milk and can transmit the infection to the suckling calves. The infected milk can transmit the FMD virus through men and materials, so the milk may be boiled before use.

Kerala: A total of 50 outbreaks were recorded in the state. The outbreaks were caused by serotype O and were recorded in the districts of Thiruvananthapuram (04), Kollam (07), Alappuzha (03), Pathanamthitta (02), Kottayam (01), Idukki (04), Ernakulam (01), Thrissur (05), Palakkad (04), Malappuram (04), Kozhikkode (05), Wayanad (05), Kannur (02) and Kasaragod (03).

Investigation of FMD outbreaks in Kerala (9-12 October 2013)

The investigation of FMD in Kerala revealed that the introduction of animals from neighboring states might have introduced FMD in the state. The scheduled vaccination against FMD was delayed by two months and several cattle and buffaloes were not vaccinated due to different reasons. Disease was observed in unvaccinated cattle and pigs. Severity of the disease was low. The animals were not vaccinated against Haemorrhagic Septicaemia under routine vaccination programme leading to the death of few FMD affected adult animals due to HS as a secondary infection.

The following observations were made after visit to the affected villages in Allapuzha, Kottayam and Kollam districts of Kerala.

- FMD started in the last week of July 2013, further spread in August 2013 and was continued sporadically.
- All the 14 districts of Kerala are covered under FMD Control Programme.The previous vaccination was carried out in January 2013, followed by the next phase of vaccination during August and September 2013, leading to the delay

in the vaccination of more than two months and making the animals susceptible to infection and disease due to large scale movement of partially vaccinated animals from neighboring states.

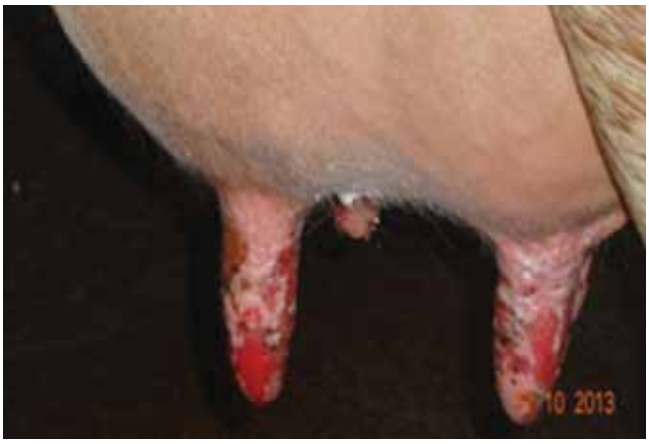
- In some villages the owners refused vaccination with the misconception of reduction in milk production, and termination of pregnancy.
- Most of the affected animals attended by the team were found unvaccinated. The lesions were observed in oral cavity, feet and teats.
- Out of 14 districts, 12 were affected with FMD with the attack rate of 0.125 % (1800 Affected / 15 lakhs population) and mortality of 5.25% in the affected animals. Cattle, buffalo, goats and pigs were affected simultaneously.
- 95% of the livestock affected were high yielding and highly susceptible cross bred animals. In some cases, there were reared in mix farming system along with pigs and goats.
- The animals were not vaccinated against Hemorrhagic Septicemia (Pasteurelosis) as a practice. Two dead animals were confirmed of HS, so it was suggested to vaccinate the animals against HS regularly as per the schedule.
- There was unrestricted influx of animals from Karnataka and Tamilnadu where there was occurrence of FMD.
- Severity of the disease was less in large and small ruminants but it was severe in unvaccinated pigs. The outbreaks resulted in more than 70% loss in milk production affecting the livelihood of the farmers.
- Mastitis and Pasteurelosis were found to be the major secondary complications.
- The organized farms following regular vaccination schedule were not affected with FMD.
- The post vaccinated animals of the organized farms showed optimum sero conversion but it was poor in the field condition indicating lapses in the vaccination coverage.
- The cases of FMD in Kerala were caused by Serotype O FMD virus of lineage 2001 that was also circulating in Karnataka. The virus of Kerala and Karnataka are genetically similar

Symptoms and lesions of FMD in cattle and pigs in Kerala

Salivation in the affected cattle

FMD lesions in the hoof

Hoof lesion in a Cow



Hoof lesion in a Cow

FMD lesions in the snout of affected pig

Severe FMD lesions in the legs of affected pig

5.2 Northern Region

Haryana: There was no incidence of FMD in the state during 2012-13. However two sporadic incidences were recorded in the district Rewari in the month of February 2014. The district share border with Rajasthan, and virus might have been introduced by animal movement. The virus could not spread further owing to high level of surrounding herd immunity and application of effective biosecurity measures.

Punjab: The state remained free of FMD during the period. Last incidence was recorded in the state during 2010-11.

Himachal Pradesh: Four incidences of FMD due to serotype O was recorded in the state, one each in the months of May, June, November and February. The cases were recorded in Solan, Chamba, Shimla and Mandi districts in cattle and buffaloes. There was no incidence of FMD in the state during 2007-09. During 2009-13, only seven sporadic incidences were recorded.

Uttar Pradesh: Twelve outbreaks were confirmed in the state. The incidences were recorded in Agra (01), Buland Sahar (01), Mainpuri (01), Mujaffarnagar (02), Bareilly (02), Hapur (01), Bijnor (01), Moradabad (01), Varanasi (01) and Saharanpur (01) districts. Serotype O was responsible for these incidences. The incidences were recorded in the months of June (01), September (01), November (02), December (06) and March (02)

Jammu and Kashmir: Five incidences of FMD owing to serotype O was recorded in the state. The cases were detected in the districts of Leh (02), Pulwama (01), Budgam (01) and Srinagar (01). Two incidences were recorded in the month of June, and one each in the months of July, September and November. Serotypes A and Asia1 have not been detected in the state during last six years (2006-2012).

Uttarakhand: During 2013-14 nine FMD outbreaks due to serotype O was recorded in the state. These incidences were recorded in the districts of Almora, Nainital, Udham Singh Nagar and Haridwar. These outbreaks were recorded in the months of September, October (2), November (01), December (04) and January (01).

5.3 Central Region

Madhya Pradesh: During this period, thirty four FMD outbreaks/cases were recorded in the state.

Disease was recorded in the districts of Shivpuri (05), Chhaterpur (01), Hoshangabad (02), Khargaon (02), Seoni (05), Sagar (04), Bhopal (03), Betul (05), Sehore (01), Hoshangabad (01), Mandla (01), Chhindwara (02), Raisen (01) and Narsinghpur (01). The outbreaks were recorded in the months of March (09), June (01), July (01), August (01), September (03), October (04), November (07), December (04), January (02) and February (02). Serotype O accounted for all the outbreaks.

5.4 Western Region

Maharashtra: During the year, 07 outbreaks/ cases of FMD were recorded in the state and all the outbreaks were caused by serotype O. The disease was recorded in the districts of Solapur, Kolhapur, Satara and Osmanabad. Five outbreaks were recorded in the month of October and one each in November and December. Serotype Asia1 which has been regularly prevailing in the state could not be detected during the current period.

Gujarat: During the year, only 3 outbreaks/cases of FMD were recorded in the state in the months of November, December and January. Two outbreaks were caused by serotype O and one was due to serotype A. The serotype O outbreaks were recorded in Navsari and Rajkot districts, and serotype A incidence was reported from Surat.

Rajasthan: During the year, 04 outbreaks of FMD were recorded. Outbreaks were recorded in the months of November, December, January and March. Serotype O was responsible for all outbreaks. The disease was recorded in the districts of Jhunjhunu, Alwar, Udaipur and Churu.

5.5 Eastern Region

Odisha: Twenty Five outbreaks/cases were recorded in the state. All the outbreaks were caused by Serotype O. Maximum outbreaks were recorded in the district Cuttack (07) followed by 5 in Khurda, 3 each in Koraput and Ganjam, 2 in Kendrapara and 1 each in Balesore, Puri, Nayagarh, Dhenkanal and Bhadrak districts. Outbreaks were recorded in the months of March (01), April (01), July (01), August (02), September (01), October (07), November (05), December (03), January (03) and February (01).

Bihar: During the period under report, 35 outbreaks/cases of FMD due to serotype O were recorded in the state. Outbreaks were observed in the months of March (01), October (06), November (09), December (13), January (03) and February (03). Highest number of outbreaks were recorded in Begusarai (09) followed by Samastipur (07), Patna (06), Champaran (03), Arwal (2), Shekhpura (01), Sahebpur Kamal (1), Nawada (1) and Munger (1). Serotypes A and Asia1 have not been recorded in the state for last five years.

West Bengal: Forty two FMD outbreaks/cases were recorded during the period in the state. Highest number of FMD outbreaks were in Bankura (08), Birbhum (06) followed by five in Paschim Medinipur. Rest of the outbreaks were recorded in Hooghly (04), Purulia (03), Burdwan (03), South 24 Parganas (03), Jalpaiguri (03), Nadia (02), Howrah (02), North 24 Parganas (01), Dakshin Dinajpur (01) and Murshidabad (01) districts. Serotypes O dominated the scenario with 31 outbreaks followed by serotype Asia 1 in 9 and serotype A in 2 incidences. Outbreaks occurred almost throughout the year in the months of March (1), April (1), May (2), July (2), August (1), September (2), October (2), November (16), December (9), January (4) and February (2).

5. 6 North Eastern Region

Assam: Twenty Four outbreaks/cases of FMD were recorded in Assam. Outbreaks were widespread and occurred in fourteen districts of the state including Kamrup (12), Karbi Anglong (4), Bongaigaon (3), Nalbari (2), Darrang (2) and Golaghat (1). Three outbreaks were diagnosed in retrospect. Serotype O accounted for all the outbreaks. Outbreaks were recorded during the months of March (01), May (01), June (02), September (01), October (03), November (03), December (03), January (07) and February (03).

Sikkim: During the year, one outbreak/case of FMD was recorded in the state in the month of

December 2013. The outbreak was caused by serotype O and recorded in Gangtok district.

Manipur: During the year, 3 outbreaks/cases of FMD due to serotype O were recorded. All three outbreaks were recorded in Imphal West district. The outbreaks occurred in the months of October (02) and November (01). Serotypes A and Asia1 are not detected since 2006-07.

Tripura: During the period under report, 11 outbreaks/cases of FMD due to serotype O were recorded in the state. Outbreaks were recorded in the months of July (01), August (02), November (2), December (2) and January (3). Outbreaks were recorded in Latoogaon, Kalashi, Betaga, Bhurachar, Lalilla, Chanipur, Govt. Cattle Farm, Khejurbagan, Lakhmilung, Kathalia and Rajnagar one in each district.

Mizoram: During the period under report three incidence/outbreaks of FMD was recorded in the state. All the three outbreaks were caused by serotype O. Outbreaks were recorded in the months of April, May and June. The outbreaks were recorded in the districts of Aizawl (02) and Serchhip (01).

6 Molecular typing of foot-and-mouth disease virus during 2013-14

6.1 Serotype O FMD Virus

FMDV serotype O is the most predominant serotype in India and cause around 80% of the outbreaks encountered in the country. Globally eleven topotypes namely Cathay, Middle East-/South Asia (ME-SA), South-East Asia (SEA), Europe-/South America (Euro-SA), Indonesia-1 (ISA-1), Indonesia-2 (ISA-2), East Africa (EA) 1-4 and West Africa have been described. Serotype O isolates from India belong to the Middle East-South Asia (ME-SA) topotype with less than 15% nucleotide divergence among them. Six genetic groups of the virus with more than 5% nucleotide divergence at 1D region designated as Branch A, B, C-I, C-II, C-III (Ind2001), C-IV(Pan Asia I) have been identified. Last outbreaks due to Branch A and B were recorded during 1994 and 2003, respectively. The Indian vaccine strain (INDR2/1975) belongs to the lineage Branch B. Pan Asia virus which caused worldwide pandemic in the year 2001 has been in circulation in the country since 1982. The 'Ind2001' lineage was first identified in 2001 as the major cause of type O outbreaks and since then this lineage has been causing sporadic outbreaks in the country. This lineage showed 5-11% nucleotide difference from Pan Asia viruses. Later, with in Pan Asia, a divergent strain (Pan Asia II) emerged in the year 2002. During 2006-07 to 2010-11, epidemiological scenario in serotype O has been largely influenced by Pan Asia and Ind2001 strains. A new genetic group in serotype O appeared in the year 2011 with 9.8 to 14.8% and 9.7 to 12.8% nucleotide divergence from contemporary viruses of Ind2001 and PanAsia lineages circulating in India, respectively. This new genetic cluster was named as Ind2011 lineage. This group of virus was not detected in subsequent years.

During 2013-14, a total of 146 serotype O field isolates were subjected to complete 1D/VP1 region sequence analysis. Neighbor-joining (NJ) tree was reconstructed using MEGA 5.05 software package. In the NJ tree, 145 of 146 isolates grouped within O/ME-SA/Ind2001 lineage indicating its complete dominance in the field. The lineage, which re-emerged in the year 2008, continued its supremacy in the field by displacing the then prevalent O/ME-SA/PanAsia lineage. Since its initial identification in the year 1997, the lineage has diversified globally in to at least four sub-lineages (Ind2001a, b, c and d) (Fig.1). The isolates of O/ME-SA/Ind2001 lineage currently circulating in the country groped precisely in sub-lineage Ind2001d. The sub-lineage Ind2001d also prevails in neighboring countries including Bangladesh, Bhutan and Nepal.

The sub-lineage Ind2001d was distributed widely covering many states including Karnataka, Kerala, Andhra Pradesh and Tamilnadu (Southern region); Uttar Pradesh, Uttarakhand and Jammu & Kashmir (Northern region); Gujarat and Maharashtra (Western region); Odisha, West Bengal and Bihar (Eastern region); Madhya Pradesh (Central region) and Assam and Manipur (North Eastern region) (Fig.2). One isolate, which is grouped within O/ME-SA/PanAsia lineage, was collected from the state of Odisha in an outbreak recorded during November 2013. The emerging Ind2011 lineage could not be detected in any of the outbreak during 2013-14, probably due to infection immunity or natural extinction. The isolates collected during 2013-14 differed from currently used vaccine strain INDR2/1975 by 11.3 to 13.7% at nucleotide level at 1D genomic region and 6.1 to 8.3% from prototypic Ind2001 lineage isolate collected during 2001. The genetic diversity of Ind2001 isolate collected during 2013-14 varied from 0.00 to 8.7% at VP1 region and mean genetic diversity was estimated at 3%, which indicate high genetic homology between the virus isolates.

Within sub-lineage Ind2001d, the genetic cluster responsible for extensive outbreaks in the southern region during 2013 was detected as early as in December 2012 from the state of Gujarat (Fig.4). The sub-lineage, which caused sporadic incidence in the first half of 2013 took upper hand since June 2013 coinciding with monsoon and caused many outbreaks. Besides southern peninsula, the sub-lineage was also detected in the state of Uttar Pradesh, Uttarakhand, Maharashtra, Odisha, Madhya Pradesh, Haryana, Bihar and far away in Assam and Manipur (Fig.3). This sub-lineage was the major cause serotype O outbreaks during 2013-14 and was highly homogenous at VP1 genomic region.

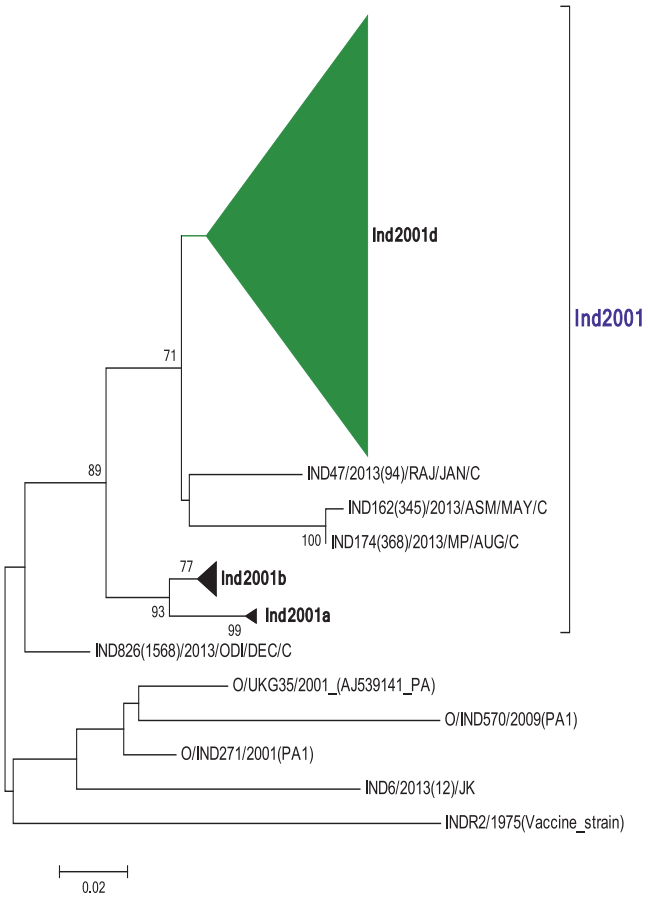


Fig. 1: Neighbor-Joining phylogenetic tree at VP1 coding region of Indian serotype O FMD virus isolates during 2013-14. The tree shows complete dominance O/ME-SA/Ind2001d sub-lineage in India during 2013-14.

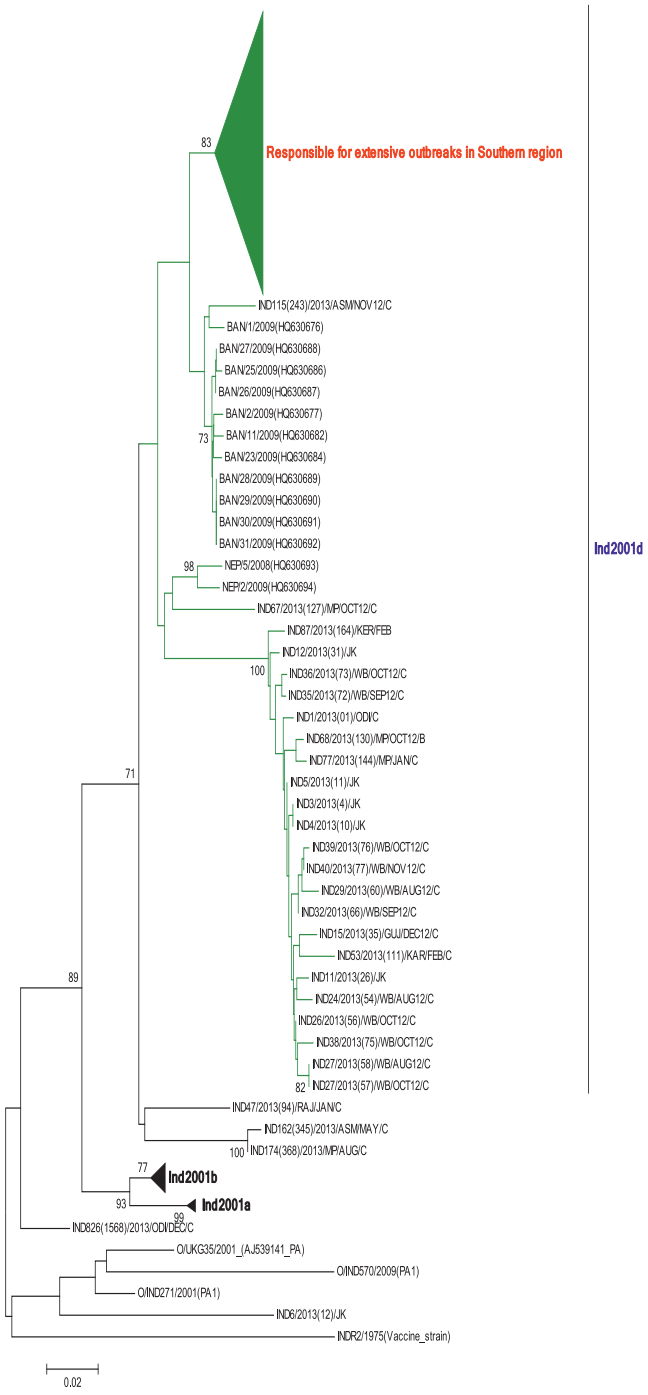


Fig. 2 : Neighbor-Joining phylogenetic tree (expanded) at VP1 coding region of O/ME-SA/Ind2001 lineage of Indian serotype O FMD virus isolates that were isolates during 2013-2014. The genetic data indicate dominance of Ind2001 lineage in major parts of the country. The lineage first emerged in the year 2001 and dominating serotype O outbreaks since 2009.

