

वार्षिक प्रतिवेदन ANNUAL REPORT 2010-11

परियोजना निदेशालय खुरपका एवं मुंहपका रोग
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उत्तराखण्ड (भारत)

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Nainital, Uttarakhand (India)



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Front Cover Page

Himalaya Views from Mukteswar, Uttarakhand (India)

Back Cover Page

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खुरपका एवं मुंहपका रोग परियोजना निदेशालय (पी.डी. एफ.एम.डी.) भारतवर्ष का एक प्रमुख संस्थान है, जिसकी स्थापना सन् 1968 में ए.आई.सी.आर.पी. के रूप में हुआ था। पिछले चार दशकों के दौरान निदेशालय का व्यापक विस्तार हुआ तथा सन् 2001 में परियोजना निदेशालय का दर्जा प्राप्त हुआ। पूरे भारतवर्ष में इस निदेशालय के अंतर्गत 23 प्रादेशिक तथा क्षेत्रीय इकाई हैं। खुरपका एवं मुंहपका रोग (एफ.एम.डी) रोग गो वशीय पशुओं में तीव्र गति से फैलने वाला विषाणु जनित संक्रामक रोग है। खुरपका एवं मुंहपका रोग भारतवर्ष में 'ओ', 'ए' तथा एशिया-1 सिरोटोइप द्वारा पूरे वर्ष होता है। यह रोग केवल भारतवर्ष ही नहीं बल्कि समूचे विश्व की अर्थव्यवस्था के लिए महत्वपूर्ण है।

परियोजना निदेशालय में एफ.एम.डी. के निदान, सर्वेक्षण एवं शोध क्षेत्रों में पारंपरिक कौशल के साथ-साथ आधुनिक तकनीक के प्रयोग होता है। निदेशालय इस रोग की पहचान, सर्वेक्षण एवं रोकथाम की दिशा में कार्य करते हुए उनके उन्मूलन की अंतिम लक्ष्य रखती है। साथ ही साथ निदेशालय एफ.ए.ओ. के दक्षिण पूर्व एशिया के प्रमुख प्रयोगशाला केंद्र के रूप में भी कार्य कर रहा है तथा ओ.आई.ई./एफ.ए.ओ. के एफ.एम.डी. वैश्विक नेटवर्क का भी सहयोगी है। निदेशालय भारतवर्ष एवं दक्षिण पूर्व एशिया के अन्तर्गत देशों में खुरपका मुंहपका रोग की रोकथाम की योजना के लिए तकनीकी एवं वैज्ञानिक सहयोग प्रदान करती है।

निदेशालय विश्वस्तरीय पर होने वाली टीका मिलान (वैक्सिन मैचिंग) परियोजना में भाग ले रहा है।

भारत सरकार के पशुपालन एवं मत्स्य विभाग द्वारा सन् 2003-04 से चलाए गये खुरपका एवं मुंहपका रोग रोकथाम के कार्य में परियोजना निदेशालय अहम भूमिका निभाती है।

सन् 2010-11 में भारत में कुल 180 खुरपका एवं मुंहपका रोग संक्रमण अंकित किये गये हैं जो कि गत वर्ष (2009-10 में 711) की तुलना में बहुत कम है। इसके पहले लगभग 4000 खुरपका एवं मुंहपका रोग संक्रमण इस देश में होता था, परंतु सन् 2006-07 के बाद इस योजना के सफलता के कारण खुरपका एवं मुंहपका रोग संक्रमण एवं इस रोग की तीव्रता में

बहुत कमी आई है। देश के कुछ भाग में खुरपका एवं मुंहपका रोग संक्रमण की संख्या में 50 से 80 प्रतिशत तक की कमी हुई है। परियोजना निदेशालय द्वारा चुने गये सही खुरपका एवं मुंहपका रोग विषाणु द्वारा विकसित टीका का पूरे देश में नियमित प्रयोग के साथ-साथ पशुओं एफ.एम.डी. विषाणु के प्रतिरोधक क्षमता की जांच एवं सही निदान हेतु प्रयोगात्मक नैदानिक कीट (एल.पी.बी.ई. एवं दीवा) के द्वारा ये संभव हुई।

सन् 2010-11 में समस्त आंचलिक क्षेत्र में खुरपका एवं मुंहपका रोग संक्रमण की संख्या में कमी आई है। ज्यादा संख्या में खुरपका एवं मुंहपका रोग की संक्रमण भारत के दक्षिण एवं उत्तर-पूर्व क्षेत्र में पाये गये हैं। उसके पश्चात् पूर्व, मध्य एवं पश्चिम क्षेत्र में तथा सबसे कम उत्तर क्षेत्र में पाये गये। इस वर्ष 622 नमूने जांच के लिए एकत्रित किये गये, जिसके सैण्डविच एलाइजा एवं एम.पी.सी.आर. द्वारा जांच के पश्चात् 377 नमूने खुरपका एवं मुंहपका रोग विषाणु ग्रसित पाये गये, जिसमें सिरोटोइप ओ-314; सिरोटोइप ए-18 तथा सिरोटोइप एशिया 1-45 पाए गए। सन् 2010-11 में सिरोटोइप-0 खुरपका एवं मुंहपका रोग विषाणु मुख्यतः देश के सभी क्षेत्र में पाया गया। परंतु सिरोटोइप ए-दक्षिण एवं उत्तर-पूर्व क्षेत्र में तथा सिरोटोइप-एशिया 1 – पश्चिम एवं पूर्व में मुख्यतः पाया गया। ये बिमारी मुख्यतः गो/महिषादी पशुओं के साथ-साथ सुअर, भेड़, बकरी, याक एवं मिथुन में भी पाया गया।