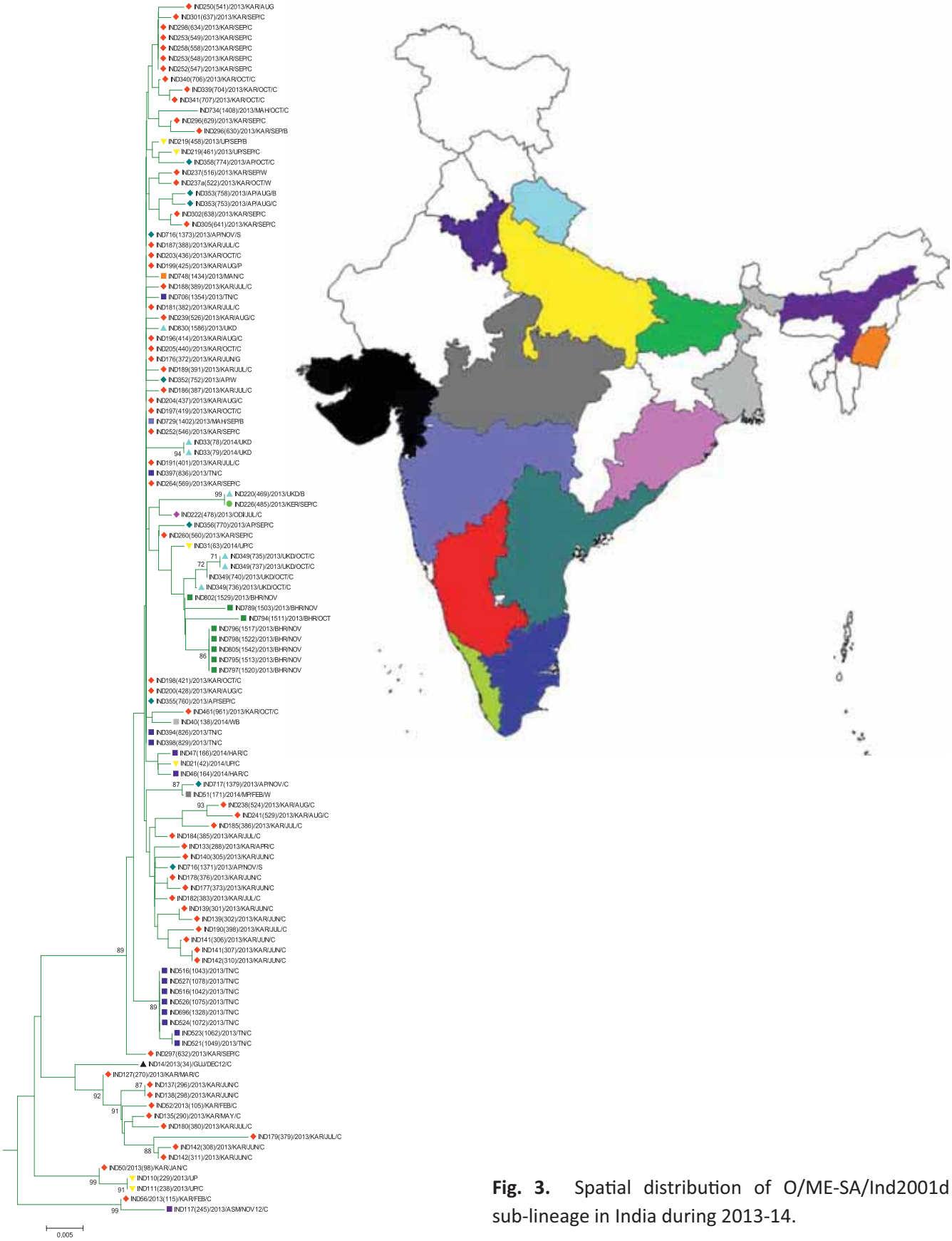


Fig. 3. Spatial distribution of O/ME-SA/Ind2001d sub-lineage in India during 2013-14.

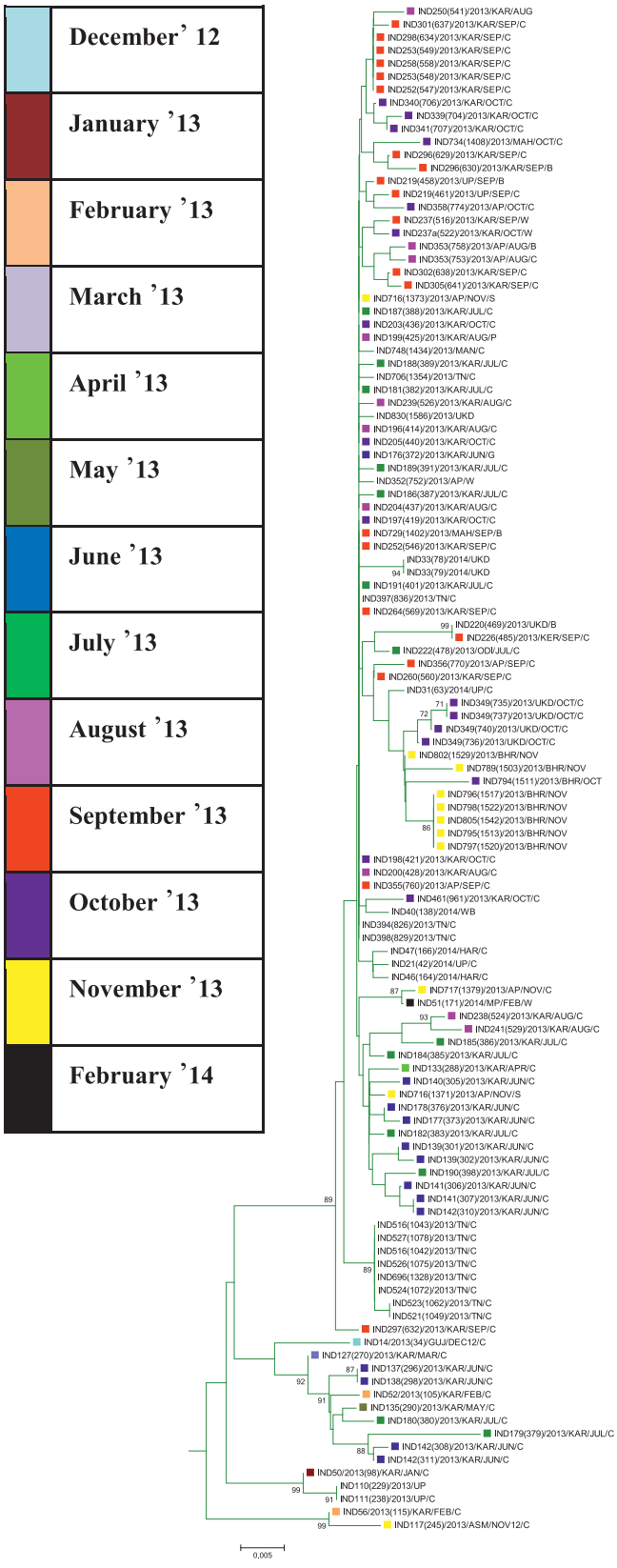


Fig. 4. Temporal distribution of O/ME-SA/Ind2001d sub-lineage in India during 2013-14.

6.2 Serotype A FMD Virus

Among the three serotypes prevalent in India, serotype A virus population is found to be genetically and antigenically most heterogeneous in nature. VP1 coding (1D) region based molecular phylogeny has established circulation of four genotypes {showing more than 15% nucleotide (nt) divergence among them at 1D region} of so far in India. Since 2001, genotype 18 has been exclusively responsible for all the field outbreaks and has out-competed all other genotypes. Within the currently circulating genotype 18, a divergent and unique lineage emerged in late part of 2002, which showed an amino acid (aa) deletion at 59th position of VP3 (VP359-deletion group) and dominated the field outbreak scenario currently. This single aa deletion is at an antigenically critical position in the structural protein VP3, which is considered to be a major evolutionary jump probably due to immune selection. During 2013-14, structural protein coding region (P1) sequence for 10 field viruses of serotype A recovered from field outbreaks in Jharkhand and Goa were sequenced for molecular epidemiological analysis. The determined 1D sequences were aligned with other Indian sequences available in the database of the Institute. The viruses from Jharkhand were found to cluster within clade 18c of the VP359-deletion lineage in the maximum likelihood tree, while the viruses from Goa clustered in a genetically divergent subcluster in the nondeletion group of genotype 18 (Fig.5). Therefore, it may be inferred that both deletion and nondeletion variants are cocirculating in the country.

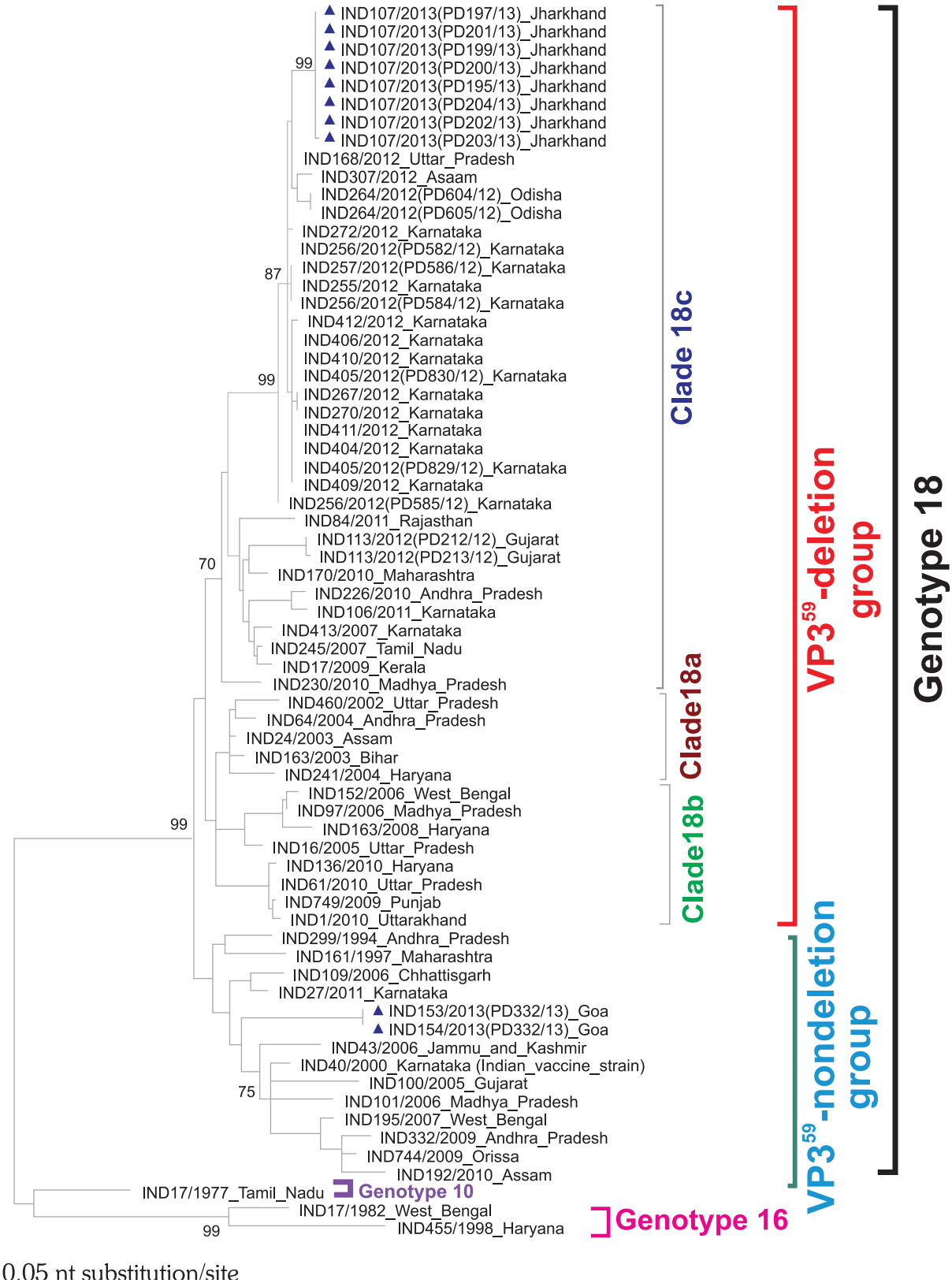


Fig.5 VP1 sequence based maximum likelihood tree depicting phylogenetic relationship among serotype A viruses. Viruses isolated during 2013-14 are marked with blue triangles.

6.3 Serotype Asia1 FMD Virus

Previous studies on 1D/VP1 gene based phylogeny demarcated Indian serotype Asia1 field isolates in to three major lineages namely B, C and D. Lineage B which include currently used serotype Asia1 vaccine strain, IND63/1972 was last recorded in the year 2000. The isolates of lineage D emerged late in 2001 and dominated the period between 2002 and 2004. The lineage C dominated the Asia1 field outbreak between 1998 and 2002, although disappeared between year 2001 and 2004, and re-emerged as the predominating lineage from 2005 onwards.

Outbreaks owing to serotype Asia1 were much less during 2013-14 compared to the previous year. During 2013-14, 10 serotype Asia 1 field isolates (2 isolates collected during 2013 and rest of the isolates sampled during 2012) were sequenced at 1D/VP1 region and subjected to phylogenetic analysis using Neighbor Joining algorithm implemented in MEGA 5.05 software package. All the isolates were found to cluster within lineage C indicating its supremacy in the field since the year 2005 (Fig.6). During 2013, serotype Asia1 isolates were obtained from the states of Maharashtra and Chhattisgarh in the months of May and June, and were found to cluster within lineage C indicating its supremacy in the field since the year 2005.

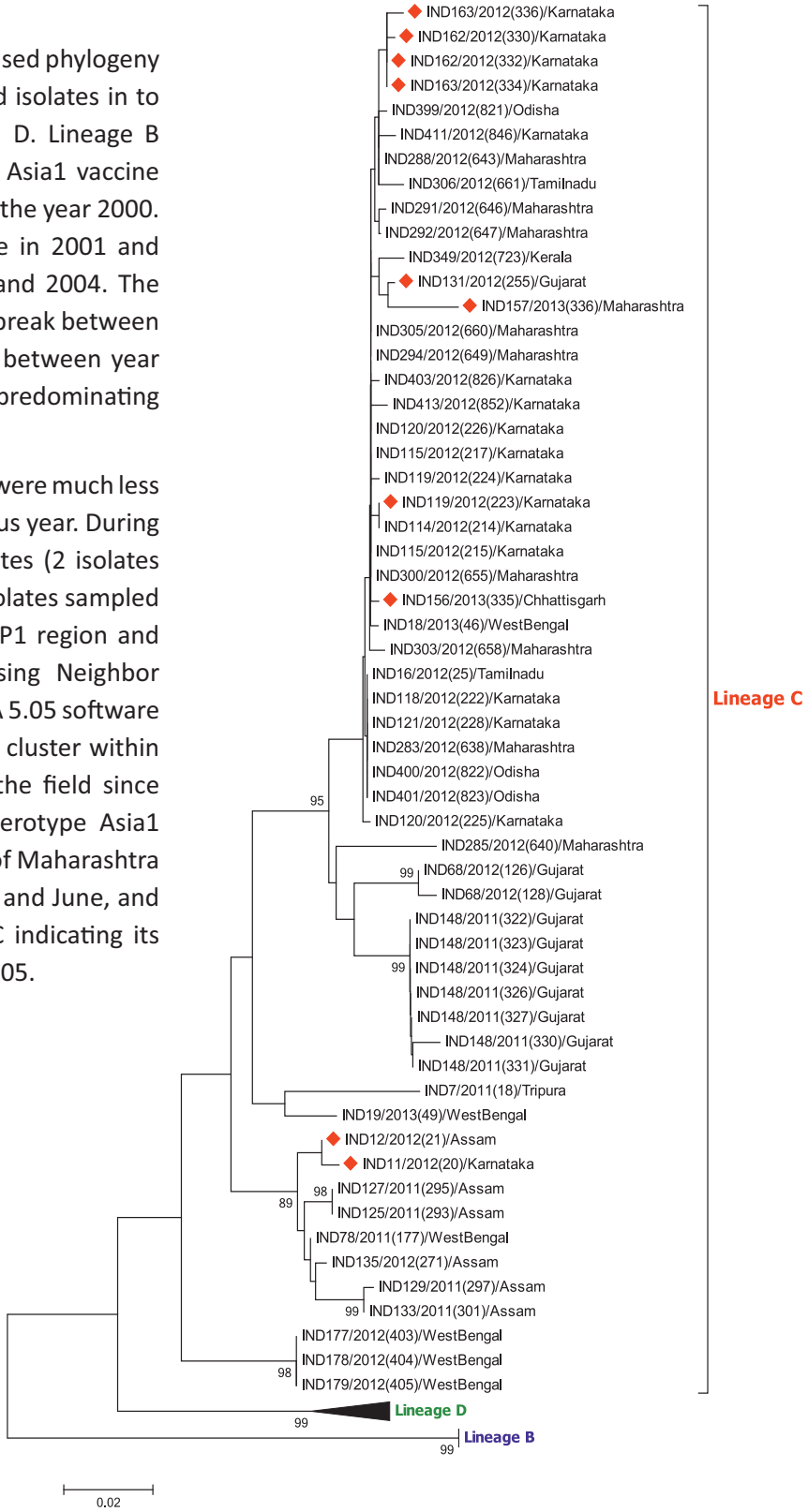


Fig. 6 Neigbhour-Joining phylogenetic tree at VP1 coding region of FMD virus isolates of serotype Asia1 during 2012-2013. Lineage C is in circulation in the country since 2005.

7 Vaccine matching of FMD virus field isolates

7.1 FMDV Serotype O

The antigenic relationships of serotype O field isolates to the currently used vaccine strain INDR2/1975 is shown in Fig.7. The test results were interpreted as per criteria set by Rweyemamu, (1984). A total of 43 isolates were subjected to vaccine matching exercise by 2D-MNT using bovine vaccinate serum during 2013-14. From the result, it can be seen that 80% of the isolates showed an r1 value of >0.3 with currently used vaccine strain INDR2/1975 and 20% had an r1 value of <0.3. Emergence of antigenic variant in an endemic country is a normal phenomenon and the currently used vaccine strain INDR2/1975 still is able to provide near optimal antigenic coverage to the field isolates. The situation is being monitored carefully to see whether the few variants emerged during 2013 epidemic will be able to persist in the future. Further four candidate vaccine strains (IND408/2007 and IND320/2007 (PanAsia II), IND271/2001 (PanAsia) and IND120/2002 (Ind2001) representing different lineages are being evaluated with the field isolates to find an alternate strain for use in case any emergency/ necessity.

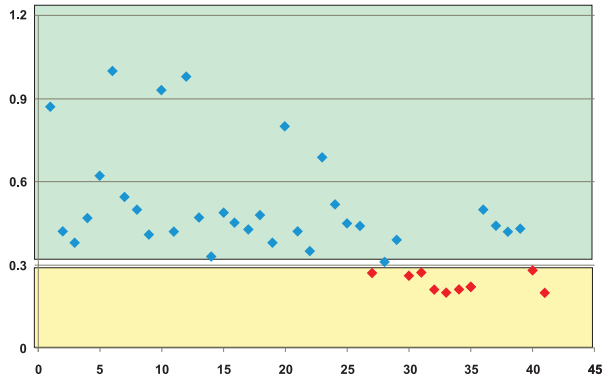


Fig.7 Antigenic relationship values FMD virus serotype O field isolate collected during 2013-2014 in relation to currently used vaccine strain INDR2/1975. Isolates data point filled with blue had r-value of >0.3.

7.2 FMDV Serotype A

A total number of 10 serotype A FMD viruses isolated from 2 field outbreaks in two states (Jharkhand and Goa) during 2013-2014 were subjected to 2D-VNT using bovine vaccinate serum against the vaccine strain, A IND 40/2000. The r1 values were < 0.3. This finding suggests recent emergence of an antigenically divergent group of viruses in the field. Hence in the current scenario, it is expected that the vaccine strain A IND 40/2000 may not offer optimum antigenic coverage at the face of natural outbreaks and warrants a search for a better vaccine strain with broader match potential. In spite of this divergence, there has been limited circulation of serotype A virus in the country.

Considering the emerging antigenic diversity, a panel of 8 candidate vaccine strains representing various lineages that have circulated in India in recent past were selected. Anti-146S hyperimmune sera were raised against the candidate strains in rabbits (three rabbits immunized per strain) and antigenic matching was conducted against 84 field isolates recovered since 2000 in 2D-virus neutralization test. The test was repeated two times and the mean titers were considered for estimation of r1 values. Based on the proportion of isolates showing r1 values > 0.3

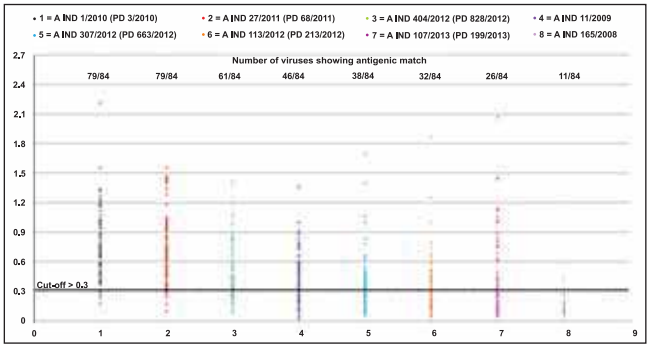


Fig. 8 Proportion of isolates showing r1 values > 0.3 with candidate vaccine strains

and giving emphasis to their antigenic relatedness with the currently existing lineages, three candidate strains could be shortlisted such as A IND 1/2010 Uttarakhand, A IND 404/2012_Karnataka and A IND 27/2011_Karnataka in that order of priority for raising bovine vaccinal serum (Fig.8).

7.3 FMDV Serotype Asia1

The antigenic relationship of two serotype Asia1 field isolates (IND156/2013 and IND157/2013) with the vaccine strain IND63/1972 was deduced during 2013-14. Both the isolates had an antigenic relationships of >0.3 with the vaccine strain. During 2012-13, 8 out of 32 isolates analysed had less antigenic match (<0.3) with currently used vaccine strain. Currently used serotype Asia1 vaccine strain, IND63/1972 has been in use for decades in the country. This vaccine strain belongs to lineage B that was in circulation only until 2000. The isolates collected during 2012-13 differed from vaccine strain by 15.1 to 18.9% at the nucleotide level and 9.6 to 12.1% at the amino acid level. Almost 25% of the isolates collected during 2012-13 had poor antigenic match with the currently used vaccine strain. A vaccine candidate panel [IND13/2001, IND78/2011 and IND68/2012] has been identified and evaluation is under progress.

8 New Research Output

8.1 Diagnostic potential of recombinant nonstructural protein 3B to detect antibodies induced by FMD in bovines

In India, the 'National FMD Control Programme' (FMDCP) is mainly implemented by enforcing preventive biannual vaccination of domestic large ruminants. Nonstructural protein (NSP)-based immunoassays with the ability to discriminate between antibodies evoked by FMD virus (FMDV) infection and vaccination play a key role in serosurveillance in a vaccinated population, and antibody against 3ABC polyprotein region is believed to be the most reliable differential serological marker of infection. Nevertheless, not all infected animals can be guaranteed to seroconvert against a particular NSP, and it was therefore proposed in an international NSP test validation workshop held at Brescia, Italy, that use of more than one NSP test would improve the prospect of detecting or confirming the infected animals. To this end, indirect ELISAs (I-ELISAs) using a host of recombinant NSPs such as 3AB, 3ABC, 2C and 2B have been developed at Project Directorate on FMD (PDFMD), Mukteswar. r3AB3 I-ELISA has been adopted as the screening test for countrywide serosurvey, while all other NSP ELISAs are being used in conjunction with r3AB I-ELISA only under specific circumstances to enhance the efficiency of detection of infection. However, occasional NSP-antibody responses induced after multiple administrations of inacti-vated vaccine preparations remain a major stumbling block on the way to unequivocal identification of infected animals.

FMDV is unique among picornaviruses with respect to coding for three nonidentical tandem copies of 23- or 24-amino-acid-long 3B polypeptides, each with a molecular weight of about 2.6 kDa. ELISAs using 3B peptides or recombinant proteins, when applied to samples derived from repeatedly vaccinated animals, exhibited relatively higher orders of diagnostic specificity (DSp) compared to other bigger NSPs. Additionally, the diverse array of antigenic sites presented by long recombinant FMDV proteins are presumed to result in nonspecific reactions with antibodies induced against other related picornaviruses. More importantly, the 3B region has been shown to have a high density of differentially reactive linear B-cell epitopes, and its antigenic region has been found to be conserved among all seven serotypes of FMDV. Therefore, in all likelihood, an assay exploiting a small protein 3B can fundamentally be expected to have exquisite specificity to its advantage while upholding substantive diagnostic sensitivity (DSn). In the present study, all three contiguous copies of NSP 3B were expressed in Escherichia coli (E. coli), and its diagnostic potential to distinguish infection from vaccination was investigated by optimizing an I-ELISA. The performance attributes of the developed recombinant 3B I-ELISA (r3B I-ELISA) were compared to those of the validated screening assay (r3AB3 I-ELISA).

The apparent molecular weight of the His-tagged recombinant 3B protein (~17.5 kDa) in SDS-PAGE differed from the calculated weight of about 13.5 kDa (including the fusion tags) by approximately 4 kDa. Such a deviation between the theoretical and the observed size of 3B protein could be ascribed to its high content of hydrophilic amino acids, which are known to retard the mobility of proteins in SDS-PAGE gels. At the decided cutoff of 40 percent positivity, the diagnostic sensitivity and specificity of the r3B I-ELISA were estimated to be 92.1 % (95 % CI: 89.0–94.5) and 98.1 % (95 % CI: 96.9–98.8), respectively, as compared to 97.04 % and 95.04 % for r3AB3 I-ELISA. Although r3B I-ELISA displayed lower sensitivity compared to the screening assay, which could possibly

be attributed to additional relevant B-cell epitopes in the carboxy-terminal half of the 3A protein, the former achieved considerably higher specificity on repeatedly vaccinated animals. NSP antibodies could be detected from 10 to as late as 998 days postinfection in experimental calves. Substantial agreement in the test results (90.6 %) was found between the two ELISAs.

In conclusion, considering the extent of variation observed in the serological response to individual NSPs in the infected animals, screening of samples in r3B I-ELISA with higher specificity and in r3AB3 I-ELISA with higher sensitivity as an integrated system could be considered as a reasonable option in India to increase the confidence of detecting infected herds. Although the absence of antibodies to any or all of the NSPs does not indicate absolute freedom from FMDV infection, simultaneous testing of the 3AB- and 3B-Ab profile would definitely help in obtaining a conclusive result. Such a combined test system would have much applicability, particularly in situations where the epidemiological picture does not correlate with the screening test results, or where the quality of the vaccines in terms of NSP content is unknown. The diagnostic strategy implemented in South America combines 3ABC indirect ELISA for screening and a

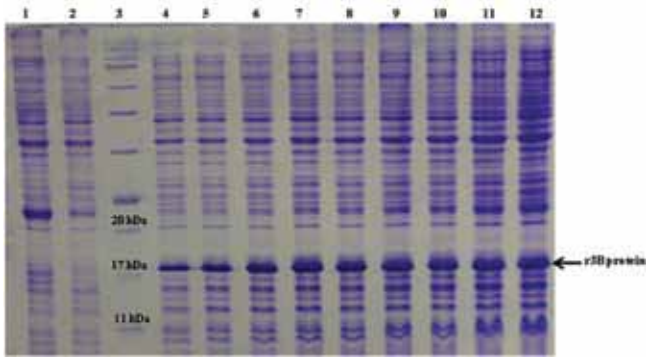


Fig. SGS-PAGE profile showing the effect of the time of induction on the level of expression of recombinant 3B protein (~17.5 kDa). Lane 1, *E. coli* BL21 (DE3) pLysS lysate; lane 2, uninduced *E. Coli* BL21 (DE3) pLysS lysate; lane 3, BLUeye prestained protein ladder (Biochem lifesciences, New Delhi, India); lane 4–12, *E. coli* BL21 (DE3) pLysS lysate after induction for 2 h, 3 h, 4 h, 5 h, 6 h, 7 h, 8 h, 9 h and 10 h, respectively

confirmatory immunoelectrotransfer blot (EITB) assay for detecting antibodies against a panel of NSPs. Because the EITB assay is too cumbersome to handle high-throughput samples, an antibody-profiling ELISA utilizing multiple NSPs is being developed at PDFMD as an alternative system. The r3B protein, expressed in this study, can potentially become a constituent of that panel.

8.2 Efficient rescue of foot-and-mouth disease virus in cultured cells transfected with RNA extracted from clinical samples

RNA transfection using lipofectamine 2000 to rescue FMDV from clinical materials was optimized and its potential application as an alternative to the conventional cell culture isolation was investigated. Although this technique has been published for positive sense RNA viruses like classical swine fever virus and FMDV (Hofmann et al., 2000 and Belsham et al., 2011), those involved collection of clinical materials in special RNA preserving agents and electroporation to introduce viral genomic RNA into BHK-21 cell. The present study extends the usefulness of transfection technique to clinical samples collected and transported in PBS-glycerol medium without any RNA preservative, a standard practice for transport of FMD suspected clinical materials to diagnostic laboratory. Furthermore, the chemical transfection approach has the advantage of convenience of handling more number of samples at a time in a multi-well tissue culture plate without requiring for any sophisticated instrument like electroporator. A significantly higher rate of virus regenaration from clinical materials regardless of their detection status in ELISA or multiplex PCR was achieved by RNA tansfection (62%; 118 out of 190 samples) compared to the conventional cell culture isolation (16%; only 30 out of 190 samples). FMD virus of the three prevalent serotypes (O, A and Asia 1) was rescued from clinical materials (ELISA negative) by RNA transfection. However, no virus could be rescued from multiplex PCR negative clinical samples (n=40). RNA extracted from all these clinical samples were positive for β-actin amplification suggesting, a genuine absence of viral genomic RNA. Chemical method of

transfection is likely to have better adaptability to various cell lines over electroporation. However, in the present study, chemical transfection was successfully adapted to rescue live virus in three different cell lines (BHK-21, LFBK and IB-RS-2) using small number (n=10) of clinical samples, suggesting the usefulness of the method.

Comparison of the results of virus isolation by conventional cell culture vis-a-vis RNA transfection from the clinical samples stored at different temperatures and pH for up to five weeks time revealed that the later technique outperformed the former in all storage conditions applied in the study suggesting stability of viral genomic RNA under inappropriate conditions that break down viral capsid. This observation relates to the requirement of stringent cold chain and maintenance of near neutral pH in the transport medium, if the clinical material is to be used for antigen diagnosis by ELISA and isolation of the virus in cell culture, and impairment in cold chain and pH conditions to a certain degree can still lead to virus recovery from viral genomic RNA. TRIzol® (Invitrogen) and RNAlater (Ambion) have been used as suitable long-term storage medium for virus containing biological materials allowing rescue of infectious progeny virus through RNA transfection even after four weeks of storage at ambient temperature. Storage and shipment in these virus inactivating and RNA preserving agents was considered as an economical and safe alternative to deep-freezing if the samples are to be used for genome detection by RT-PCR or for rescuing infectious virus from archived samples. Hence, further recommendation from this work would be to collect duplicate samples from each suspected case, one in PBS-glycerol and the other in an RNA stabilizing agent such as Trizol® or RNAlater as a standard operating procedure so that antigen detection ELISA can be applied and virus isolation can be made through conventional cell culture or RNA transfection. Such complementary approach is not only expected to improve the efficiency of diagnosis but also can obviate to certain extent the ill effects of improper storage and transport conditions on clinical materials. Transportation of clinical materials under stringent cold chain is a daunting task and is not practically feasible in case of remote locations in the country. Hence, the possibility of virus recovery by RNA transfection from clinical materials transported without application of cold chain is of immense significance for FMD surveillance and diagnosis programmes in a vast, subtropical country like India. Availability of rescued viruses from every single case/outbreak would enable comprehensive characterization of the virus strains circulating in the country including matching of the field isolates with the vaccine strains instead of confining only to genome sequencing in the absence of live virus isolate. This would ultimately aid in monitoring both antigenic and genome makeup of the virus strains causing each single case of FMD.

Table 1: Summary of viruses rescued from clinical samples by RNA transfection and conventional cell culture

Category of sample	No. of samples processed	No. of viruses rescued by transfection	No. of viruses isolated in
Both antigen detection ELISA and mPCR positive	98	56	21
Antigen detection ELISA negative but mPCR positive	92	62	09
Total	**190**	**118**	**30**

8.3 Truncated recombinant non-structural protein 2C-based indirect ELISA for FMD sero-surveillance

India has entered into the third stage of the Progressive Control Pathway for FMD. Under the state-run FMD-CP ~120 million cattle and buffalo are biannually vaccinated with the inactivated trivalent oil adjuvanted FMD vaccine (serotype O, A and Asia1). Sero-surveillance studies during last three years in

India revealed decrease in NSP-Ab prevalence in the regions covered under FMD-CP. Under such a scenario, to differentiate infected from vaccinated animals, NSP ELISA based surveillance strategy is needed. In an earlier study it has been observed that although majority of the vaccines used in India are free from NSP contamination, a few vaccine batches may contain substantial NSPs to interfere with the DIVA assay. Hence, selection of an NSP which remain largely absent in the purified vaccine preparations is critical for DIVA assay. For successful DIVA, 3AB and 3ABC polyprotein NSP ELISAs are used in India. It has been reported that anti-2C antibody response is observed in bovine serum from convalescent animals irrespective of serotype but absent in the serum obtained from vaccinated animals, thus 2C protein can be used as an indicator of the FMD infection. In addition, as the protein 2C remains membrane associated it usually do not form the part of the purified vaccine unlike many other NSPs. Hence in this study, protein 2C of FMDV was chosen to develop NSP-based ELISA for DIVA in bovines. The 2C NSP gene was truncated to facilitate the level of expression of the recombinant protein in the prokaryotic system and the performance of 2Ct I-ELISA was compared with that of the r3AB3 I-ELISA to validate the assay for use as an adjunct in specific cases during serosurveillance.

The assay was optimized and validated by testing a serum panel drawn from infected, vaccinated and naive animals. The cut-off was estimated at 40 PP by ROC analysis with DSn and DSp values of 92.9% and 94.0%, respectively. The false positives in naïve sera as well as uninfected vaccinated sera were observed in this study. This could be attributed to the non-specific reactivity as the analytical specificity of the assay could not be estimated due to the non-availability of serum samples. However this anti-2C response perishes from 14.5% (21 days post-vaccination serum samples) to 1.4% in 180 days post-vaccination serum samples. About 1.2% false positivity was observed in samples collected from FMD-CP areas which could be ascribed either to the cross-reactivity or to the residual anti-NSP response from past infections. It was observed that the 2Ct I-ELISA had low sensitivity (92.2%) in comparison to the in-house r3AB3 I-ELISA (98.8%). The probable reasons of lower DSn could be the less persistent anti-2C response and inherent lower immunogenicity of protein 2C in comparison to 3AB. This is further corroborated by the finding that for 21-1000 dpi samples 2Ct I-ELISA showed lower sensitivity (73.03%) in comparison to 21-365 dpi samples (92.9%) which further increased to 97.0% when only 21-180 dpi samples were considered. The ASn of 2Ct I-ELISA was two-fold lower when compared to r3AB3 I-ELISA and that could also be attributed to the lower immunogenicity of NSP 2C thereby dictating lower population of antibodies against 2C than 3AB.

The earliest time point of detection of antibodies to any NSP is the foremost requirement for adopting the control measures. Although, there has been variation in time regarding initial appearance and detection of seroconversion against different. Precise onset of seroconversion could not be predicted in this study as no intermediate serum samples between 5 and 12 dpi were available for analysis. It has been reported that sero-conversion against most of the NSPs has been evident at 7-12 dpi (Mackay et al. 1998; Sorensen et al. 1998), and the apparent sero-conversion observed in this study with the available samples was at 12 dpi. Serum from the FMDV serotype A intradermolingually inoculated calf remained positive up to 720 days followed by the intermittent seropositivity till 900 dpi and then became consistently negative. But the anti-2C antibodies in rest of the three experimentally infected calves persisted for a shorter duration of time-period, that is, 450 dpi, 387 dpi and 327 dpi for FMDV serotype A contact-infected, FMDV serotype Asia 1 intradermolingually inoculated and FMDV serotype Asia 1 contact-infected, respectively. The anti-2Ct response in two contact-infected calves was significantly lower persistent than corresponding two intradermolingually inoculated calves. This variation in persistence might have been a result of differences in the virus load in the system. On comparison of anti-SP and anti-2Ct NSP response, it was observed that all the experimentally infected animals show

early seroconversion at 5 dpi against SPs and the response was persistent for a longer period of time comparatively. To sum up, in all the infected calves, irrespective of route or strain of infection, SP-Ab response was found to be more durable than 2Ct-Ab response.

On testing of the random bovine samples collected across the country, the apparent sero-prevalence of 2Ct NSP-Ab was calculated as 23.7% in comparison to 30.9% against 3AB NSP. The lower prevalence of antibodies against 2Ct may be due to its lower antigenicity compared to 3AB leading to less persistent 2C-Ab response post-infection rather than the lower proportion of sero-conversion among infected animals.

Vaccination based FMD-CP operational in India has been expanded from 54 districts to 221 districts of the country since its launch in 2003, thus leading to the progressive increase in the vaccinated livestock and reduction in the occurrence of FMD outbreaks. Thus, reliability on single-NSP results would not be adequate under such circumstances. It has also been resolved in an international workshop held at Bressica, Italy that no single NSP test satisfies all the required criteria

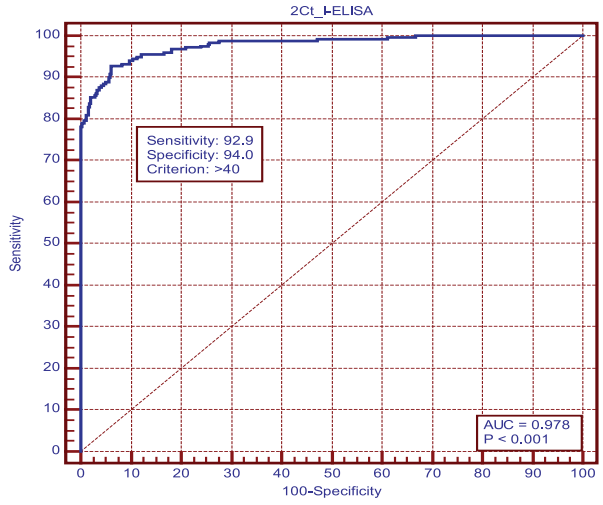


Fig. 1 ROC analysis for estimation of cut-off value of 2Ct I-ELISA (a) Sensitivity over (100-specificity); at different criterion values. Each point on the graph indicates sensitivity and specificity value at a particular cut-off value

of sero-surveillance and hence, more than one NSP should be used for a fool-proof surveillance. Therefore, the 2Ct-ELISA kit developed and validated will be an adjunct to the r3AB3-ELISA kit being currently used in India for increasing sensitivity of detection in low FMD prevalence zones.

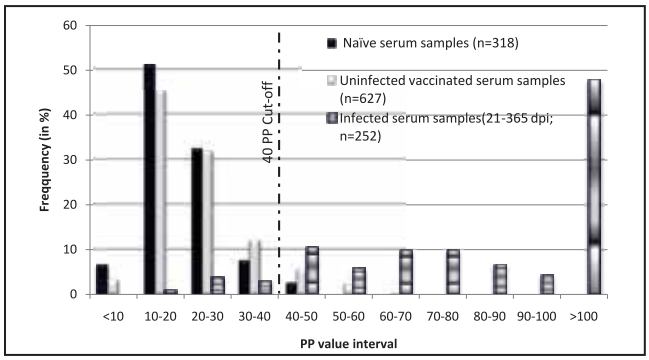


Fig Percent frequency distribution of PP values of serum from different infection/vaccination status for 2Ct I-ELISA

8.4 Detection of Foot-and-mouth disease virus infection specific antibody using recombinant non-structural protein 2B based indirect ELISA

NSP ELISAs have become an essential part of vaccination based control and serosurveillance policy in many FMD endemic countries. Further, non-endemic countries are seriously debating for the advantage of vaccinating animals in order to reduce the need to stamp out susceptible in-contact animals under 'vaccinate-to-live' policy. In India, vaccination based FMDCP was launched with an aim to create disease-free zones. Under such scenario, it is imperative to have the information on FMD virus exposure in domesticated large ruminants irrespective of vaccination status. For this purpose, in India large scale national FMD serosurveillance has been carried out by determining seroconversion against 3AB3 NSP using an in-house r3AB3 based I-ELISA. However, as per the suggestions made at an international NSP test validation workshop at Brescia, Italy, there is a need to use more than one NSP assay to increase the efficiency of detection. Further, when the epidemiological picture does not correlate with the screening test results,

in particular because of vaccinal NSP response, it is important to establish the reliability of the screening test results through the evaluation of the profiling of multiple NSP antibodies in the serum samples.