टीका मिलान (वैक्सिंग मैचिंग) कार्य द्वारा प्रमाणित किया गया कि देश में इस वर्ष संक्रमित समस्त खुरपका एवं मुंहपका रोग विषाणु सभी देश में उपलब्ध टीका द्वारा नियंत्रित किया जा सकता है।

एकत्रित नमूनों के विषाणुओं आनुवांशिकी निर्धारण कर न्यूक्लियोटाइड विभिन्नताओं की जांच पर पाया गया कि सिरोटोइप ओ. विषाणु मुख्यतः आईएनडी 2001, लिनिएज़ के अंतर्गत है, जोकि 2008 से नियमित रूप से पाया जा रहा है। इसके पश्चात् पैन एशिया 2 लिनिएज़ के विषाणु, कुछ संक्रमण में पश्चिम बंगाल, अरुणाचल प्रदेश, महाराष्ट्र एवं पंजाब में पाये गये हैं। सिरोटोइप ए. के समस्त विषाणु जीनोटाइप 18

(vii) के अंतर्गत है। सिरुटाइप एशिया 1 विषाणु लिनिएज सी के अंदर है जो कि 2005 से लगातार पाया जा रहा है। वर्तमान सिरुटाइप ए. विषाणु का जीनोटाइप VII के अंदर एक अलग विषाणु पाया गया है, जिसमें VP3 जीन में 59 न्यूक्लियोटाइड स्थान पर बदलाव पाया गया है जिसको VP3⁵⁹ डिलिसन वर्ग में जाना जाता है एवं यह विषाणु रोग संक्रमण में अहम विस्तार कर रही है। जिसकी सही एवं समय पर पहचान आवश्यक है। यह निदेशालय न्यूक्लियोटाइड विभिन्नताओं का निर्धारण किया जो कि समय पर सापेक्ष है। एक एम.पी.सी.आर. की जांच सुविधा विकसित किये है जो कि नमूने में खुरपका एवं मुंहपका रोग विषाणु का तुरंत पहचान कर लेता है।

निदेशालय में विकसित पी.सी.आर. तकनीक द्वारा गो/महिषादी वीर्य (सीमेन) में खुरपका एवं मुंहपका रोग विषाणु का पहचान किया जा सकता है। इसकी सहायता से कृत्रिम प्रजनन द्वारा खुरपका एवं मुंहपका रोग संक्रमित होने वाले पशुओं की संख्या में कमी आ सकती है। निदेशालय में एक जांच के द्वारा ये साबित हुआ कि खुरपका एवं मुंहपका

रोग विषाणु संक्रमण के बाद एक संक्रमित सांड के वीर्य में आठ महीना तक पाया जा सकता है।

राष्ट्रीय खुरपका एवं मुंहपका रोग सिरुसरवीलेन्स के अंतर्गत 31049 यदिछा संग्रहित सिरम नमूने का डीवा जांच किया गया, जिसमें 27 प्रतिशत पशु खुरपका एवं मुंहपका रोग विषाणु का वाहक के रूप में पाया गया।

निदेशालय में स्थापित राष्ट्रीय खुरपका मुंहपका रोग विषाणु संग्रहालय में अब तक 1712 आइसोलेट (ओ.-1102, ए.-276, सी.-15 एवं एशिया 1-319) उपलब्ध है।

ए.आई.सी.आर.पी.ऑन एफ.एम.डी. के अंतर्गत प्रादेशिक एवं क्षेत्रीय प्रयोगशाला के वैज्ञानिक अधिकारियों को परियोजना निदेशालय में खुरपका मुंहपका रोग विषाणु की सिरुटाइपिंग, डीवा, एल.पी.बी.ईलाइसा., एम.पी.सी.आर. आदि के बारे में नियमित रूप से प्रशिक्षण दिया जाता है। निदेशालय इन प्रयोगशाला को निरंतर समस्त डाइग्नोस्टिक किट्स आपूर्ति करता रहा है।

बी. पटनायक

Project Director's Report

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 the last four decades of its existence the scope of the project was expanded considerably 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.

This year witnessed a drastic reduction in number of FMD outbreaks across the country. During this period, a total of 180 outbreaks were recorded as against 799, during the previous year 2009-10 (Table 1). Nearly 4000 FMD outbreaks used to occur every year prior to beginning of FMD control programme by Government of India in 2003-04. Since 2006-07, there has been

gradual reduction in the occurrence of the disease, and as of today, there has been significant reduction in the severity of clinical sickness. Number of FMD outbreaks/cases has dropped by 50-80% in different regions of the country. This has been possible due to regular vaccination in those areas with a vaccine carrying appropriate vaccine strains and seromonitoring by sensitive and specific companion diagnostics (LPB-ELISA and 3AB3DIVA). Further, awareness of the owners/keepers has increased in favour of regular 6 monthly vaccinations.

This year, in all the regions, progressive decline in the number of outbreaks was evident. Maximum outbreaks were recorded in the southern region followed by north eastern region. Maximum incidence of disease was recorded during December to March (Fig 1).