Using a set of serum samples from naïve, uninfected vaccinated bovines and known infected populations a cut-off value of 50 PP was determined for Δ2B I-ELISA. At the cut-off value of 50 PP, a sensitivity of 95.3% and specificity of 94.3% was determined for the Δ2B I-ELISA. Besides, preliminary precision studies based on PP values from intra-plate, inter-plate and inter-day replicates showed the CV values to be within the acceptable limits in support of the repeatability of the optimised assay. The diagnostic sensitivity and diagnostic specificity of indirect r3AB3 I-ELISA for bovines were found to be 96% and 96.4% respectively. As the performance of the recently developed recombinant Δ2B I-ELISA is comparable to the r3AB3 I-ELISA, the Δ2B assay has the potential to be used either as a screening or confirmatory assay in conjunction with the r3AB3 I-ELISA. During the current analysis, a good overall concordance has been observed between 3AB3 and Δ2B assay. Therefore, a third option can be proposed to run both the assays (3AB3 I-ELISA and Δ2B I-ELISA) in parallel to each other.

The earliest time of detection of FMD post-exposure through anti-NSP antibody assay is of paramount importance for adopting the control measures. However, there has been variation of time with respect to early detection of antibodies against various NSPs. Although the precise time of onset of anti-2B antibodies could not be detected in this study, all the four calves were found sero-converted to 2B protein by 10 dpi. While studying the post-infection kinetics of anti-2B antibodies in serum samples collected from four experimentally infectioned calves, a variation in the persistence of anti-2B antibodies was detected. This variation in antibody response could be attributed to the route of infection and load of virus challenge in respective calves. However, all the four calves were found consistently positive until 400 days post infection, with a peak in antibody response between two-three months post-infection. Therefore, in case of a field outbreak, serosurveillance using Δ2B I-ELISA could be carried out at two-three months post infection for increasing the chance of detection of infected animals. The findings on the post-infection kinetics of anti-2B antibody is in agreement with the earlier report on 2B peptide based ELISA, where the authors reported that, 2B peptide based ELISA showed a higher efficiency than other NSP tests in the detections of both early and late after infection. Though, in the current study, a relationship between the persistence of anti-2B antibody and detection of known FMD carrier animals has not been conducted, from the earlier findings of others it can be proposed that recombinant Δ2B NSP based ELISA could be used for the detection of persistently infected animals.

While testing the random bovine serum samples collected from different parts of the country in Δ2B I-ELISA, an overall seroconversion rate of 29.2% was detected. This anti-2B NSP antibody apparent prevalence estimated in the current work is close to that against 3AB3 NSP (~26.41%) which has been determined under the national FMD serosurveillance programme. As amino acid sequence of 2B NSP region is known to be highly conserved among the different serotypes of FMD virus, the recombinant 2B protein

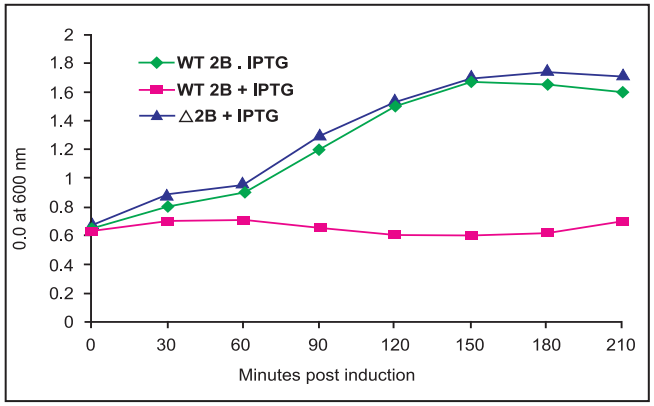


Fig. 1 Cytotoxic activity FMDV 2B protein in E.coli. The optical density at 600 nm of uninduced (diamonds), full length 2B (squares) and Δ2B-expressing cultures (triangles) were determined at 30 minute intervals for 3.5 hours. The optical density of E.coli culture medium containing the full length 2B protein was found to decrease significantly after induction.

assay is expected to detect infection specific antibodies against all the seven serotypes of FMDV. At the same time, it can be emphasised that a significant difference between the 2B protein sequences of various genera of Picornaviridae does exist. Therefore, it could be assumed that there may be less chance of cross reaction of 2B protein with antibodies against other picornaviruses that cause clinically similar vesicular diseases in cattle. In conclusion, the recombinant Δ2B NSP based I-ELISA, which has been developed for the first time, could be used for serological detection of FMD virus circulation.

9 National FMD Virus Repository

The Central FMD laboratory of the Project Directorate maintains the National FMD Virus Repository that is upgraded annually with addition of latest/new virus isolates. The virus repository has served the cause of the country by providing isolates for molecular epidemiological studies, evaluation of antigenic relatedness between the field and vaccine strains and selection of new candidate vaccine strains whenever required. A total of 73 virus isolates (61 type O, 10 type A and 2 Asia 1) were added to the repository during the reported period. At present the National FMD virus Repository holds a total of 1924 isolates (O-1241, A-308, C-15 and Asia 1-360).

Table 9.1 Year-wise details of the virus isolates added to National FMD Virus Repository during last five years

Isolates revived	O	A	Asia1	Total
2009-10	97	12	24	133
2010-11	06	17	02	25
2011-12	46	03	13	62
2012-13	32	19	26	77
2013-14	61	10	2	73

State-wise distribution of FMD virus (serotype O, A, C and Asia 1) Isolates (*n*=1924) preserved in National FMD virus Repository, PD on FMD, Mukteswar

For sixteen isolates, state of origin is not available and one isolate each is from Nepal & Bhutan

State-wise distribution of serotype O Isolates (*n*=1924) preserved in National FMD virus Repository, PD on FMD, Mukteswar

For eleven isolates state of origin is not available and one isolate each is from Nepal

State-wise distribution of serotype A Isolates (*n*=308) preserved in National FMD virus Repository, PD on FMD, Mukteswar

For eleven isolates state of origin is not available and one isolate each is from Nepal

State-wise distribution of serotype Asia 1 Isolates (*n*=360) preserved in National FMD virus Repository, PD on FMD, Mukteswar

For eleven isolates state of origin is not available and one isolate each is from Bhutan

State-wise distribution of serotype C Isolates (*n*=15) preserved in National FMD virus Repository, PD on FMD, Mukteswar

10 National FMD Serosurveillence:

10.1 DIVA (Antibody against NSPs; Percent Infected):

Seroconversion against NSPs (3AB3) is observed since 10-14 days after FMD virus infection. Whereas, if the animal is 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. This differential induction of anti-NSP antibody is exploited in DIVA ELISA to discriminate between infected and vaccinated animals. In this DIVA test reactivity of anti-3AB3 antibodies present in the serum of an infected animal (bovine species only) was assessed using purified recombinant 3AB3 (~38 kD) NSP in an indirect ELISA. The test is to be considered to be valid provided the mean absorbance of the positive control wells is not less than 0.8. Likewise a plate has to be rejected if the mean absorbance of the supplied negative control serum is > 0.3. The O.D. in back ground control wells should also be less than 0.1. To reduce inter-run variation due to differences in absolute absorbance between runs/tests, final results for each test serum is expressed as the PP value [(test serum sample mean OD/positive control serum mean OD) x 100] i.e., percent positivity value or PP value. The results are interpreted based on the following cut-off zones:

1. 3AB3 NSP reactivity positive: If PP value is more than 40%
2. 3AB3 NSP reactivity negative: If PP value is less than 40%

During the year, a total of 52,224 bovine serum samples collected at random from various parts of the country were tested in r3AB3 NSP-ELISA for assessing NSP-antibody (NSP-Ab) response, which is an underlying indicator of FMD virus exposure regardless of vaccination status. The test revealed overall seropositivity in ~ 25.1% samples/animals (Table 1). The test also included serum samples from recent suspected outbreak areas

Sl. No.	Place of origin	Host	Total serum samples tested	Total positive	%3AB3 reactors
Southern Region					
1	Andhra Pradesh	Bovine	2186	936	42.8
2	Karnataka	Bovine	2991	631	21.1
3	Kerala	Bovine	1260	559	44.4
4	Tamilnadu	Bovine	3050	609	20.0
Central Region					
5	Madhya Pradesh	Bovine	4558	1336	29.3
Western Region					
6	Rajasthan	Bovine	5600	2094	37.4
7	Gujarat	Bovine	2500	1512	60.5
8	Maharashtra	Bovine	2962	1434	48.4

Eastern Region					
9	West Bengal	Bovine	360	117	32.5
10	Bihar	Bovine	2251	820	36.4
11	Odisha	Bovine	3326	1381	41.5
12	Jharkhand	Bovine	556	188	33.8
Northern Region					
13	Haryana	Bovine	4200	87	2.1
14	Uttarakhand	Bovine	447	143	32
15	Uttar Pradesh	Bovine	1023	577	56.4
16	Himachal Pradesh	Bovine	1200	154	12.8
17	J & K	Bovine	1401	261	18.6
18	Punjab	Bovine	1900	230	12.1
19	Delhi	Bovine	200	10	5
North Eastern Region					
20	Asom	Bovine	2600	459	17.7
21	Arunachal Pradesh	Bovine	891	257	28.84
22	Manipur	Bovine	900	255	28.3
23	Mizoram	Bovine	550	41	7.45
24	Nagaland	Bovine	3027	977	32.28
25	Tripura	Bovine	1146	95	8.3
Island					
26	Andaman	Bovine	1139	105	9.2
	Total	**Bovine**	**52,224**	**15,268**	**29.2**

Table 2. Summary of r3AB3 NSP-ELISA During 2008-09 to 2013-14; the prevalence has been around 27%

Year	Total samples tested	Total positive	% DIVA reactors
2008-09	18,326	5,120	27.94
2009-10	29,763	8,303	27.90
2010-11	31,042	8,341	26.87
2011-12	37,467	10,410	26.09
2012-13	40,934	10,811	26.41
2013-14	52,224	15,268	29.20
Total	**2,09,756**	**58,253**	**27.80**

10.2 LPB-ELISA (Percent protected):

During the year under report, a total of 32,202 serum samples were subjected to LPB ELISA for determination of antibody level against structural protein (SPs) of serotypes O, A and Asia1.

Table 3. Summary of LPBE result obtained on Random serum samples.

Sl. No.	Name of place/State	Species	Total no. of samples	Protective Titre ≥1.8		
				O	A	Asia 1
Southern Region						
1.	Andhra Pradesh	Bovine	2186	1385(63.3)	1401(64.2)	1442(65.9)
2.	Karnataka	Bovine	2969	1142(38)	1658(56)	2119(71)
3.	Kerala	Bovine	1166	319(27.4)	284(24.4)	550(47.2)
4.	Tamilnadu	Bovine	3050	2176(71.3)	2230(73.1)	2238(73.4)
Central Region						
5.	Madhya Pradesh	Bovine	4558	1259(27.6)	742(16.3)	1017(22.3)
Western Region						
6.	Maharashtra	Bovine	3154	1983(63)	2295(73)	2011(64)
7.	Rajasthan	Bovine	957	300(31.3)	351(36.7)	528(55.2)
Northern Region						
8.	Haryana	Cattle+ Buffalo	275	163(59.3)	147(53.5)	206(74.9)
9.	Uttar Pradesh	Bovine	1023	442(43.2)	437(42.7)	465(45.5)
10.	Himachal Pradesh	Bovine	1200	783(65.3)	512(42.6)	633(52.8)
11.	Jammu & Kashmir	Bovine	1401	347(24.8)	284(20.3)	511(36.5)
Eastern Region						
12.	West Bengal	Bovine	545	244(44.8)	122(22.4)	154(28.3)
13.	Bihar	Bovine	1420	315(22.2)	96(6.8)	37(2.7)
14.	Odisha	Bovine	2587	723(27.9)	708(27.4)	1039(40.2)
North Eastern Region						
15.	Asom	Bovine	2600	478(18.4)	178(6.8)	260(10)
16.	Manipur	Bovine	1000	536(53.6)	481(48.1)	399(39.9)
17.	Mizoram	Bovine	154	114(74)	90(58.4)	56(36.4)
18.	Tripura	Bovine	954	231(24.2)	287(30.1)	292(30.6)
19.	Meghalaya	Bovine	141	45(31.9)	39(27.7)	21(14.9)
20.	Arunachal Pradesh	Bovine	32	22 (68.8)	4 (12.5)	6 (18.8)
21.	Nagaland	Bovine	380	166 (43.7)	137(36.1)	216(56.8)
Island						
22.	Andaman & Nicobar	Bovine	450	187(41.6)	159(35.3)	301(67)

10.3 Surveillance and Monitoring of FMD in ovine, caprine and porcine species in India:

During the period of the project, a total serum samples of 4027 animals (sheep = 1804, goat = 2223) were collected from various places of 11 states (Maharashtra, Andhra Pradesh, Tamil Nadu, Karnataka, Assam, Madhya Pradesh, Andaman and Nicobar Islands, Uttar Pradesh, Jammu & Kasmir, Uttarakhand, Rajasthan) of India. Out of 1804 ovine serum samples tested in 3AB-NSP-ELISA, 341 (18.90%) were found to be 3AB-NSP reactors. Out of 2223 caprine serum samples tested in 3AB-NSP-ELISA, 207 (9.31%) were found to be 3AB-NSP reactors. Out of 835 ovine serum samples tested in LPB-ELISA, only 1 (0.11%) showed log10 titre of ≥1.8 against all three

prevalent FMDV serotypes of O, A and Asia 1. Out of 419 caprine serum samples tested in LPB-ELISA, only 4 (0.95%) showed log10 titre of ≥1.8 against all three prevalent FMDV serotypes of O, A and Asia 1. A total of 21 clinical materials from 16 sheep and 5 goats (tongue epithelium/oral swab/Inter-digital cleft swab/gum pad swab etc) were collected from several states like Uttar Pradesh, Karnataka and Tamil Nadu during FMD outbreaks. Total 4 samples (2 sheep and 2 goats) were typed to be serotype O in sandwich ELISA and multiplex PCR. Two isolates e.g. PD372/2013 and PD463/2013 from goats could be partially sequenced at VP1 coding region for phylogenetic reconstruction.

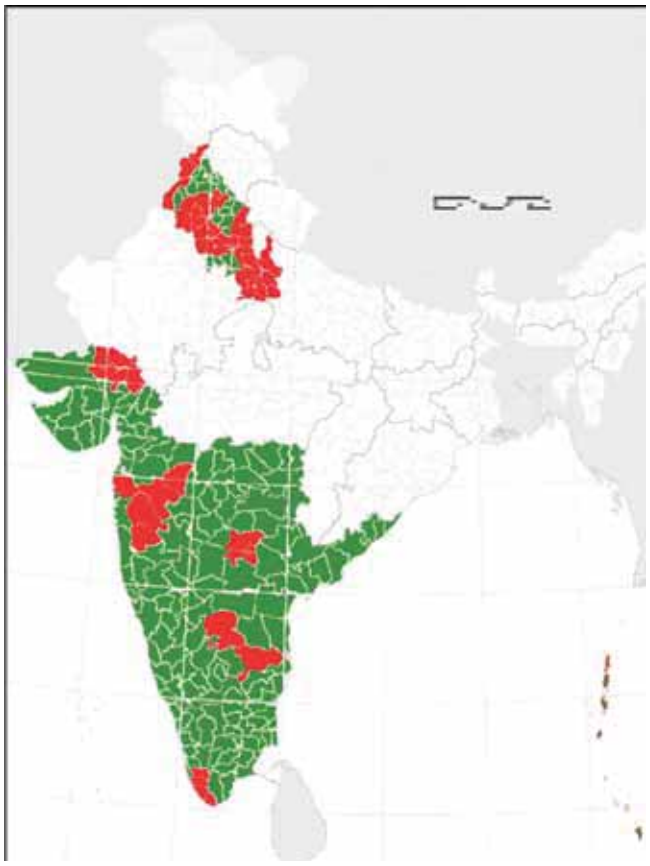
11 Post Vaccinal Seroconversion Studies

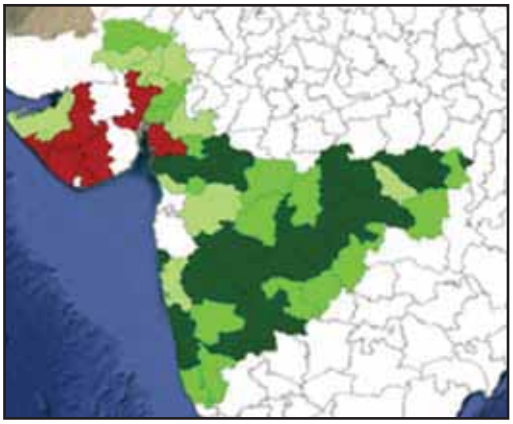
11.1 Sero-monitoring of FMD Control Programme (FMD-CP)

A vaccination based FMD Control Programme (FMD-CP) has been initiated by the Government of India since August 2003-04 covering 54 specified districts in the country. This involves 6 monthly vaccinations with a trivalent O, A and Asia1 vaccine of all cattle and buffaloes against FMD. Serum samples before vaccination and 21 to 30 days post vaccination are collected by the respective state AH department and submitted to testing centres of PD-FMD for estimation of level of serotype specific neutralizing antibodies by Liquid Phase Blocking ELISA (LPBE) developed by PD-FMD. The Regional Centers, Network Units and Central FMD laboratory of the Project Directorate participate in this post vaccinal sero-conversion under FMD-CP. Since 2011-12, Central Agricultural Research Institute, Port Blair has been included as a testing laboratory for sero-monitoring of FMD in A & N Islands. All reagent and training to conduct LPB ELISA are provided by the institute. The test was compared with SNT, and it is recommended that LPB ELISA titer (in serum) of ≥ log10 1.8 indicates protection against FMD. Due to initial success, additional 167 districts (another 80-90 million cattle and buffalo) have been included under the programme in 2010-11. Currently, this programme includes 221 districts of the country covering all the states of Southern peninsula (Kerala, Tamilnadu, Puducherry, Karnataka and Andhra Pradesh), Maharashtra, Goa, Daman and Diu, Gujarat, Punjab, Haryana, Delhi, Dadra and Nagar Haveli, Andaman & Nicobar Islands, Lakshadweep and 16 districts in Uttar Pradesh (Fig 4), and targeting ~120 million cattle and buffalo.

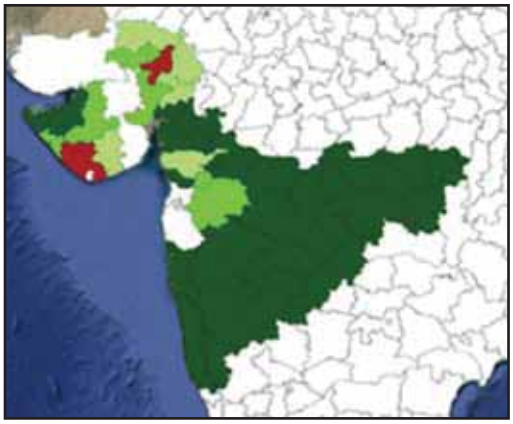
During 2013-14, a total of 1,89,159 pre and post vaccinated serum samples were tested and of which, 48,377 serum samples were from first phase FMDCP districts representing XIII, XIV, XV and XVI phases of vaccinations and remaining 1,41,782 serum samples were from expanded FMD CP districts of 2010-11 representing Phases II, III, IV V and VI.

Fig.1 Regions covered under FMD-CP. Fifty four districts in which control programme started in 2003-04 are marked red. One sixty seven districts in which the control programme started in 2010-11 are marked green.

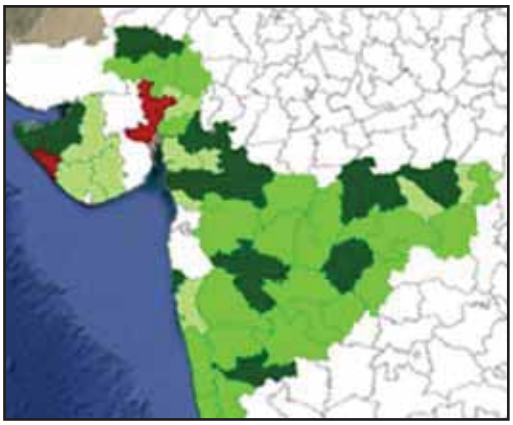




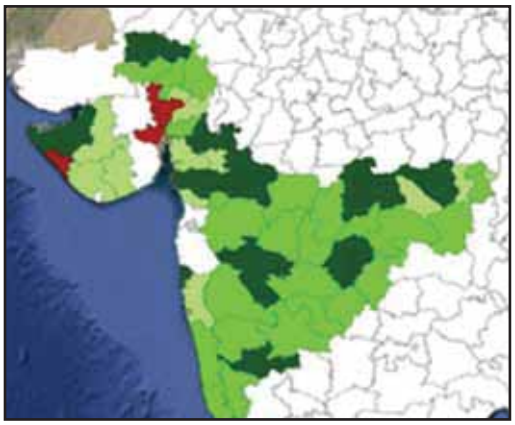
Pre Vaccinated Serotype A



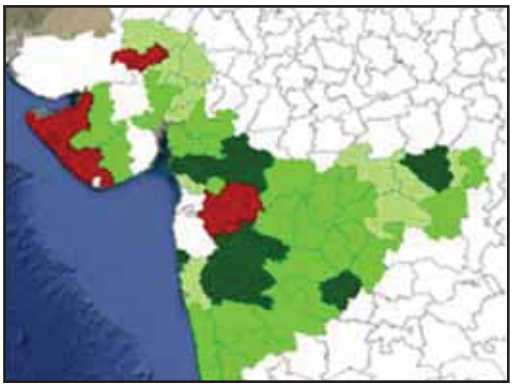
Post Vaccinated Serotype A



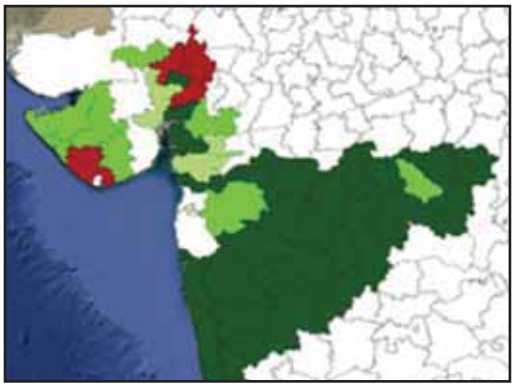
Pre Vaccinated Serotype O



Pre Vaccinated Serotype O



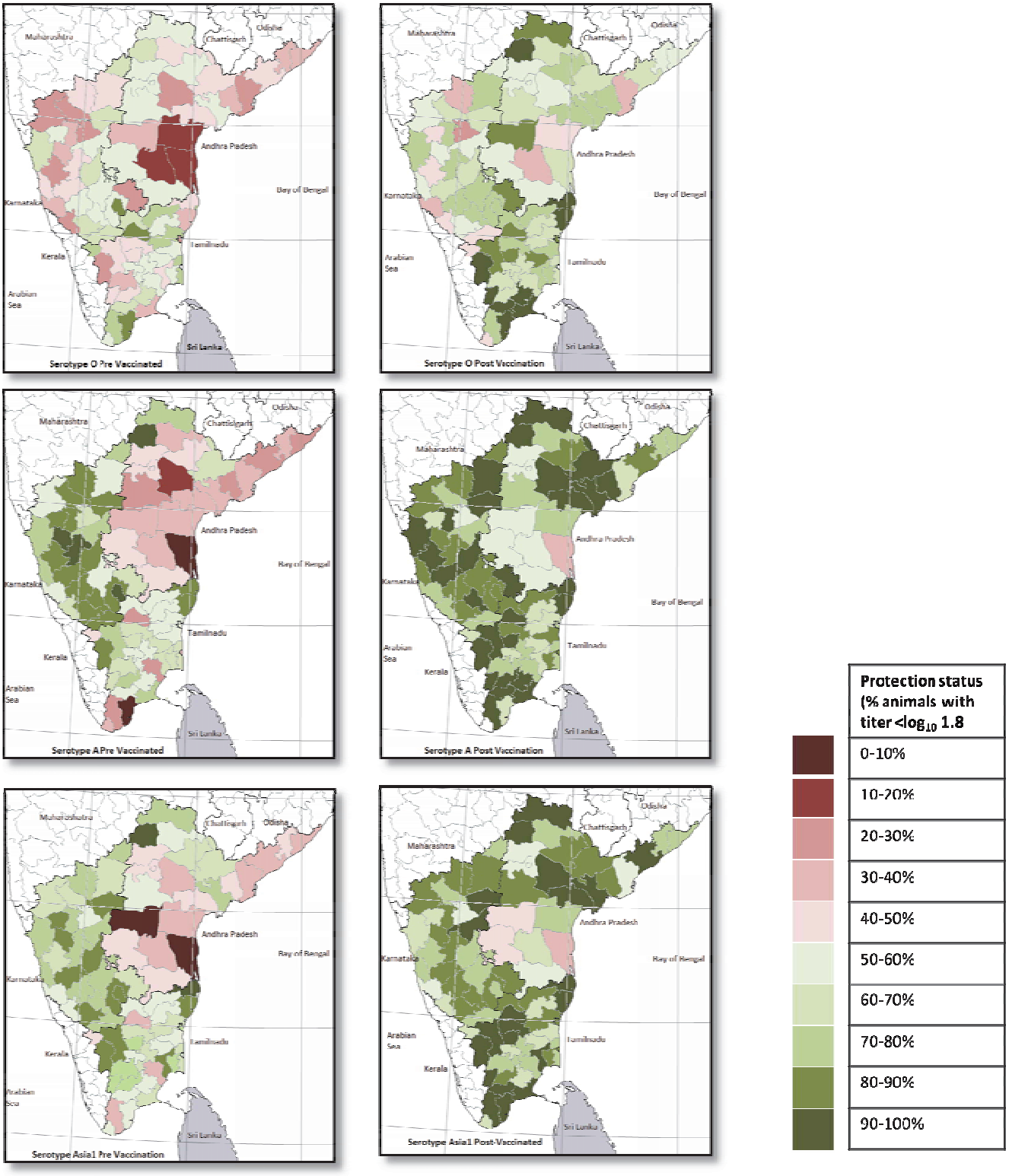
Pre Vaccinated Serotype Asia1



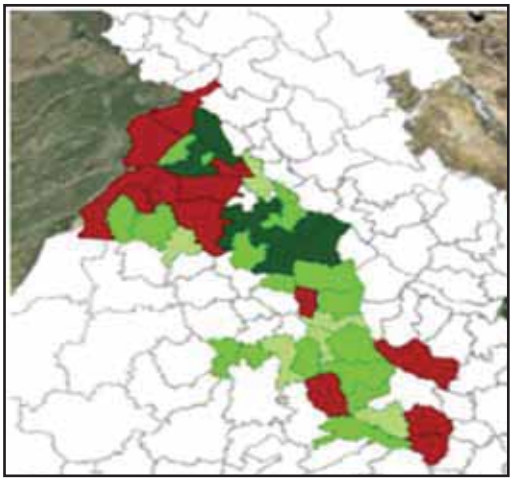
Pre Vaccinated Serotype Asia1

Pre and Post Vaccination monitoring of three serotype of FMDV in FMDCP areas (Gujarat and Maharashtra)

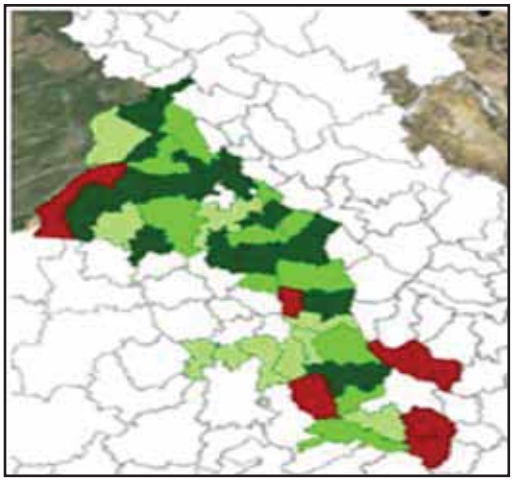
Color Coding	Percentage of Population Protected
	>70%
	40-70%
	20-40%
	0-20%
	No information available



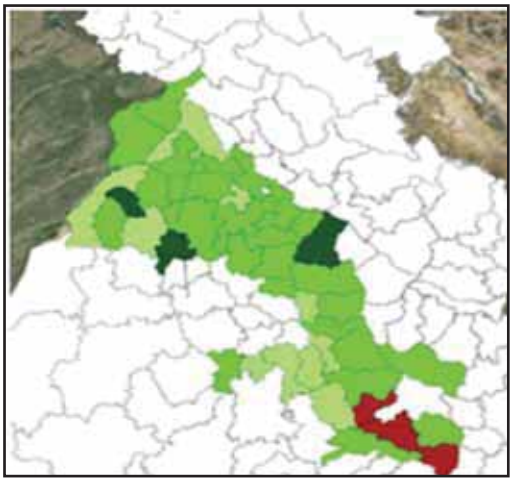
Pre and Post Vaccination monitoring of three serotype of FMDV in FMDCP areas (Southern Peninsular region of the country



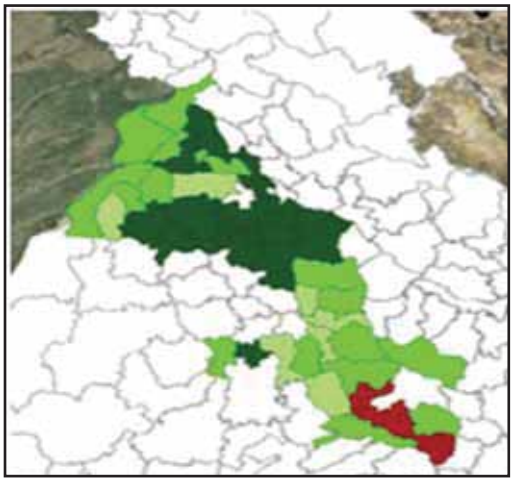
Pre Vaccinated Serotype A



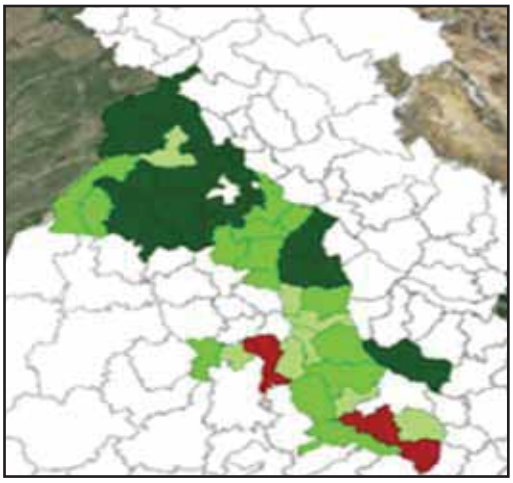
Post Vaccinated Serotype A



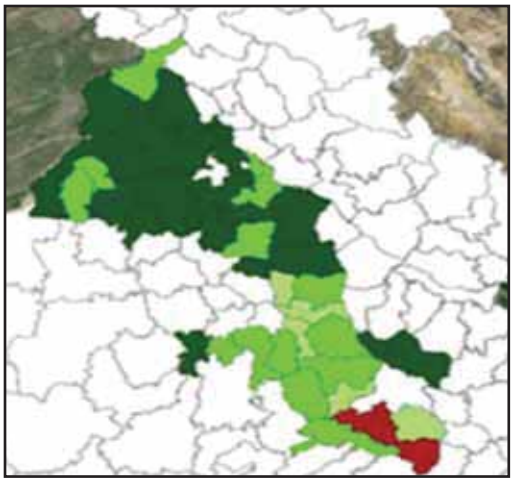
Pre Vaccinated Serotype O



Pre Vaccinated Serotype O



Pre Vaccinated Serotype Asia1



Pre Vaccinated Serotype Asia1

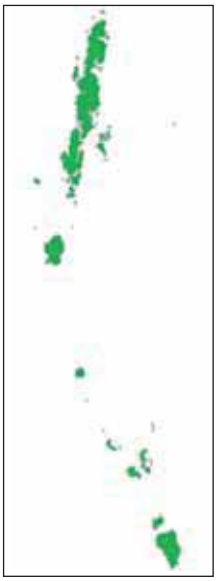
Pre and Post Vaccination monitoring of three serotype of FMDV in FMDCP areas
(Haryana, Punjab and Parts of Uttar Pradesh)

Sero-monitoring in Andaman & Nicobar Island

Initially, eight villages of Andaman & Nicobar were covered under FMDCP in 2003-04 and later in 2010-11, entire Andaman & Nicobar Island was included. Central Agricultural Research Institute, Port Blair is undertaking the sero-monitoring of animals covered under the programme in A&N Islands.

Table 1: Result of seroconversion in Andaman & Nicobar Islands (2003-04)

Phase	Vaccination		Number & % animals showing titres ≥1.8 log10 against FMDV					
			Type O		Type A		Type Asia 1	
	Pre	Post	Pre-vac	Post-vac	Pre-vac	Post-vac	Pre-vac	Post-vac
III	154	162	40(25.9)	97(60.0)	5(2.8)	37(20.3)	52(34.0)	118(73.6)
IV	149	146	50(33.5)	94(64.6)	50(33.5)	96(65.9)	35(23.4)	101(67.6)
V	126	122	72(57.2)	68(55.8)	62(50.8)	64(52.5)	54(44.3)	62(50.8)
VI	270	270	50 (18.5)	80 (29.6)	66 (24.4)	104 (38.4)	28 (10.2)	36 (13.2)
VII	265	265	112 (42.3)	174 (65.7)	82 (30.9)	110 (41.5)	56 (21.1)	66 (24.9)
VIII	251	251	53(21.11)	102(40.63)	18(7.17)	49(19.52)	47(18.72)	85(33.86)
IX	228	228	73(32.01)	69(30.26)	31(13.59)	35(15.35)	56(24.56)	42(18.42)
XII	180	180	36(20.0)	49(27.22)	19(10.56)	40(22.22)	11(6.11)	30(16.67)
XIII	283	283	26(9.2)	78(27.6)	12(4.2)	52(18.4)	15(5.3)	44(15.5)
XIV	794	593	144(18.1)	279(47)	100(12.6)	214(36.1)	77(10)	194(32.7)
XV	1445	1109	308(21.3)	550(49.9)	333(23)	584(52.6)	433(29.9)	674(60.7)



Overall herd immunity and seroconversion was good till Phase V and thereafter decline in herd immunity has been observed.

Sero-monitoring in Tamil Nadu

Only district Kanyakumari, was covered under FMDCP in 2003-04 (filled red) and later in 2010-11, rest of the districts (filled green) was included in the control programme.

Table: 2 Result of seroconversion in Tamil Nadu (2003-04)

Phase	Vaccination		Number & % animals showing titres ≥1.8 $log10_{10}$ against FMDV					
			Type O		Type A		Type Asia 1	
	Pre	Post	Pre-vac	Post-vac	Pre-vac	Post-vac	Pre-vac	Post-vac
I	100	100	28(28)	51(51)	29(29)	57(57)	24(24)	54(54)
II	100	100	23(23.0)	63(63.0)	24(24.0)	40(40.0)	18(18.0)	61(61.0)
III & IV	180	330	59(32.7)	246(74.5)	61(33.8)	201(60.9)	45(25.0)	216(65.4)
VI	160	130	30(18.7)	99(76.1)	31(23.8)	109(83.8)	28(21.5)	103(79.2)
VII	300	300	35(11.7)	210(70)	34(11.3)	231(77)	36(12)	226(75.3)
VIII	100	100	34(34)	74(74)	40(40)	60(60)	25(25)	78(78)
IX	100	100	40(40)	58(58)	45(45)	64(64)	33(33)	74(74)
X	100	100	32(32)	62(62)	45(45)	63(63)	41(41)	70(70)
XI	200	200	38(19)	144(72)	31(15.5)	87(43.5)	29(14.5)	83(41.5)
XIV	200	200	71(35.5)	116(58)	93(46.5)	137(68.5)	92(46)	128(64)
XV	200	200	92(46)	199(99.5)	115(57.5)	198(99)	120(60)	194(97)

Increase in herd immunity and seroconversion has been observed in the district

Table: 3 Result of seroconversion in Tamil Nadu (2010-11).

Phase	Vaccination		Number & % animals showing titres ≥1.8 $log10_{10}$ against FMDV					
			Type O		Type A		Type Asia 1	
	Pre	Post	Pre-vac	Post-vac	Pre-vac	Post-vac	Pre-vac	Post-vac
I	5440	5440	1860(34.2)	3417(62.8)	1351(24.8)	2561(47.1)	115(20.5)	2209(40.6)
II	5040	5240	1383(27.4)	3504(66.9)	684(13.6)	2433(46.4)	245(4.9)	979(18.7)
III	4600	4600	789(17.2)	2788(60.6)	396(8.6)	1801(39.2)	1030(22.4)	3361(73.1)
IV	5801	5843	2570(44.3)	4547(77.8)	3296(56.8)	4826(82.6)	3643(62.8)	5066(86.7)
V	6099	5697	3503(57.4)	5075(89.1)	3704(60.7)	5201(91.3)	3790(62.1)	5185(91)

Increase in herd immunity and seroconversion has been observed

Table: 4 Result of seroconversion in Puducherry (2010-11).

Phase	Vaccination		Number & % animals showing titres ≥1.8 $log10_{10}$ against FMDV					
			Type O		Type A		Type Asia 1	
	Pre	Post	Pre-vac	Post-vac	Pre-vac	Post-vac	Pre-vac	Post-vac
I	30	55	16(44.4)	24(66.66)	9(25)	20(55.55)	5(13.88)	11(30.55)
II	38	38	16(42.1)	20(52.6)	10(26.3)	14(36.8)	0(0)	18(21.1)
III	46	46	21(45.7)	29(63)	7(15.2)	20(43.5)	26(56.5)	30(65.2)

Sero-monitoring in Kerala

Three districts of Kerala namely, Trivandrum, Kollam and Pathanamthitta were covered under FMDCP in 2003-04 (filled red) and later in 2010-11; eleven districts (filled green) was included

Table: 5 Result of seroconversion in Kerala (2003-04).

Phase	Vaccination		Number & % animals showing titres ≥1.8 $log10_{10}$ against FMDV					
			Type O		Type A		Type Asia 1	
	Pre	Post	Pre-vac	Post-vac	Pre-vac	Post-vac	Pre-vac	Post-vac
I & II& IV	483	496	158(32.7)	255(51.4)	140(29.0)	236(47.5)	165(34.2)	280(56.4)
V	290	290	67(23.1)	197(67.9)	52(17.9)	171(58.9)	61(21.0)	211(72.7)
VI	70	70	49 (20.4)	185(77.1)	41(17.1)	169(70.4)	38(15.8)	171(71.3)
VII	300	300	48 (16.0)	208(69.3)	43 (14.3)	213 (71	52 (17.3)	210(70.0)
VIII & IX	600	600	226(37.6)	395(65.8)	265(44.2)	341(56.8)	260(43.3)	397(66.2)
X	400	100	160(40)	59(59)	145(36.3)	66(66)	150(37.5)	53(53)
XI	352	315	122(19)	122(19)	122(19)	115(17.2)	96(14.4)	88(13.2)
XII	500	500	59(11.8)	202(40.4)	73(14.6)	197(39.4)	63(12.6)	153(30.6)
XIII	150	150	11(7.3)	42(28)	13(8.7)	39(26)	13(8.7)	41(27.3)
XIV	546	526	73(13.4)	74(14.1)	108(20)	123(23.4)	123(22.5)	200(38)
XV	598	553	129(21.6)	286(51.7)	190(31.8)	327(59.1)	313(52.3)	432(78.1)

Overall herd immunity is poor in Kerala

Table: 6 Result of seroconversion in Kerala (2010-11)

Phase	Vaccination		Number & % animals showing titres ≥1.8 $log10_{10}$ against FMDV					
			Type O		Type A		Type Asia 1	
	Pre	Post	Pre-vac	Post-vac	Pre-vac	Post-vac	Pre-vac	Post-vac
II	676	180	84(12.4)	65(36.1)	105(15.5)	65(36.1)	65(9.6)	61(34)
III	1631	1474	199(12.2)	525(35.6)	178(10.9)	484(32.8)	135(8.3)	376(25.5)
IV	2378	2109	308(13)	526(25)	362(15.2)	633(30)	404(17)	735(35)
V	2043	1941	400(20)	991(51.1)	505(24.7)	1135(58.5)	922(45.1)	1364(70.3)

Overall herd immunity is poor in Kerala

Table: 7 Result of seroconversion in Lakshadweep (2010-11)

Phase	Vaccination		Number & % animals showing titres ≥1.8 $log10_{10}$ against FMDV					
			Type O		Type A		Type Asia 1	
	Pre	Post	Pre-vac	Post-vac	Pre-vac	Post-vac	Pre-vac	Post-vac
I	107	107	45(42.1)	80(74.8)	16(15)	63(58.9)	35(32.7)	50(46.7)

Sero-monitoring in Andhra Pradesh

Four districts of Andhra Pradesh namely, Ananthapur, Chitoor, Medak and Rangareddy are covered under FMDCP in 2003-04 (filled red) and rest of the districts (filled green) were included in 2010-11.