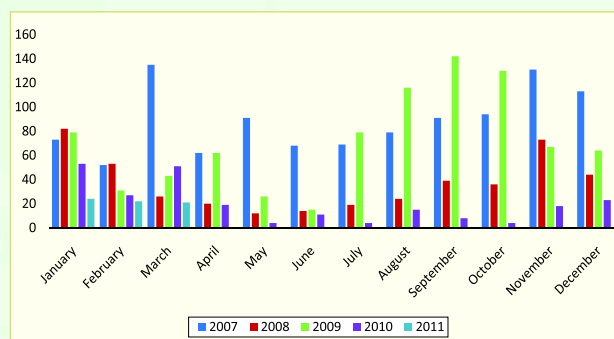


Fig. 1 Month wise occurrence of FMD outbreaks. During 2010-11, FMD outbreaks occurred round the year with maximum during December to March

Table. 1 Number of FMD outbreaks in different years in different geographical region of the country

Year	South	North	Central	West	East	North East	Total
2005-06	2117	314	52	59	355	65	2962
2006-07	697	18	32	29	611	80	1467
2007-08	631	42	41	42	353	102	1211
2008-09	263	42	33	19	102	52	511
2009 -10	85	61	20	33	498	102	799
2010-11	51	9	29	20	31	40	180

Table 2. Number of FMD outbreaks diagnosed during the period and serotypes involved

			Serotype				
			O	A	Asia1	Total	NVD
Number of tissue samples	2007-08	2258	1042	136	91	1269	982*
	2008-09	640	334	26	16	376	264*
	2009-10	1624	991	38	38	1067	557*
	2010-11	622	314	18	45	377	245*
Number of suspected Outbreaks	2007-08	1211	721	65	56	842	369
	2008-09	511	198	19	24	241	270
	2009-10	799	568	33	9	610	189
	2010-11	253	154	10	16	180	73

*Many of them are duplicate samples collected from same outbreak at different times

A total of 622 clinical specimens were collected from 253 suspected outbreaks of which 180 outbreaks could be confirmed as FMD. Using sandwich ELISA and multiplex PCR, virus could be diagnosed in 377 samples. More than one clinical sample was collected from same outbreak in many occasions. The details of virus serotype confirmation are shown in Table 2. Dominance of type O virus in causing the disease continued in the country.

During 2010-11, in all the geographical regions, serotype O was most prevalent. Increase in serotype O incidence was noticed in all regions except North East and East. Serotype A which was prevalent in all the geographical area except North East last year was absent in Northern, Western and Eastern regions during 2010-11. Serotype Asia 1 was absent in Southern, Central and Northern regions. Incidence of Asia1 serotype is constantly noticed in Western and Eastern regions (Table 3). Though majority of the outbreaks involved cattle, disease also occurred in buffaloes, pigs, sheep, goats, Yak and Mithun.

Vaccine matching exercise is imperative for analyzing antigenic relationship of field isolates with currently used vaccine strains. Studies of antigenic relationship of the field outbreak strains with in-use vaccine strains is a regular exercise conducted to monitor antigenic variation, if any, occurring in the field, and appropriateness of in-use vaccine strains. Selected virus isolates were subjected to one-way antigenic relationship

analysis with respective vaccine strains. All of them showed close antigenic match with respective vaccine strain except two type A isolates. Such occasional emergence of antigenically divergent strains within the VP3⁵⁹-deletion group has been observed earlier that supports within the group antigenic diversity. In the scenario of 80% of the outbreak strains exhibiting close antigenic match with respective vaccine strains, it is satisfied that the current type A vaccine strain, IND 40/2000 offer optimum antigenic coverage over the field virus strains.

Phylogenetic analysis based on 1D region is routinely carried out to assess genetic variations, inter-strain relationships and track movement of the virus. This year phylogenetic analysis of type O virus shows that 'Ind2001' strains, which reemerged in late part of the year 2008, spread to majority of states in Northern, Eastern, North-Eastern and Southern India. In ten out of 12 states where disease due to type O was experienced were traced back to this lineage. Next to 'Ind2001' lineage, Pan Asia II was responsible for sporadic outbreaks/cases in West Bengal, Arunachal Pradesh and Maharashtra. There were cases of FMD in cattle in Punjab caused by PanAsia I lineage. In case of type A, all the isolates were found to cluster within genotype 18 (VII), but grouped both in the nondeletion and the VP3⁵⁹-deletion sub-lineages. The Asia1 field isolates were grouped with lineage C reiterating the supremacy of this lineage since 2005.

Table. 3 Distribution/incidence of FMD virus serotypes in different geographical regions of the country during 2005-06 to 2010-11. There is progressive decline in the occurrence of the disease and dominance of serotype O virus in the country

	South			North			Central			West			East			North East			Total Material serotyped
	O	A	Asia1	O	A	Asia1	O	A	Asia1	O	A	Asia1	O	A	Asia1	O	A	Asia1	
2005-06	686 (91%)	61 (8%)	11 (1%)	50 (75%)	13 (19%)	4 (6%)	54 (70%)	17 (22%)	6 (8%)	87 (71%)	9 (7%)	27 (22%)	362 (85%)	18 (4%)	48 (11%)	62 (53%)	35 (30%)	21 (17%)	1571
2006-07	85 (85%)	7 (7%)	8 (8%)	19 (100%)	0 (0%)	0 (0%)	27 (66%)	0 (0%)	14 (34%)	68 (65%)	8 (8%)	29 (27%)	409 (60%)	97 (14%)	182 (26%)	17 (16%)	6 (6%)	80 (78%)	1207
2007-08	493 (91.8%)	38 (7.1%)	6 (1.1%)	21 (67.7%)	7 (22.5%)	3 (9.7%)	32 (39.5%)	39 (48%)	10 (12.5%)	98 (88.2%)	1 (0.9%)	12 (10.8%)	315 (80.5%)	47 (12%)	29 (7.5%)	83 (70.3%)	4 (3.3%)	31 (26.3%)	1269
2008-09	80 (86%)	13 (14%)	0 (0%)	12 (80%)	0 (0%)	3 (20%)	60 (70.6%)	0 (0%)	24 (29.4%)	18 (69.3%)	2 (7.7%)	6 (23%)	104 (91.2%)	4 (3.5%)	6 (5.3%)	60 (88.2%)	7 (10.3%)	1 (1.53%)	401
2009-10	127 (91.4%)	12 (8.6%)	0 (0%)	37 (66.1%)	13 (23.2%)	6 (10.7%)	42 (93.3%)	3 (6.4%)	0 (0%)	40 (57%)	2 (3%)	28 (40%)	619 (99%)	5 (0.8%)	1 (0.16%)	114 (100%)	0 (0%)	0 (0%)	1049
2010-11	98 (98.9%)	1 (1.1%)	0 (0%)	21 (100%)	0 (0%)	0 (0%)	60 (93.7%)	4 (6.3%)	0 (0%)	44 (83%)	0 (0%)	13 (17%)	34 (77%)	0 (0%)	10 (23%)	57 (61.9%)	13 (14.2%)	22 (23.9%)	377