Table: 8 Result of seroconversion in Andhra Pradesh (2003-04)

Phase	Vaccination		Number & % animals showing titres ≥1.8 $log10_{10}$ against FMDV					
			Type O		Type A		Type Asia 1	
	Pre	Post	Pre-vac	Post-vac	Pre-vac	Post-vac	Pre-vac	Post-vac
I	800	800	83 (10.3)	340 (42.5)	43 (5.3)	244 (30.5)	92 (11.5)	340 (42.5)
II	800	800	N.A.	434 (54.2)	N.A.	498 (62.3)	N.A.	438 (54.7)
III	800	800	210 (26.2)	286 (35.7)	395 (49.3)	532 (66.5)	306 38.2)	422 (52.7)
IV	800	800	281 (35.1)	374 (46.8)	465 (58.1)	617 (77.1)	329 41.1)	518 (64.8)
V	800	800	247 (30.8)	440 (55)	466 (58.2)	574 (71.8)	343(42.8)	450 (56.3)
VI	800	800	275 (34.3)	490 (61.3)	554 (69.2)	690 (86.3)	446 55.7)	634 (79.3)
VII	800	800	274 (34.0)	483 (60.3)	349 (44.0)	540 (67.5)	391(48.8)	518 (64.7)
VIII	800	800	356 (44.5)	594 (74.0)	415 (51.8)	624 (78.0)	333(41.6)	527 (65.8)
IX	800	800	422 (52.8)	673(84.1)	329 (41.1)	534 (66.8)	287(35.9)	534 (66.8)
X	800	800	502(62.7)	635(79.3)	368(46)	575(71.8)	411(51.3)	602(75.2)
XI	800	800	398(49.75)	617(77.1)	356(44.5)	600(75)	333(41.6)	568(71.5)
XII	800	800	387(48.4)	568(71)	266(33.25)	483(60.4)	177(22.1)	367(45.9)
XIII	800	800	537(67.1)	654(81.8)	438(54.8)	602(75.3)	315(39.3)	498(62.3)
XIV	800	800	366(45.7)	634(79.2)	186(23.3)	446(54.7)	100(12.5)	389(48.6)
XV	800	800	464(58)	578(72.2)	605(75.6)	733(91.6)	626(78.2)	726(90.7)
XVI	800	800	503(62.8)	680(85)	675(84.3)	773(96.6)	711(88.8)	796(99.5)

Table: 9 Result of seroconversion in Andhra Pradesh (2010-11)

Phase	Vaccination		Number & % animals showing titres ≥1.8 $log10_{10}$ against FMDV					
			Type O		Type A		Type Asia 1	
	Pre	Post	Pre-vac	Post-vac	Pre-vac	Post-vac	Pre-vac	Post-vac
I	3600	3600	1043(29)	2396(66.5)	648(18)	2030(56.4)	419(13.1)	1709(47.5)
II	3480	3480	1435(41.2)	2381(68.4)	1026(29.5)	2054(59)	595(17.1)	1499(43.1)
III	3600	3600	1392(38.6)	2498(69.3)	750(20.8)	1661(46.1)	393(10.9)	1162(32.2)
IV	3600	3600	1364(38)	2354(65.4)	1356(37.7)	2821(78.4)	1663(46.2)	2788(77.4)
V	3600	3600	1546(42.9)	2478(68.6)	2292(63.6)	3153(87.5)	2574(71.5)	3239(89.9)
VI	400	400	235(58.7)	314(78.5)	334(83.5)	369(92.2)	315(78.5)	365(91.5)

Overall herd immunity and sero-conversion is very good in Andhra Pradesh

Sero-monitoring in Karnataka

State of Karnataka was included under FMDCP in 2010-11

Table: 10 Result of seroconversion in Karnataka (2010-11).

Phase	Vaccination		Number & % animals showing titres ≥1.8 $log10_{10}$ against FMDV					
			Type O		Type A		Type Asia 1	
	Pre	Post	Pre-vac	Post-vac	Pre-vac	Post-vac	Pre-vac	Post-vac
I	4587	4266	1817(40)	2383(56)	687(15)	1722(40)	426(9)	1049(24.5)
II	5401	4632	2718(50)	3101(67)	1471(27)	2161(47)	1577(39)	2354(51)
III	3864	3075	2118(54.8)	1855(60.3)	1129(29.2)	1289(41.8)	2376(61.5)	2158(70.2)
IV	5053	5225	2439(48.3)	3245(62.1)	3977(78.7)	4493(86)	3834(76)	4294(82.2)
V	5916	5853	1954(33)	3470(59)	3047(52)	3957(68)	3795(64)	4734(81)

Overall herd immunity and sero-conversion is very good in Karnataka

Sero-monitoring in Maharashtra

Six districts of Maharashtra namely, Ahmadnagar, Aurangabad, Pune, Satara, Mumbai and Thane were covered under FMDCP in 2003-04 (filled red) and later in 2010-11, twenty nine districts (filled green) was included

Table : 11 Result of seroconversion in Maharashtra (2003-04).

Phase	Vaccination		Number & % animals showing titres ≥1.8 $log10_{10}$ against FMDV					
			Type O		Type A		Type Asia 1	
	Pre	Post	Pre-vac	Post-vac	Pre-vac	Post-vac	Pre-vac	Post-vac
I	844	761	173 (20.5)	456 (59.9)	151(17.9)	437 (57.4)	192 (22.8)	466 (61.2)
II	834	834	N.A.	508 (60.9)	N.A.	490 (58.6)	N.A.	553 (66.2)
III	753	799	184 (24.4)	438 (54.8)	351 (46.8)	580 (72.7)	262 (34.7)	534 (66.9)
IV	789	797	191 (24.2)	417 (52.3)	517 (65.6)	679 (85.3)	278 (35.2)	509 (63.9)
V	802	772	142 (17.7)	271 (35.1)	353 (44.2)	477 (62.3)	121 (15.0)	245 (31.8)
VI	901	928	404 (44.9)	663 (71.4)	622 (69)	853 (91.9)	245 (27.2)	446 (48.1)
VII	1000	1000	446 (44.6)	692 (69.2)	701 (70.1)	893 (89.3)	431 (43.1)	667 (66.7)
VIII	1000	1000	646 (64.6)	904 (90.4)	574 (57.4)	848 (84.8)	198 (19.8)	452 (45.2)
IX	1000	1000	730(73)	951(95.1)	524(52.4)	817(81.7)	324(32.4)	695(69.5)
X	1000	1000	785(78.5)	978(97.8)	686(68.6)	935(93.5)	607(60.7)	846(84.6)
XI	1000	1000	558(55.8)	916(91.6)	534(53.4)	871(87.1)	403(40.3)	837(83.7)
XII	980	980	590(60.2)	894(91.2)	468(47.75)	823(83.97)	341(34.79)	730(74.48)
XIII	950	1050	418(44)	727(69.2)	75(7.9)	332(31.6)	58(6.1)	277(26.4)
XIV	1040	1037	496(48)	881(85)	400(38.5)	839(81)	426(41)	831(81)
XV	1098	1098	382(34.8)	902(82.1)	598(54.5)	999(91)	661(60.2)	1018(92.7)
XVI	1055	1051	702(66.5)	978(93.1)	774(73.4)	991(94.3)	709(67.2)	986(93.8)

Table: 12 Result of seroconversion in Maharashtra (2010-11)

Phase	Vaccination		Number & % animals showing titres ≥1.8 $log10_{10}$ against FMDV					
			Type O		Type A		Type Asia 1	
	Pre	Post	Pre-vac	Post-vac	Pre-vac	Post-vac	Pre-vac	Post-vac
I	5988	6018	1687(28.2)	4390(72.9)	941(15.7)	3080(51.2)	382(6.4)	2310(38.4)
II	7208	7341	1849(25.7)	4890(66.6)	481(5.8)	2530(34.5)	491(6.8)	2279(31)
III	4721	4723	938(20)	2674(56.6)	1444(30.6)	2933(62.1)	2674(31.6)	3096(65.6)
IV	5250	5305	1673(31)	3746(70.6)	2641(50.3)	4429(83.5)	2809(53.5)	4513(85.1)
V	4891	4891	3027(61.9)	4523(92.5)	3466(70.9)	4619(94.4)	2701(55.2)	4307(88.1)

Overall herd immunity and sero-conversion is very good in Maharshtra

Table: 13 Result of seroconversion in Goa (2010-11).

Phase	Vaccination		Number & % animals showing titres ≥1.8 $log10_{10}$ against FMDV					
			Type O		Type A		Type Asia 1	
	Pre	Post	Pre-vac	Post-vac	Pre-vac	Post-vac	Pre-vac	Post-vac
I	391	381	47(12)	244(86.8)	8(2)	92(24.1)	11(2.8)	92(24.1)
II	383	378	159(41.5)	316(84)	59(15.4)	234(62)	175(46)	331(88)
III	384	368	182(47.4)	302(82.1)	241(64.3)	317(86.1)	209(54.4)	316(86)
IV	379	376	171(45.1)	289(77)	222(58.5)	323(86)	215(57)	320(85.1)

Sero-monitoring in Gujrat

Four districts of Gujarat namely, Banaskantha, Sabarkantha, Mehsana and Patan were covered under FMDCP in 2003-04 (filled red) and later in 2010-11; rest of the districts (filled green) was included

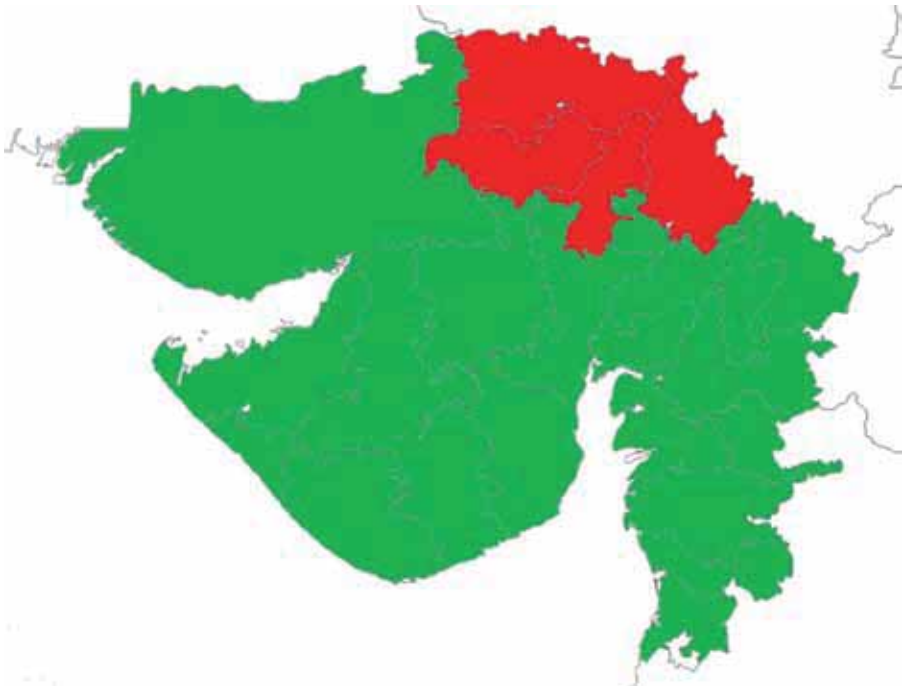


Table: 14 Result of seroconversion in Gujarat

Phase	Vaccination		Number & % animals showing titres ≥1.8 $log10_{10}$ against FMDV					
			Type O		Type A		Type Asia 1	
	Pre	Post	Pre-vac	Post-vac	Pre-vac	Post-vac	Pre-vac	Post-vac
I	382	259	50 (19.1)	116 (44.7)	59 (24.5)	128 (48.7)	42 (16.1)	114 (43.5)
III	442	357	123 (27.8)	171 (47.9)	171 (39.2)	268 (58.3)	51 (12.4)	149 (35.4)
IV	497	456	113 (22.7)	277 (60.7)	184 (40.7)	355 (81.2)	73 (14.6)	218 (46.8)
V	195	202	46 (23.6)	99 (49.0)	126 (66.1)	179 (91.6)	44 (26.5)	92 (51.3)
VI	395	395	119 (30.1)	223 (56.4)	249 (63.0)	317(80.2)	195 (49.3)	240 (60.7)
VII	800	800	434 (54.3)	630 (78.8)	385 (48.1)	559 (69.9)	344 (43.0)	556 (69.5)
VIII	800	800	191 (23.9)	394 (49.3)	197 (24.6)	357 (44.6)	264 (33.0)	403 (50.4)
IX	800	800	230(28.7)	618(77.2)	284(35.5)	572(71.5)	326(40.7)	595(74.4)
X	800	800	356(44.5)	620(77.5)	286(35.7)	525(65.6)	276(34.5)	535(66.9)
XI	800	800	55(27.5)	76(38)	44(22)	71(35.5)	29(14.5)	49(24.5)
XII	800	800	104(52)	105(52.5)	80(40)	67(33.5)	56(28)	25(12.5)
XIII	2007	2029	589(29.4)	1009(49.7)	407(20.3)	784(38.6)	670(33.4)	1011(49.8)
XIV	1555	1201	742(47.7)	641(53.4)	513(33)	491(41)	557(35.8)	384(32)
XV	800	800	641(80.1)	582(77.1)	559(70)	626(78)	647(81)	612(76.5)

Phase	Vaccination		Number & % animals showing titres ≥1.8 $log10_{10}$ against FMDV					
			Type O		Type A		Type Asia 1	
	Pre	Post	Pre-vac	Post-vac	Pre-vac	Post-vac	Pre-vac	Post-vac
II	3194	3600	1323(41.4)	2132(59.2)	1065(33.3)	1906(60)	1191(37.3)	1940(54)
III	3900	3908	2011(51.6)	2582(66.1)	1678(43)	2320(59.4)	1598(41)	2142(54.8)

Overall herd immunity and sero-conversion is very good in Gujarat

Sero-monitoring Haryana

Eight districts of Haryana namely, Bhiwani, Fatehabad, Hisar, Jhajjar, Jind, Rohtak, Sirsa and Sonipat were covered under FMDCP in 2003-04 (filled red) and later in 2010-11; rest of the districts (filled green) were included

Overall post-vac response is very good at 80% against all the three serotypes, and this has been well reflected as drastic reduction in occurrence of the disease in the state.

Table: 15 Result of seroconversion in Haryana (2003-04).

Phase	Vaccination		Number & % animals showing titres ≥1.8 $log10_{10}$ against FMDV					
			Type O		Type A		Type Asia 1	
	Pre	Post	Pre-vac	Post-vac	Pre-vac	Post-vac	Pre-vac	Post-vac
II	1558	1558	NA	1065(68.3)	NA	859 (55.1)	NA	831 (53.3)
III	1585	1585	NA	1146(72.3)	NA	1007(63.6)	NA	1005(63.4)
IV	1589	1552	953 (60.1)	1222(78.7)	668 (42.1)	887 (57.1)	844(53.2)	1170(75.3)
V	1600	1599	955 (59.7)	1352(84.5)	813 (50.8)	1274(79.6)	941(58.8)	1353(84.5)
VI	1496	1499	995 (66.5)	1306(87.1)	895 (59.8)	1229(82.0)	844(56.4)	1118(74.6)
VII	1562	1574	856(54.8)	1296 (82.3)	1021(65.3)	1380(87.6)	888 (56.8)	1317 (83.6)
VIII	1547	1540	949(61.3)	1289 (83.7)	877 (56.6)	992 (64.4)	765 (49.4)	1101 (71.4)
IX	1497	1476	647(43.2)	1140(77.2)	590(39.4)	1022(69.2)	410(27.4)	879(59.6)
X	1420	1439	851(59.9)	1350(93.8)	615(43.3)	1003(69.7)	587(41.3)	1145(79.5)
XI	1500	1464	734(48.9)	1302(88.9)	546(36.4)	1180(80.6)	455(30.3)	1109(75.8)
XII	1360	1210	593(43.6)	975(80.6)	520(38.2)	989(81.7)	474(34.9)	896(74.1)
XIII	1590	1600	925(58.2)	654 (82.8)	218(27.6)	630(79.8)	185(23.4)	616(78.0)
XIV	1580	1580	627(39.7)	1327(84.0)	594(37.6)	1279(81.0)	536(33.9)	1272(80.5)
XV	1600	1600	963(60.2)	1286(80.4)	856(53.5)	1207(75.4)	724(45.3)	1182(73.9)
XVI	1600	1600	913(57.1)	1335(83.4)	813(50.8)	1351(84.4)	983(61.4)	1409(88.1)

Seroconversion is likely to improve after subsequent vaccinations.

Table: 16 Result of seroconversion in Haryana (2010-11).

Phase	Vaccination		Number & % animals showing titres ≥1.8 $log10_{10}$ against FMDV					
			Type O		Type A		Type Asia 1	
	Pre	Post	Pre-vac	Post-vac	Pre-vac	Post-vac	Pre-vac	Post-vac
I	3086	2354	1049(43.9)	1790(76.1)	988(41.4)	1789(76.0)	715(30.0)	1469(62.4)
II	2586	2594	1081(41.8)	1876(73.5)	986(38.1)	727(28.1)	986(38.1)	1537(60.2)
III	2555	2562	1092(42.5)	1809(71.2)	1113(43.3)	1856(73.1)	650(25.3)	1576(62.1)
IV	2565	2575	1043(40.1)	2049(79.5)	893(34.8)	1811(70.3)	840(32.7)	1700(66)
V	2600	2600	1210(46.5)	1867(71.8)	1178(45.3)	1638(63)	1010(39)	1709(66)

Sero-monitoring in Delhi

Delhi was included under FMDCP in 2003-04

Districts included in 2003-04 (Red)

- Herd immunity is very good at >80%.

Table: 17 Result of seroconversion in Delhi (2003-04)

Phase	Vaccination		Number & % animals showing titres ≥1.8 $log10_{10}$ against FMDV					
			Type O		Type A		Type Asia 1	
	Pre	Post	Pre-vac	Post-vac	Pre-vac	Post-vac	Pre-vac	Post-vac
I	50	50	26 (53)	50 (100)	13 (26)	47 (94)	17 (34)	48 (96)
II	24	24	22 (91)	23 (96)	12 (40)	15 (62)	23 (95)	22 (86)
III	50	50	47 (94)	49 (98)	30 (60)	40 (80)	43 (86)	46 (92)
IV	50	46	38 (76)	38 (82.6)	14 (28)	40 (86.9)	27 (54)	41 (89.1)
V	44	53	26 (59)	47 (88.6)	23 (52.2)	37 (69.8)	32 (72.7)	41 (77.3)
VI	98	98	76 (77.5)	97 (98.9)	60 (61.2)	93 (94.9)	71(72.4)	97 (98.9)
VII	50	50	39(78)	44(88)	33(66)	43(86)	25(50)	41(82)
VIII	100	100	92 (92)	100 (100)	66 (66)	86 (86)	83 (83)	98 (98)
IX	100	NA	57(57)	NA	65(65)	NA	33(33)	NA
XI	200	NA	172(86)	NA	100(50)	NA	91(45.5)	NA
XIII	100	100	98(98)	98(98)	95(95)	100(100)	87(87)	100(100)
XIV	NA	200	NA	170(85)	NA	179(89.5)	NA	153(76.5)
XV	200	200	157(78.5)	171(85.5)	124(62)	158(79)	143(71.5)	156(78)

Sero-monitoring Punjab

Eight districts of Punjab namely, Amritsar, Bhatinda , Fatehgarh Sahib, Ferozpur , Mansa , Sangrur, Patiala and Gurdaspur were covered under FMDCP in 2003-04 (filled red) and later in 2010-11, rest of the districts (filled green) was included included

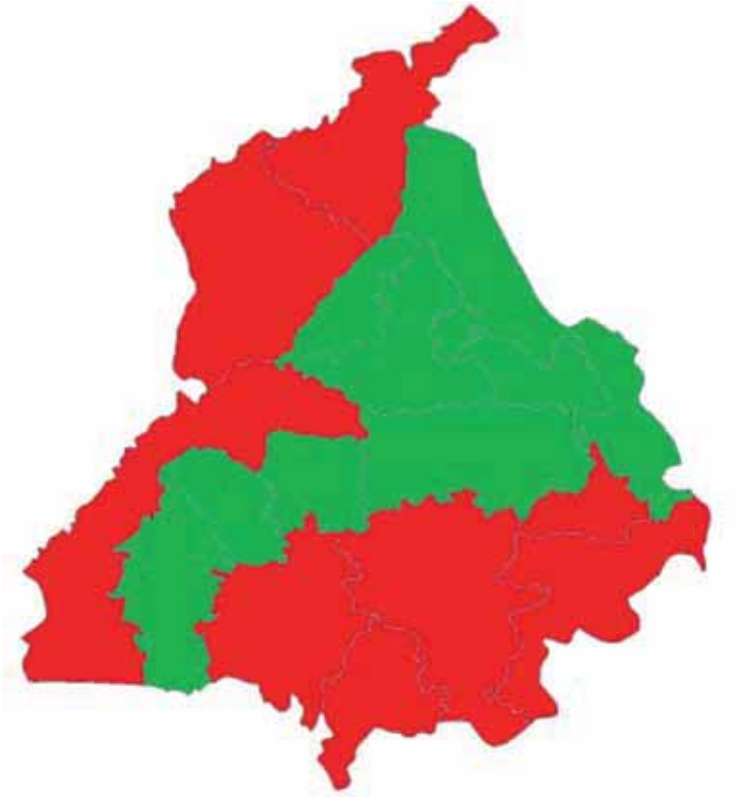


Table: 18 Result of seroconversion in Punjab (2003-04).

Phase	Vaccination		Number & % animals showing titres ≥1.8 $log10_{10}$ against FMDV					
			Type O		Type A		Type Asia 1	
	Pre	Post	Pre-vac	Post-vac	Pre-vac	Post-vac	Pre-vac	Post-vac
I	-	742	N.A.	187(25.2)	N.A.	90(11.5)	N.A.	273(49.5)
II	-	500	N.A.	219(43.8)	N.A.	113(20.9)	N.A.	279(58.1)
III	1084	1365	915(84.4)	1175(86.1)	816(75.3)	1007(73.8)	437(40.2)	573(42.0)
IV	1291	978	988(76.5)	792 (81.0)	794(61.5)	627 (64.1)	694 (53.8)	356(36.4)
V	1370	1139	477(34.8)	621(54.5)	445(32.8)	630(53.7)	513(38.5)	690(60.1)
VI	1509	1568	653 (43.3)	944 (60.2)	654 (43.3)	921 (58.7)	496 (32.9)	743 (47.4)
VII	1265	1432	520 (41.1)	898 (62.7)	356 (28.1)	639 (44.6)	448 (35.4)	696 (48.6)
VIII	984	1125	580(58.9)	825(73.33)	410(41.7)	643(57.2)	452(45.9)	741(65.9)
IX	1558	1546	1035(66.4)	1193(77.1)	831(53.3)	978(63.4)	926(59.4)	1132(73.2)
X	1592	1592	1030(64.7)	1231(77.3)	904(56.8)	1098(67.0)	970(61.0)	1156(72.6)
XI	1600	1600	991(61.9)	1186(74.1)	881(55.1)	1075(67.2)	965(60.3)	1142(71.4)
XII	1600	1556	1033(64.5)	1115(71.6)	914(57.1)	1026(65.9)	897(56.1)	NT
XIII	3320	3210	2002(60.3)	1920(59.8)	2048(61.7)	1868(58.2)	2114(63.7)	2494(77.7)
XIV	1998	1853	1061(53.1)	1333(72)	1214(61)	1099(59.3)	1520(76.1)	1553(83.8)

Districts included in 2010-11(Green)

Overall seroconversion and herd immunity is good.

Table: 18 Result of seroconversion in Punjab (2003-04).

Phase	Vaccination		Number & % animals showing titres ≥1.8 $log10_{10}$ against FMDV					
			Type O		Type A		Type Asia 1	
	Pre	Post	Pre-vac	Post-vac	Pre-vac	Post-vac	Pre-vac	Post-vac
I	1800	1800	797(44.3)	978(54.3)	704(39.1)	825(45.8)	615(34.2)	874(48.6)
II	1800	1782	1002(55.6)	1096(61.5)	902(50.1)	978(54.8)	904(50.2)	NT
III	2872	2390	1880(65.5)	1690(70.7)	1880(65.5)	1690(70.7)	1806(62.9)	1979(82.8)
IV	1917	1657	1094(57.1)	1125(68.7)	1317(69.3)	659(40)	1329(69.3)	1363(82.3)

Overall seroconversion and herd immunity is good, and this has been well reflected as drastic reduction in occurrence of the disease in the state.

Sero-monitoring in Uttar Pradesh

Sixteen districts of UP (Agra, Aligarh, Budaun, Bulandsahar, Etah, Ferozabad, Gautam Bhuddha Nagar, Gaziabad, Hatras, J.P.Nagar, Mathura, Meerut, Baghpat, Saharanpur, Muzaffarnagar and Muradabad) are covered under FMDCP in 2003-04 (Red). No new districts included during the expansion in 2010-11.

Seroconversion is very poor.

Table: 20 Result of seroconversion in Uttar Pradesh (2003-04).

Phase	Vaccination		Number & % animals showing titres ≥1.8 $log10_{10}$ against FMDV					
			Type O		Type A		Type Asia 1	
	Pre	Post	Pre-vac	Post-vac	Pre-vac	Post-vac	Pre-vac	Post-vac
II	139	407	0(0)	180(44.2)	0(0)	155(38.1)	0(0)	293(72.0)
III	1155	1584	399(34.5)	780(49.2)	494(42.7)	910(57.4)	490(42.4)	1138(71.8)
IV	1910	1770	344(18.0)	537(30.3)	610(31.9)	866(48.9)	519(27.2)	808(45.6)
V	1440	1591	516(35.8)	715(44.9)	625(43.4)	802(50.4)	684(47.5)	786(49.4)
VI	1488	1579	514(34.5)	968 (61.3)	520 (34.9)	826 (52.3)	400 (26.9)	838 (53.1)
VII	2833	2075	706 (24.9)	911 (43.9)	597 (21.1)	808 (38.9)	740 (26.1)	930 (44.8)
VIII	1904	2744	707(37.1)	1550(56.5)	502(26.4)	1310(47.7)	617(32.41)	1288(46.9)
IX	2762	3002	927(33.5)	1198(39.9)	617(22.34)	1095(36.5)	597(21.6)	1072(35.7)
XI	643	2206	47(7.3)	481(21.8)	68(10.6)	321(14.6)	385(59.9)	1103(50)
XII	1934	1535	184(9.5)	270(17.6)	252(13)	524(34.1)	424(21.9)	773(50.6)
XIII	983	2946	146(15)	955(32.4)	69(7.7)	780(26.5)	220(22.4)	1054(35.8)
XIV	4041	3800	2473(61.2)	2522(66.4)	2501(62)	2139(56.3)	2501(62)	1107(29)

Summary of overall sero conversion against each serotype and impact of vaccine (54 districts).

The herd immunity has progressively increased with minor aberrations that speak for positive impact of vaccination for last 6-7 years. Incidence/occurrence of the disease has also progressively declined in the southern region and also down to near zero in the states of Haryana and Punjab. There has been case of FMD in FMD-CP districts affecting very limited number of animals and did not spread due to surrounding herd immunity. Further, there has been reduction in severity of clinical sickness. Of late, due to delay in vaccination there have been a few sporadic incidences in vaccinated areas. There have been certain problems in maintaining 6 month interval between successive vaccinations. This problem can be circumvented/ compensated by using a vaccine having at least 6-8 PD50/dose. The results have been encouraging and should be further strengthened by constituting a National FMD Control Commission.

Table 21: Percent animals showing post vaccinal antibody titers of ≥1.8 log10 against FMD virus (2003-04, 54 districts)

Phase	Type O		Type A		Type Asia 1	
	Pre-vac	Post-vac	Pre-vac	Post-vac	Pre-vac	Post-vac
I	27.3	53.5	22.0	49.5	23.8	57.6
II	36.7	60.2	23.3	48.4	36.8	63.5
III	43.7	64.3	43.7	61.5	39.1	62.6
IV	41.2	62.3	42.4	67.5	36.2	61.1
V	38.0	39.3	46.3	65.6	40.8	59.4
VI	38.9	67.9	46.6	73.9	36.8	62.6
VII	39.7	68.5	39.4	67.1	35.1	62.8
VIII	42.3	68.7	37	58.6	33.5	57
IX	63.7	85.6	52	73.3	52.6	73
X	63.4	87.4	50.6	74.7	48.9	76.7
XI	44.1	57.8	37.8	51.5	39.3	59.3
XII	36.6	55.3	31.8	54.9	30	39.3
XIII	44.0	48.8	26.8	41.4	30.4	46.3
XIV	48.2	67.7	45.5	58.9	47.3	52.7
XV	46.5	71.6	50.1	76.0	54.4	78.5
XVI	62.2	87.3	59.8	58.4	63.7	90.3

Table 22: Percent animals showing post vaccinal antibody titers of ≥1.8 log10 against FMD virus (2010-11, 167 districts)

Phase	Type O		Type A		Type Asia 1	
	Pre-vac	Post-vac	Pre-vac	Post-vac	Pre-vac	Post-vac
I	33.4	65.3	21.4	50.7	10.9	40.7
II	37.5	66.5	23.5	46.3	20.5	38.2
III	36.5	63.1	28.3	52.1	34.2	56.1
IV	39.4	66.8	50.5	75.3	53.7	77.8
V	45.9	74.1	57.3	81.1	60.4	84.4

Table: 23a Summary of total number of serum samples tested under FMD CP (2003-04)

State/UT	Phase I		Phase II		Phase III		Phase IV		Phase V		Phase VI		Phase VII	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Andaman & Nicobar	-	-	-	-	154	162	149	146	126	122	270	270	265	265
Andhra Pradesh	800	800	-	800	800	800	800	800	800	800	800	800	800	800
Delhi	50	50	24	24	50	50	50	46	44	53	98	98	50	50
Gujarat	382	259	-	-	442	357	497	456	195	202	395	395	800	800
Haryana	-	-	-	1558	-	1585	1589	1552	1600	1599	1496	1499	1562	1574
Kerala	483 (pre) and 496(post) of Phase I, II and IV								290	290	70	70	300	300
Maharashtra	844	761	-	834	753	799	789	797	802	772	901	928	1000	1000
Punjab	-	742	-	500	1084	1365	1291	978	1370	1139	1509	1568	1265	1432
Tamilnadu	100	100	100	100			-	-	160	130	300	300		
Uttar Pradesh	-	-	139	407	1155	1584	1910	1770	1440	1591	1488	1579	2833	2075
subTotal	2659	2712	759	4223	4618	6702	7405	6545	6667	6568	7187	7337	9175	8596
Total	5371*		4982*		11320*		13950*		13235		14524		17771	

Table: 23b Summary of total number of serum samples tested under FMD CP (2003-04)

State/UT	Phase I		Phase II		Phase III		Phase IV		Phase V		Phase VI		Phase VII	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Andaman & Nicobar	251	251	228	228	-	-	-	-	180	180	283	283	794	593
Andhra Pradesh	800	800	800	800	800	800	800	800	800	800	800	800	800	800
Delhi	100	100	100	-	-	-	200	-	-	-	100	100	-	200
Gujarat	800	800	800	800	800	800	800	800	800	800	2007	2029	1555	1201
Haryana	1547	1540	1497	1476	1420	1439	1500	1464	1360	1210	1590	1600	1580	1580
Kerala	600 (pre)		600(post)		400	100	352	315	500	500	150	150	546	526
Maharashtra	1000	1000	1000	1000	1000	1000	1000	1000	980	980	950	1050	1040	1037
Punjab	984	1125	1558	1546	1592	1592	1600	1600	1600	1556	3320	3210	1998	1853
Tamilnadu	100	100	100	100	100	100	200	200	-	-	-	-	200	200
Uttar Pradesh	1904	2744	2762	3002	88	-	643	2206	1934	1535	983	2946	4041	3800
subTotal	8086	8460	9445	8952	6200	5831	7095	8385	8154	7561	10183	12168	12554	11790
Total	16546*		18397*		12031		15480		15715		22351		24344	

Table: 23c

State/UT	Phase XV		Phase XVI	
	Pre	**Post**	**Pre**	Post
Andaman & Nicobar	651	516		
Andhra Pradesh	800	800	800	800
Delhi	200	200		
Gujarat	800	800		
Haryana	1600	1600	1600	1600
Kerala	598	553		
Maharashtra	1098	1098	1055	1051
Punjab				
Tamilnadu	200	200		
Uttar Pradesh				
subTotal	5947	5767	3455	3451
Total		**11714**		**6906**
Grant total		**224634**		

* excluding the samples of Phase I, II, IV, VIII and IX from Kerala; Phase III and IV from Tamilnadu as samples of this phases were mixed up at the level of collection and labeling

**this includes all the samples tested

Table: 25 Summary of total number of serum samples tested under extended FMD CP (2010-11)

State/UT	Phase I		Phase II		Phase III		Phase IV		Phase V		Phase VI	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Andhra Pradesh	3600	3600	3480	3480	3600	3600	3600	3600	3600	3600	400	400
Haryana	3086	2354	2586	2594	2555	2362	2565	2575	2600	2600		
Karnataka	4587	4266	5401	4632	3864	3075	5053	5225	5916	5853		
Maharashtra	5988	6018	9435	9698	4721	4723	5250	5305	4891	4891		
Goa	381	391	383	378								
Punjab	1800	1800	1800	1782	2872	2390	1917	1657				
Gujarat	-	-	3194	3600	3900	3908						
Kerala			676	180	1631	1474	2378	2109	2043	1941		
Tamilnadu	5440	5440	5040	5240	4600	4600	5801	5843	6099	5697		
Puducherry	30	55	38	38	46	46						
Lakshadweep	107	107	-	-								
Sub total	25019	24031	32033	31622	22999	26178	26564	26314	25149	24582	400	400
Total		**49050**		**63655**		**53967**		**52878**		**49731**		**800**
Grant total	**265291**											

Serum testing under FMD Control Programme and Phase wise result

State	2005-06	2006-07	2007-08	2008-09	2009-10	2010-11	2011-12	2012-13	2013-14
Andaman& Nicobar	-	-	Phase III, IV & V	Phase VI	Phase VII	Phase VIII & IX	Phase XII	Phase XIII	Phase XIV & XV
Andhra Pradesh	Phase I	Phase I	Phase II, III, IV, V & VI	Phase VI	Phase VII, VIII & IX	Phase IX	Phase XI & XII | Ext Phase I &II	Phase XIII & XIV Ext Phase I, II, III & IV	Phase XV & XVI Ext Phase IV, V & VI
Karnataka								Ext Phase I, II & III	Ext Phase III, IV & V
Delhi	Phase I	Phase I	Phase II, III, IV, V & VI	Phase VI	Phase VII & VIII	Phase IX & XI	-	Phase XIII	Phase XIV & XV
Gujarat	Phase I	Phase I	Phase III, IV, V & VI	Phase VI	Phase VII & VIII	Phase IX & X	Phase XI & XII	Phase XIII & XIV	Phase XIV & XV Ext Phase II & III
Haryana	Phase II	-	Phase III, IV, V & VI	-	Phase VII & VIII	Phase IX & X	Phase XI & XII	Phase XIII & XIV Ext Phase I, II & III	Phase XV & XVI Ext Phase IV & V
Kerala	Phase I	Phase I	Phase II, IV, V & VI	Phase VI	Phase VII	Phase VIII, IX & X	Phase XI Ext Phase I	Phase XII & XIII Ext Phase I, II, III & IV	Phase XIV & XV Ext Phase IV & V
Maharashtra	Phase I	Phase I & II	Phase III, IV, V & VI	Phase VIII	Phase VI & VII	Phase IX & X	Phase XI & XII Ext Phase I	Phase XIII Ext Phase I & II	Phase XIV, XV & XVI Ext Phase III, IV & V
Goa								Ext Phase I	Ext Phase I, II, III & IV
Punjab	Phase I	Phase I & II	Phase III, IV, V & VI	Phase VI & VII	Phase VII	Phase VII, VIII & IX	Phase X & XI Ext Phase I	Phase XI & XII Ext Phase I & II	Phase XIII & XIV Ext Phase III & IV
Tamil Nadu	Phase I	Phase I	Phase II, III, IV & VI	Phase VII	Phase IX	Phase VIII & X	Phase XI Ext Phase I	Ext Phase II & III	Phase XIV & XV Ext Phase III, IV & V
Pondicherry								Ext Phase I, II & III	
Lakshadweep								Ext Phase I	
Uttar Pradesh	**Phase II**	**Phase II**	**Phase II, III, IV & VI**	**Phase VII**	**Phase VI & VII**	**Phase VIII & IX**	**Phase IX**	**Phase XI & XII**	**Phase XIII & XIV**

11.2 Sero-monitoring of post vaccinal immunity in animals vaccinated under ASCAD/RKVY programmes: sampling was done at random, and not as per FMD-CP format

State	Number of sample tested	Species	Number & % animals showing titres ≥1.8 $log10_{10}$ against FMDV					
			Type O		Type A		Type Asia 1	
			Pre-vac	Post-vac	Pre-vac	Post-vac	Pre-vac	Post-vac
Madhya P	6556+5277	Bovine	1185 (10.1)	2051(17.3)	818(6.9)	1487(12.6)	1090(9.2)	2002(16.9)
Arunachal P	269+269	Bovine	79(29.3)	228(84.7)	62(23)	206(76.5)	51(18.9)	184(68.4)
Bihar	528+456	Bovine	133(25.2)	248(54.4)	54(10.2)	172(37.7)	94(17.8)	268(59)
Tripura	1379+1265	Bovine	249(18.1)	750(59.3)	358(26)	820(64.8)	380(27.6)	853(67.4)
Asom	900+900	Bovine	187(20.8)	577(64.1)	106(11.8)	382(42.4)	102(11.3)	374(41.6)
Himachal P	300+300	Bovine	182(60.7)	227(75.7)	127(42.3)	179(59.7)	185(61.7)	177(59)
Odisha	78+130	Bovine	33(42.3)	79(61)	18(23.1)	43(33.1)	33(42.3)	77(59.2)
J&K	50+63	Bovine	18(36)	43(68.3)	7(14)	35(55.6)	9(18)	44(70)
Mizoram	160+160	Bovine	22(13.8)	77(48.1)	7(4.3)	49(30.1)	0(0)	16(10)
Manipur	406+406	Bovine	186(45.8)	369(91)	178(43.8)	348(85.7)	96(23.6)	322(79.3)
Nagaland	110+110	Bovine	42(38.2)	101(91.2)	51(46.4)	96(87.3)	63(57.7)	106(96.4)
West Bengal	32+32	Bovine	22(68.8)	31(96.9)	17(53.1)	29(90.6)	10(31.3)	31(96.9)

Percentage serum samples having protective titre against serotypes O, A and Asia 1 is given in parenthesis

12 Production, Standardization and Supply of Diagnostic Reagents

For production of reagents, the vaccine virus strains {O (IND R2/75), Asia1 (IND 63/72),) and A (IND 40/00)} were bulk produced in roller culture vessels and purified by density gradient centrifugation. Antibodies against purified virus was raised and titrated against homologous as well as heterologous virus. Freeze dried and standardized serum antibodies (rabbit and guinea pig) and known positive antigen (killed) of all three serotypes were supplied to all the centres and network units for use in virus serotyping ELISA and LPB-ELISA. Recombinant 3AB3 NSP was produced as per requirement.

During the period, r3AB3 DIVA Kit for FMD to test a total number of 93,900 serum samples was produced. Similarly, virus serotyping Kits for 27,000 tests and LPB-ELISA Kits for 2,51,500 were produced. The kits have been supplied to the AICRP units FMD Regional centers/network units for sero-surveillance and monitoring of FMD and SAARC Countries. Further they were also provided to vaccine manufacturing companies and government's establishments.