Serotype A FMD virus has been most variable in the nature. In order to understand the global character of FMDV, a more comprehensive global genotyping to determine the extent of genetic diversity of serotype A was carried out. A total of 26 regional genotypes within 3 continental topotypes, based on 15% nucleotide divergence cut-off criteria, could be identified. These genotypes correlated with distinct evolutionary lineages in the maximum-likelihood phylogeny. In India, four genotypes [genotype I (2), IV (10), VI (16) and VII (18)] have been documented. Besides, comparative complete genome analysis of seventeen serotype A Indian field isolates representing different genotypes and sub-lineages was carried out, and details presented later.

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 25 virus isolates were added to the repository during the period. At present the National FMD virus Repository holds a total of 1712 isolates (O-1102, A-276, C-15 and Asia 1-319).

At present an antigenically heterogeneous, unique lineage within the genotype VII of type A dominated the field scenario. This genetic cluster has amino acid deletion at position 59 of VP3 (VP3⁵⁹-deletion group), considered to be antigenically critical. The emergence of this group warrants rapid and accurate detection to facilitate early planning and implementation of an effective control policy. A rapid multiplex PCR assay was developed for detection of the dominating VP3⁵⁹-deletion group even before generating sequence data and confirmatory phylogenetic analysis. This method has helped in detecting deletion group lineage virus in the FMD suspected clinical tissue materials directly.

A PCR assay for detection and identification of FMDV in semen was standardized in the form of kit. Validation of assay was performed by testing 112 semen samples collected from cattle bull farms having recent history of FMD. It was established that infected cattle bull may secrete FMD virus up to 8 months in semen. Keeping Indian conditions in mind where maintaining cold chain during transport is difficult, the kit has been made relatively thermostable. The reagents required for performing reverse transcription and multiplex polymerase chain reactions are supplied as freeze dried master mix in the kit. Just before use, the contents have to be dissolved and used directly for diagnosis. The kit is equipped with a self explanatory instruction manual. Recombinant 3ABC non structural protein based immunoassay for differentiation of FMD infected and vaccinated animals (DIVA) was developed as a companion of 3AB3-DIVA. The diagnostic sensitivity and specificity of the 3ABC-DIVA was calculated by testing a known panel of serum samples, and was found to be 96.77% and 100% respectively, at 27.66% positive percent cut off. In case of C-ELISA, diagnostic sensitivity and specificity was estimated to be 86.96% and 97.01%, respectively at >40% PI by testing a panel of known bovine serum samples. Further validation is underway.

Under National FMD serosurveillance, 31,042 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. These samples included serum collected at random. The test revealed overall DIVA positivity of ~ 27% in the country during 2010-11, similar to the previous year.

The Directorate is extending full technical and scientific/laboratory support to the FMD Control Programme being run by the Department of Animal husbandry, Dairying and Fisheries, GOI in selected 54 districts of the country since

2003-04. LPB-ELISA kits required for sero-monitoring were produced by the institute. Pre and Post-vaccinal sero-monitoring is done by Regional FMD centers, Network Units and the Central FMD Laboratory of the institute. Gradual increase in protective antibody response was observed subsequent to phase 1 vaccination. After phase X vaccination, 87.4, 74.7 and 76.7 percent of animals tested were having protective antibody level (\log_{10} 1.8 and above) against serotypes O, A and Asia-1, respectively in post-vac serum samples. The herd immunity has progressively increased with minor aberrations that speak for positive impact of vaccination for last 6-7 years. There has been gradual decline in occurrence of the disease in vaccinated areas. In recent times, there have been cases of FMD in some FMD-CP districts affecting a few animals and could not spread due to surrounding herd immunity. Further, there has been reduction in severity of clinical sickness and this warrants extensive use of genome diagnostics. There has been certain problem in maintaining 6 month interval between successive vaccinations. This problem can be circumvented/compensated by using a vaccine having 6-8 PD50/dose. The results have been encouraging and the programme should be further strengthened by constituting a National FMD Commission.

Regular training and refresher courses for the scientific staff of Regional Centers and Network units were conducted on use/application of virus typing 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. Sufficient fund was provided to all the centers and network units of the AICRP to carry out the technical programmes. Requirement of diagnostics kits in the Government was met by the institute.

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 Leading Laboratory for FMD. The institute is also now a member of GFRA (Global FMD Research Alliance). International Center for FMD will be established and commissioned by 2014. Creation of this international laboratory with state-of-the-art features of bio-safety and

bio-containment (BSL 3+) will facilitate Global participation and control of the disease in the SAARC region. I thank all my fellow scientist colleagues, administrative, accounts and laboratory staff of the institute for their sincere efforts and contribution in to accomplishing the tasks assigned to the Institute.

-B. PATTNAIK



Mandate, Objectives and Technical Programme

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.