Supply of Diagnostic kits

	LPBE	S-ELISA	DIVA
2009-10	80,000	7,000	54,485
2010-11	82,800	9,000	71,940
2011-12	1,54,600	10,000	61,670
2012-13	1,77,850	16,500	85,350
2013-14	2,36,640	21,500	87,850

13 Research Projects

S.No	Title	PI	Duration
1	Cataloging and Maintenance of National FMD virus repository during 2014-15	B. Pattnaik	2014-15
2	Production, standardization and supply of diagnostic reagents for FMD diagnosis and surveillance during 2014-15	B. B. Dash	2014-15
3	Seromonitoring of pre and post vaccinal immunity against FMD during 2014-15	B. B. Dash	2014-15
4	Random serosurveillance of FMD in India during 2014-15	B. B. Dash	2014-15
5	Antigenic and molecular characterization of serotype A FMD viruses during 2014-15	J.K. Mohapatra	2014-15
6	Genetic and antigenic analysis of foot and mouth disease virus serotype O in India during 2014-15	Saravanan S	2014-15
7	Evaluation of foot and mouth disease virus serotype O candidate vaccine panel with recent field isolates	Saravanan S	2014-15
8	Development of an online Foot and Mouth Disease Decision Support System (FMD-DSS) for control of FMD in India	G.K.Sharma	2013-15
9	Spatial and temporal distribution of foot and mouth disease in India during 2001-2011	G.K.Sharma	2012-15
10	Genetic and antigenic analysis of foot-and-mouth disease virus serotype Asia1 during 2014-15	G.K.Sharma	2014-15
11	Development of single chain variable fragment antibodies against structural proteins of FMD virus through phage display	G.K.Sharma	2014-16
12	Epidemiology of Foot-and-Mouth Disease in Sheep and Goats in India during 2014-15	M. Rout	2014-15
13	Epidemiology of Foot and Mouth Disease in pigs during 2014-15	M. Rout	2014-15
14	Development of 3B epitope deletion mutant of FMDV serotype O.	J.K.Biswal	2013-15
15	Development of improved thermo-stable FMD virus serotype O by reverse genetics technique	J.K.Biswal	2013-15
16	Recombinant Capsid protein for Immunodiagnosis of FMD Component 1 Prokaryotic expression of serotype O recombinant capsid protein and its antigenic characterization	J.K.Biswal	2014-15
17	Expression profiling of bovine Toll Like Receptors (TLRs) in response to FMD Vaccine.	R.Ranjan	2013-15
18	Understanding interactions between foot and mouth disease virus non-structural protein 2C with host proteins	S. Mahajan	2014-15
	Collaborative Projects		
1	An effective vaccination programme for the eradication of foot-and-mouth disease from India (Collaborating organization: The Pirbright Institute, UK)	B Pattnaik	2014-16
2	Understanding FMD viral ecology and landscape epidemiology towards control and eradication (Collaborating organization: The Plum Island Animal Disease Center, US)	J.K. Mohapatra	2014-16
3	Assessment of socio-economic impact of FMD and its control in India (Collaborating Institute: PD-ADMAS, ICAR, India)	G.Govindaraj	2013-15

14 Publications/ Abstracts/Presentations in Conferences

Publications in Peer Reviewed Journals

1. Bisht P, Mohapatra JK, Subramaniam S, Das B, Pande V, Biswal JK, Sharma GK, Rout M, Ranjan R, Dash BB, Sanyal A, Pattnaik B (2013). Efficient rescue of foot-and-mouth disease virus in cultured cells transfected with RNA extracted from clinical samples. Journal of Virological Methods. 196:65-70
2. Biswal J.K, Jena S, Mohapatra J.K, Bisht P, Pattnaik B (2014). Detection of antibodies specific for foot-and-mouth disease virus infection using indirect ELISA based on recombinant nonstructural protein 2B. Archive of Virology
3. G. R. Gowane, A. K. Sharma, M. Sankar, K. Narayanan, Punam Bisht, S. Subramaniam and B. Pattnaik (2014). The expression of IL6 and 21 in crossbred calves upregulated by inactivated trivalent FMD vaccine. Animal Biotechnology. 25: 108–118
4. Mahajan, S., Mohapatra, J.K., Pandey, L.K., Sharma, G.K., Pattnaik, B., 2013. Truncated recombinant non-structural protein 2C-based indirect ELISA for FMD sero-surveillance. Journal of Virological Methods.193 (2013) 405-414
5. Mohapatra J.K, Pandey L.K, Pattnaik B (2013). RNA structure disrupting G320-T transversion within the short fragment of the 5' untranslated region prevents rescue of infectious foot-and-mouth disease virus. Journal of Virological Methods. doi: 10.1016/j.jviromet.2013.11.007
6. Pandey LK, Mohapatra JK, Subramaniam S, Sanyal A, Pande V, Pattnaik B (2014) Evolution of serotype A foot-and-mouth disease virus capsid under neutralizing antibody pressure in vitro. Virus Research. 181:72-6
7. Rout M, Senapati MR, Mohapatra JK, Dash BB, Sanyal A, Pattnaik B (2014). Serosurveillance of foot-and-mouth disease in sheep and goat population of India. Preventive Veterinary Medicine. 113(2):273-7
8. Rout. M, J K Biswal, B B Dash, R Hegde, S Subramaniam and J K Mohapatra (2014). Investigation of foot-and-mouth disease outbreaks in Chikkaballapur district of Karnataka. Indian Journal of Animal Sciences. 84 (3): 231–235
9. Sarangi LN, Mohapatra JK, Subramaniam S, Sanyal A, Pattnaik B (2013).Antigenic site variation in foot-and-mouth disease virus serotype O grown under vaccinal serum antibodies in vitro. Virus Research. 176(1-2):273-9
10. Subramaniam S, Mohapatra JK, Sharma GK, Das B, Dash BB, Sanyal A, Pattnaik B (2013). Phylogeny and genetic diversity of foot and mouth disease virus serotype Asia1 in India during 1964-2012. Veterinary Microbiology. 167(3-4):280-8
11. Subramaniam S, Sanyal A, Mohapatra JK, Sharma GK, Biswal JK, Ranjan R, Rout M, Das B, Bisht P, Mathapati BS, Dash BB, Pattnaik B (2013). Emergence of a novel lineage genetically divergent from the predominant Ind2001 lineage of serotype O foot-and-mouth disease virus in India. Infection Genetics and Evolution. 18:1-7

Book chapter

1. S. Subramaniam, J. K. Mohapatra, A. Sanyal, J. Misri, K.M.L. Pathak and B. Pattnaik (2013) Emergence and re-emergence of different genotypes/lineages of Foot-and-mouth Disease virus in India. In: Pathak, K.M.L., Pattnaik, B., Rout, M., Bhasin, V., Sumption, K., Prasad, G, Misri, J.

(Eds). Topics in Foot and mouth disease. Indian council of Agricultural Research. pp. 79-94

2. M. Madhanmohan, M. Rout, J. Misri, B.B. Dash, B. Pattnaik, V. A. Srinivasan (2013). Role of Small Ruminants and Pigs in the Epidemiology of Foot and Mouth Disease. In: Pathak, K.M.L., Pattnaik, B., Rout, M., Bhasin, V., Sumption, K., Prasad, G, Misri, J. (Eds). Topics in Foot and mouth disease. Indian council of Agricultural Research. pp. 133-147
3. G K Sharma, J K Biswal, S Mahajan and R Ranjan (2013). Role of bull semen in epidemiology of Foot and Mouth Disease. In: Pathak, K.M.L., Pattnaik, B., Rout, M., Bhasin, V., Sumption, K., Prasad, G, Misri, J. (Eds). Topics in Foot and mouth disease. Indian council of Agricultural Research. pp. 148-154
4. B S Mathapati, A Sanyal, G K Sharma and S. Subramaniam (2013). Antigenic Cartography of Indian FMD Virus isolates of serotype O. In: Pathak, K.M.L., Pattnaik, B., Rout, M., Bhasin, V., Sumption, K., Prasad, G, Misri, J. (Eds). Topics in Foot and mouth disease. Indian council of Agricultural Research. pp. 170-177
5. A.K.Sharma, G.R. Gowane, G. Prasad, KML Pathak and B. Pattnaik (2013). Role of Host Genetics in Immune response to Foot-and-Mouth Disease. In: Pathak, K.M.L., Pattnaik, B., Rout, M., Bhasin, V., Sumption, K., Prasad, G, Misri, J. (Eds). Topics in Foot and mouth disease. Indian council of Agricultural Research. pp. 213-235
6. M. Rout, J.K. Biswal and G.K. Sharma (2013). Microevolution of Foot and Mouth Diseases Virus. In: Pathak, K.M.L., Pattnaik, B., Rout, M., Bhasin, V., Sumption, K., Prasad, G, Misri, J. (Eds). Topics in Foot and mouth disease. Indian council of Agricultural Research. pp. 236-256
7. M. Rout, R. Ranjan, B.B. Dash and J. Misri (2013). Methods in Diagnosis of Foot and Mouth Disease. In: Pathak, K.M.L., Pattnaik, B., Rout, M., Bhasin, V., Sumption, K., Prasad, G, Misri, J. (Eds). Topics in Foot and mouth disease. Indian council of Agricultural Research. pp. 292-330
8. M. Rout, B.B. Dash and B. Pattnaik (2013). New Generation Vaccines for Foot and Mouth Disease. In: Pathak, K.M.L., Pattnaik, B., Rout, M., Bhasin, V., Sumption, K., Prasad, G, Misri, J. (Eds). Topics in Foot and mouth disease. Indian council of Agricultural Research. pp. 394-414

Lead Papers

1. Saravanan Subramaniam, Bana.B.Dash and Bramhadev Pattnaik (2013). Scenario of Foot and Mouth Disease in India. All India conference on Livestock and Dairy Development, Gujarat, India. 11 June, 2013
2. B. Pattnaik, M. Rout, R. Ranjan, J.K. Mohapatra, B.B. Dash and A. Sanyal. Status of Foot-and-Mouth Disease in South Asia. Compendium of Veterinary Pathology Congress - 2013 during 21st - 23rd November'2013, held at College of Veterinary Science and Animal Husbandry, Bhubaneswar.

Abstract presented/published in conferences

1. Amiya Kumar Mohapatra, Jajati K. Mohapatra, Laxmi K. Pandey, Aniket Sanyal, B. Pattnaik (2013). Recombinant non-structural protein 3B based indirect ELISA for Foot-and-mouth disease sero-surveillance. Asia-Pacific Congress of Virology. Virocon-2013, 17th-20th December 2013.
2. Gaurav Kumar Sharma, Sonalika Mahajan, Rakesh Matura, Saravanan S and B Pattnik (2013). Production and Characterization of Single Chain variable fragment (scFv) antibodies against 3ABC non-structural protein in Immuno-diagnosis of FMD. Asia-Pacific Congress of Virology. Virocon-2013, 17th-20th December 2013.
3. Jitendra K Biswal, Bramhadev Pattnaik (2013). Detection of Foot-and-mouth disease virus infection specific antibody using recombinant non-structural protein 2B based indirect ELISA. Asia-Pacific Congress of Virology. Virocon-2013, 17th-20th December 2013.
4. L. Dash, G.K. Sharma and J.K. Biswal (2013). Single domain heavy chain antibodies: A new tool in

Foot-and-mouth disease diagnosis (2013). Asia-Pacific Congress of Virology. Virocon-2013, 17th-20th December 2013.

5. M. Rout, A.V. Krishna Mohan and J.K. Mohapatra. (2013). Serosurvey of Foot-and-Mouth Disease in Small Ruminants of Andhra Pradesh. Compendium of Veterinary Pathology Congress - 2013 during 21st - 23rd November'2013, held at College of Veterinary Science and Animal Husbandry, Bhubaneswar.
6. M. Rout, M.R. Senapati, A. Kanani and J.K. Mohapatra. (2013). Serological Indication of Foot-and-Mouth Disease Virus Activity in Organized Dairy Farms of Gujarat. Compendium of Veterinary Pathology Congress - 2013 during 21st - 23rd November'2013, held at College of Veterinary Science and Animal Husbandry, Bhubaneswar.
7. M. Rout, S.S. Pawar, N.S. Nair, E.D. Benjamin, A.P. Usha, K.S. Anil, J.K. Mohapatra, S.Subramaniam, B. Pattnaik (2013). Detection of foot-and-mouth disease virus infection in cattle and pig farm in Kerala. Asia-Pacific Congress of Virology. Virocon-2013, 17th-20th December 2013.
8. R. Ranjan and B. Pattnaik (2013). Reverse transcription- Loop mediated isothermal amplification assay (RT- LAMP) is a tool for rapid detection of foot and mouth disease virus. Veterinary Pathology Congress- 2013. National symposium on "advances and applications of diagnostic pathology for disease management in livestock, poultry, pet, fish, laboratory animal and wildlife" at Orissa University of agriculture and technology, Bhubaneswar, Odisha, India, 21- 23 November 2013
9. Rajeev Ranjan, Bramhadev Pattnaik (2013). Foot-and-mouth Disease: Management and control. Asia-Pacific Congress of Virology. Virocon-2013, 17th-20th December 2013.
10. Sonalika Mahajan, Gaurav K. Sharma, Bramhadev Pattnaik (2013).The autophagy regulatory protein Beclin-1 interacts with the foot-and-mouth disease virus non-structual protein 2C. Asia-Pacific Congress of Virology. Virocon-2013, 17th-20th December 2013.

15 Human Resource Development

Participation in conference/workshop

1. Scientists of the Project attended 24th Annual review Meeting (ARM) of AICRP on FMD during 21-22 Sep 2013 at Veterinary collage, Pondicherry.
2. Scientists participated in the Asia-Pacific Congress of Virology. Virocon-2013, 17th-20th December 2013, Delhi
3. Scientists participated in Veterinary Pathology Congress- 2013 held at Bhubaneswar, 21- 23 November 2013

International Meetings

1. Dr. B. Pattnaik and Dr. B. B. Dash participated in the Second Regional Workshop on FMD – PCP for South Asian Countries at Agra during 2nd to 4th October 2013, Organized by FAO at Agra, India
2. Dr. B. Pattnaik attended face to face meeting with OIE Scientific Commission for Animal Diseases on official FMD Control Programme (FMD-CP) in India at Paris, France from 12-13 February, 2014
3. Dr. B. Pattnaik and Dr. G.K.Sharma attended the third Laboratory Directors Meeting and Workshop on Biorisk Management: Enhancing Laboratory Expertise through Quality Management Systems in SAARC Countries" 5-6 March 2014, New Delhi, India.

Training Organized

Six training Programmes on sandwich ELISA, SdLPBE, LPBELISA and DIVA were organized, in which scientists from network units/regional centres of AICRP on FMD and FMD vaccine manufacturing companies,.

S. No	Title of Training	Participants
1	Training on FMD diagnosis (LPB-ELISA, Typing- ELISA and DIVA- ELISA)	AICRP Network Unit Agartala, Tripura.
2	Training on FMD diagnosis (LPB-ELISA and DIVA- ELISA)	AICRP on FMD Network Unit of Shimla, Himachal Pradesh and Regional Center of Pune, Maharashtra.
3	Training on FMD diagnosis (LPB-ELISA and DIVA- ELISA)	the AICRP on FMD of Jalandhar, Palode and Bhopal.
4	Training on FMD diagnosis (LPB-ELISA and DIVA- ELISA)	AICRP on FMD Network Unit, Ahmadabad
5	Hands on the training on throughput Single Dilution Liquid Phase Blocking ELISA (SdLPBE)	AICRP on FMD Centers Ranipet, Bangalore, Hyderabad, Mathura and Pune.
6	Hands on the training on throughput Single Dilution Liquid Phase Blocking ELISA (SdLPBE)	AICRP on FMD Centers Bangalore, Ranipet, Pune, Hyderabad, Jaipur, Imphal, Jammu, Guwahati, Bhopal, Kohima, Port-Blair, Hisar, Aizwal and Kerala

16 Reports and Recommendations

16.1 Proceedings/Recommendations of the 24th Annual Review Meeting

S.No.	Action Recommended	Action Taken
1.	Disease reporting system to be strengthened for initiating rapid response for effective surveillance and control of FMD in the country. (Action: DADF/ State AH Depts./ AICRP centers and collaborating units)	Noted and facilities of internet and Telefax have been provided to the AICRP centres for rapid disease reporting system and surveillance. However, disease reporting is the primary mandate of State AH departments.
2.	Pilot study on effect of serotype 'O' monovalent FMD vaccine with higher payload to be carried out in selected areas. (Action: DADF and PDFMD)	DADF will provide required vaccine doses and select the area for use of Serotype O monovalent FMD vaccine, followed by studies on the vaccine response.
3.	All scientific/diagnostic support would be provided to SAARC member countries for control of FMD in Indian subcontinent. (Action: PDFMD)	Noted and necessary scientific/ diagnostic support has been extended to SAARC member countries from time to time through the regional office of FAO, Kathmandu.
4.	Vaccine candidate strains to be evaluated for serotype A deletion mutant. (Action: PDFMD)	Work on Selection of serotype A vaccine candidate is in progress, looking at the dominance deletion mutants (VP3-59).
5.	Timely serum sample collection need to be done from animal above 1.5 years of age for seromonitoring under FMD-CP. (Action: DADF/ State AH Dept.)	The concerned state AH departments have been intimated to follow the guide line.
6.	In case of socioeconomic assessment studies of FMD in Tami Nadu, Erode district need to be included instead of Namakkal for evaluation. (Action: PDADMAS/NIVEDI)	Noted and PD-ADMAS/ NIVEDI has been intimated to include Erode.
7.	Vaccination against FMD needs to be done twice in a year (six-monthly) with coverage of at least 80-90% to bring down incidences of FMD. (Action: DADF/State AH dept.)	Concerned have been intimated.
8.	Sporadic incidences of FMD should not be declared as an outbreak, rather an incidence or occurrence. (Action: PDFMD/DADF/State AH Depts.)	Concerned have been intimated to follow the guide line.
9.	All the regional and collaborative centers should adopt 5 villages to actively monitor FMD vaccination and serosurveillance by themselves. (Action: PDFMD/DADF/State AH Depts.)	Noted and being implemented.
10.	Studies on the NSP-antibody prevalence status in the animals of Andaman & Nicobar Islands to be intensified as a step for making it a potential FMD-free zone. (Action: CARI, Port-Blair)	Noted and action has been initiated through CARI, Portblair.

11.	Work in Collaborative centers at Uttar Pradesh, Bihar, Rajasthan, Odisha, West Bengal, Jammu & Kashmir and Arunachal Pradesh need to be improved to meet the target. (Action: PDFMD/ respective state AH Depts.)	Noted and necessary action has been initiated
12.	The network units of AICRP on FMD to be named as collaborative centers. (Action: PDFMD)	Noted and implemented. Necessary correction has been made in the XII Plan EFC memo.
13.	There will be six regional centers and the Mathura center to be converted into collaborative center. (Action: PDFMD)	Noted and will be implemented after approval of EFC is communicated.
14.	11 new collaborative centers will be opened in the states of Goa, Uttarakhand, Jharkhand, Chhattisgarh, Andaman & Nicobar Islands, Meghalaya, Puducherry, NRC on Yak, Dirrang, Arunachal Pradesh and Lakshadweep to cover the entire country for better FMD surveillance. (Action: PDFMD)	10 new collaborative centres are approved in the XII plan EFC meeting. These are at Chhatishgarh, Jharkhand, Uttarakhand, A&N Islands, Goa, Lakshadweep, NRC on Yak, Dirang, NRC on Mithun, Jharnapani, IVRI-ERS, Kolkata and RIVER, Pondicherry. These will be functional with the final approval of the XII plan EFC.
15.	All the stakeholders involved in FMD related research across the country need to be participate in the coming ARM. A list of Scientists working on FMD in the country will be prepared. (Action: J. Misri, PS, ICAR)	Noted for implementation in the25th ARM of AICRP on FMD. List of such scientists is being prepared.
16.	Timely release of adequate funds of PDFMD for monitoring and assessment of FMD vaccine response. (Action: DADF)	Noted and DADF has been intimated.
17.	Rajasthan need to be included in the FMD-CP area immediately to check the risk of spread of FMD to the neighbouring states for creating a FMD-free zone in North India. (Action: DADF)	Noted, and DADF have been communicated.
18.	Each and every occurrence of FMD should be investigated by regional/collaborative centers of AICRP. (Action: PDFMD/ State AH Depts.)	Noted and being implemented through the AICRP centres.

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