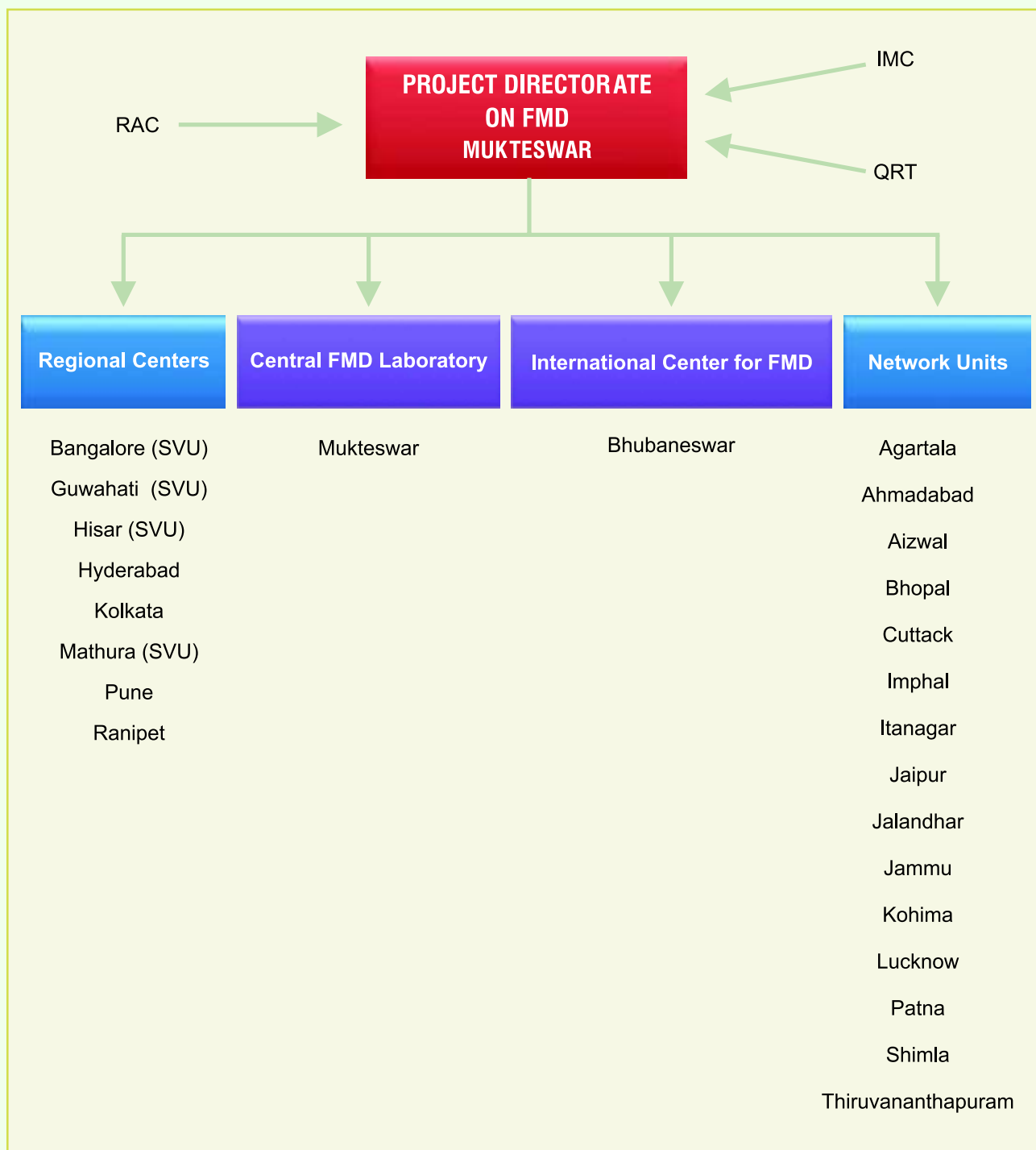
Technical Programme

1. Production and standardization of type-specific anti-146S sera, antigen and other

reagents used in sandwich and LPB ELISA, and supply to the regional Centers, network units, and other agencies to ensure uniformity in result.

2. Confirmatory diagnosis and expert advise.
3. To study molecular epidemiology of FMD in India.
4. To carryout antigenic and molecular characterization of field isolates.
5. To continue to carryout vaccine matching exercise for monitoring of appropriateness of in-use vaccine strains.
6. Maintenance of National Repository of FMD virus strains.
7. 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.
8. 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
9. Participation in FMD Control Programme with vital contribution in assessing antibody response following vaccination for assessment of individual and herd immunity level.
10. National FMD Serosurveillance
11. International collaborations in areas of interest.

Organizational Setup



Staff Position

Scientific staff

S. No.	Name of the scientist	Designation	Discipline	Joining in Current Post	Month of Leaving
1.	Dr. B.Pattnaik	Project Director	Veterinary Microbiology	December 2006	Continuing
2.	Dr. A. Sanyal	Pr. Scientist	Veterinary Microbiology	April 2009	Continuing
3.	Dr. B.B. Dash	Sr. Scientist	Veterinary Microbiology	August 2009	Continuing
4.	Dr. J. K. Mohapatra	Scientist	Veterinary Microbiology	February 2006	Continuing
5.	Dr. R.P. Tamilselvan	Scientist	Veterinary Microbiology	May 2007	Continuing
6.	Dr. Saravanan. S	Scientist	Veterinary Microbiology	May 2007	Continuing
7.	Dr. Sachin S Pawar	Scientist	Animal Biotechnology	May 2008	In study leave
8.	Dr. K. Munisamy	Scientist	Animal Biotechnology	June 2008	Continuing
9.	Dr. P. Rameshkumar	Scientist	Veterinary Pathology	June 2008	May, 2010
11.	Dr. G.K. Sharma	Scientist	Veterinary Microbiology	December 2009	Continuing
12.	Dr. M. Rout	Scientist	Veterinary Pathology	March 2010	Continuing
13.	Dr. Rajeev Ranjan	Scientist	Veterinary Pathology	September 2010	Continuing

Administrative, Technical and Supporting staff

S. No.	Name of the staff	Designation	Joining in Current Post	Month of Leaving
1.	Shri. D. N. Joshi	AAO	January 2009	Continuing
2.	Shri. Raja Ram	AF & AO	August, 2008	Continuing
3.	Shri. M. C. Meena	T-5 (Lab)	January 2007	March, 2011
4.	Shri. A. K. D. Bhatt	T-3 (Stockman)	April 1999	Continuing
5.	Shri Nayan Sanjeev	T-3 (Lab)	October 2010	Continuing
6.	Shri J.P. Bhan	S. S. Gr. IV	February 2008	Continuing

Epidemiology Report

Table 4. FMD cases/outbreaks reported/recorded and diagnosed during 2010-11 and virus serotype involved

States	Reporting Centre/Unit	No. of FMD cases/ outbreaks reported	No. of Samples tested	Outbreak wise Serotyping Results				No. of confirmed outbreaks
				O	A	Asia1	NVD	
Southern Region								
Tamil Nadu	Ranipet	13	79	13(21)	-	-	0(58)	13
Andhra Pradesh	Hyderabad	3	15	2(4)	1(1)	-	0(8)	3
Karnataka	Bangalore	37	98	27(59)	-	-	10(39)	27
Kerala	Thiruvanthapuram	42	48	8(14)	-	-	34(34)	8
Total		95	240	50(98)	1(1)	-	44(141)	51
Northern Region								
J &K	Jammu	5	2	1(2)	-	-	4(0)	1
Haryana	Hisar	2	4	2(4)	-	-	-	2
Himachal Pradesh	Shimla	1	4	1(1)	-	-	0(3)	1
Punjab	Jalandhar	7	14	3(11)	-	-	4(3)	3
Uttar Pradesh	Mathura	1	1	1(1)	-	-	0(0)	1
	CADRAD, IVRI	2	11	1(2)	-	-	1(9)	1
Total		18	36	9(21)	-	-	9(15)	9
Central Region								
Madhya Pradesh	Bhopal	29	85	27(60)	2(4)	-	0(21)	29
Total		29	85	27(60)	2(4)	-	0(21)	29
Western Region								
Gujarat	Ahmedabad	9	43	7(21)	-	1(5)	1(17)	8
Maharashtra	Pune	8	15	6(11)	-	2(2)	0(2)	8
Rajasthan	Jaipur	13	23	3(12)	-	1(6)	9(5)	4
Total		30	81	16(44)	-	4(13)	10(24)	20
Eastern Region								
Orissa	Cuttack	1	-	1	-	-	-	1(R)
Bihar	Patna	11	23	10(16)	-	-	1(7)	10
West Bengal	Kolkata	27	43	14(18)	-	4(10)	7(15)	20 (2R)
Total		39	66	27(34)	-	4(10)	8(22)	31
North Eastern Region								
Assam	Guwahati	16	23	10(13)	6(10)	-	0(0)	16
Meghalaya		6	14	3(8)	-	3(6)	0(0)	6
Arunachal	Itanagar	9	44	6(21)	-	1(1)	2(22)	7
Nagaland	Kohima	3	10	3(10)	-	-	0(0)	3
Mizoram	Aizwal	1	3	1(3)	-	-	0(0)	1
Tripura	Agartala	5	18	-	1(3)	4(15)	0(0)	5
Manipur	Imphal	2	2	2(2)	-	-	0(0)	2
Total		42	114	25(57)	7(13)	8(22)	2(22)	40
Grand Total		253	622	154(314)	10(18)	16(45)	73(245)	180

R- Outbreaks diagnosed in retrospect

Number of samples collected from FMD suspected outbreaks are given in parenthesis.

More than one clinical material was collected from many cases/outbreaks of FMD

5.1 Southern region

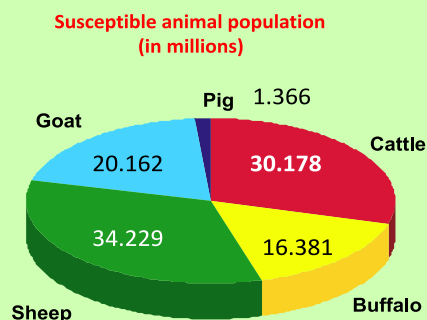
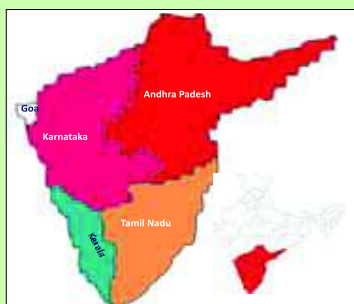
Tamilnadu (Ranipet): During the year under report, 13 FMD cases/outbreaks were recorded. Highest numbers of cases (7) were reported during the month of January followed by 2 each in the months of November and February and December. Maximum number of cases were reported from Dindugal district (3) followed by two each in Salem and Tiruppur, one each in Pondichery, Madurai, Theni, Namakkal, Tiruvannamali and Dharmapuri. Serotype O was responsible for all the 13 outbreaks/cases.

Andhra Pradesh (Hyderabad): During the year under report, 3 outbreaks/cases of FMD were recorded. One outbreak each was recorded in the months of June, October and March in the districts of Nalgonda, Guntur and Karimnagar, respectively. Involvement of serotype O in 2 outbreaks and type A in 1 outbreak was identified.

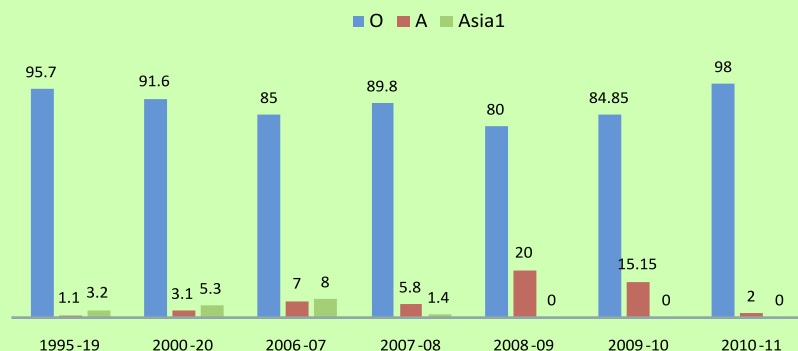
Karnataka (Bangalore): During the year under report, 27 outbreaks/cases were reported in the state, and all were caused by type O virus. Large and small ruminant were involved in the attack. Highest number of outbreaks were reported from Chikkaballapur (16) followed by 3 each in Bangalore rural, Shimoga, Dakshina Kannada, 2 each in Mandya, Gulbarga and Tumkur, and 3 each in Hassan, Udupi, Bijapur, Mysore, Yadgir and Bangalore urban. Maximum numbers of outbreaks were recorded during January (9) followed by November (5), August (4), December (3), February (2), and 1 each in April, May and March.

Kerala (Thiruvananthapuram): Eight FMD outbreaks/cases were recorded in the state. Two outbreaks each was recorded in Malappuram and Thrissur districts and one outbreak each was recorded in Kozhikode, Ernakulam, Idukki and

Scenario of FMD in Southern Region



Serotype-wise prevalence



Trivandrum. More number of outbreaks/cases were recorded during December (3) followed by January (2), July (1), August (1) and March (1). Serotype O was responsible for all the outbreaks/cases.

5.2 Northern Region

Jammu and Kashmir (Jammu): A single case was reported in the J&K State in the Kargil district in the month of October and was recorded in Yak. Serotype O virus was responsible for the disease.

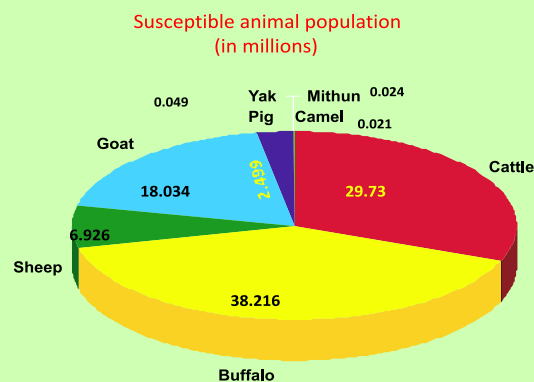
Punjab (Jalandhar): During the period under report, 3 outbreaks/cases which involved cattle and buffaloes were recorded. These occurred in the months of February and March in the districts of Jalandhar, Ludhiana and Sangrur. Serotype O virus was responsible for all the three cases.

Haryana (Hisar): Two FMD outbreaks/cases were recorded. One was recorded in the month of April from Rohtak and the other from Karnal district in March. Cross-bred cattle and buffaloes were affected. Both the cases were due to FMDV serotype O.

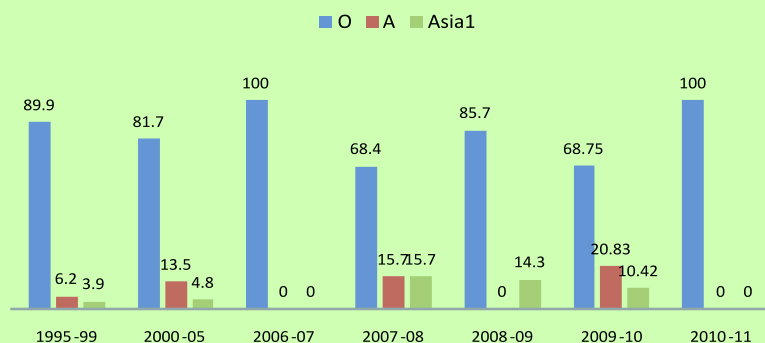
Uttar Pradesh (Mathura): During the period under report, a single outbreak which affected cattle and buffalo were recorded. The outbreak was recorded in the month of March, 2011 in Mathura dist. Serotype O was responsible for the outbreak.

Himachal Pradesh (Shimla): A single case of FMD which affected both cattle and buffalo was recorded in the district Solan, and serotype O was responsible for the disease.

Scenario of FMD in Northern Region



Serotype-wise prevalence



5.3 Western region

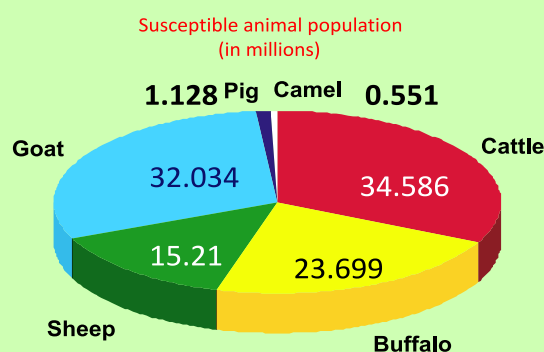
Gujarat (Ahmedabad): During the year, 8 outbreaks/cases of FMD were recorded. Two outbreaks each were recorded in the months of April, August and February, followed by one outbreak each in July, December and March. Maximum outbreaks have been reported from Gandhinagar (3) followed by 2 each in Mehesana and Panchmahal, 1 each in Banaskantha and Kheda. Ahmedabad, Rajkot and Vadodara regions recorded 6, 9 and 5 outbreaks, respectively. Serotype O virus was responsible for 5 outbreaks and Asia1 was responsible for one.

Maharashtra (Pune): During the year, 8 outbreaks/cases of FMD were recorded. Maximum numbers of outbreaks were recorded

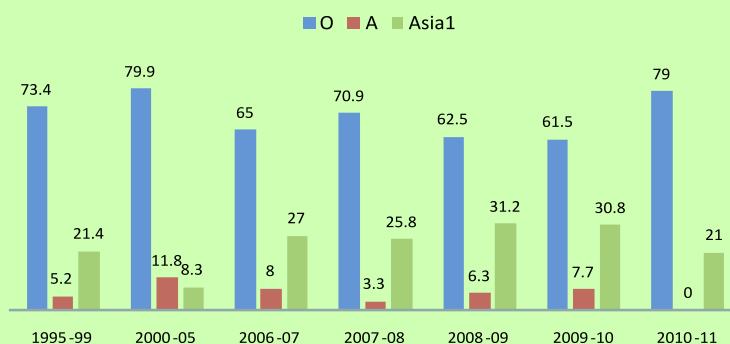
in the month of February (4) followed by April (2), June (1) and December (1). Serotype O virus was responsible for 6 outbreaks, and Asia1 was responsible for two. The outbreaks which affected cattle and buffalo were recorded in the districts of Latur, Ahmad Nagar, Pune, Sangli and Kolhapur.

Rajasthan (Jaipur): During the year under report, 4 outbreaks/cases were recorded in the state. The outbreaks were recorded in the districts of Ajmer, Kota, Jhunjhunu and Sriganga Nagar. Serotype O was responsible for three outbreaks and Asia1 is responsible for outbreak in Kota. Maximum incidence recorded in the month of March (2) followed by February (1) and December (1).

Scenario of FMD in Western Region



Serotype-wise prevalence



5.4 Eastern Region

West Bengal (Kolkata): Twenty FMD outbreaks/cases were recorded during the period in the state. Highest number of FMD outbreaks were in Hooghly (4), followed by 3 each in Dinajpur and Purulia, 2 each in Malda Birbhum and Nadia, and 1 each in south 24 Parganas, Murshidabad, Howrah and Darjeeling. Prevalence of FMD virus serotype O was maximum that caused 16 outbreaks. FMDV type Asia 1 was responsible for remaining 4 outbreaks. Highest number of outbreaks were recorded in the month of April (6), followed by March (3), 2 each in September, December, January, February, and 1 each in May, October and November.

Orissa (Cuttack): A single outbreak/case was recorded in the state in the month of September. The outbreak could be diagnosed in

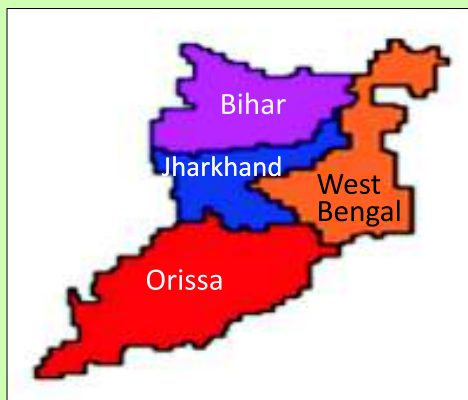
retrospect by LPB-ELISA that revealed involvement of serotype O virus.

Bihar (Patna): During the period under report, 11 outbreaks/cases of FMD were recorded of which 10 outbreaks were caused by serotype O virus. Highest number of outbreaks were recorded in the month of November (4) followed by 2 each in August, September and December, and 1 each in January, February and March.

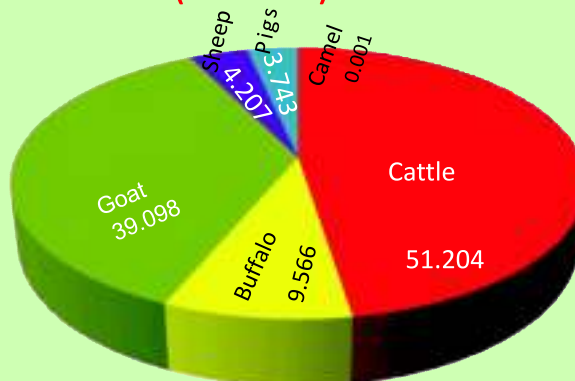
5.5 North Eastern Region

Assam (Guwahati): Sixteen outbreaks/cases of FMD were recorded in Assam. Highest number (4) of outbreaks was recorded in Goalpara district followed by Barpeta (3), Nagaon (3), Nalbari (2), Jorhat (2), Kamrup (1) and Darrang (1). Cattle and buffaloes were affected in the outbreaks. Highest number of outbreaks

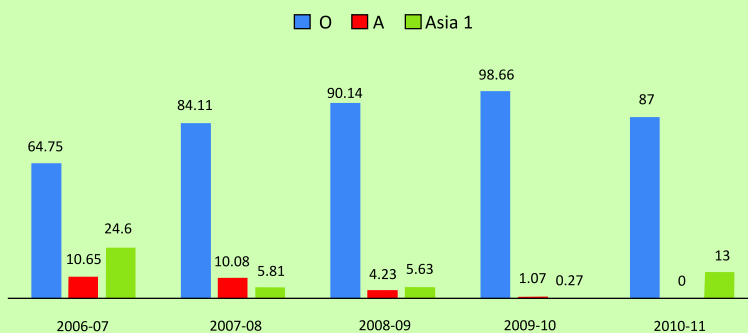
Scenario of FMD in Eastern Region



Susceptible animal population (in millions)



Serotype-wise prevalence



was recorded in the month of June (9) followed by April (4), February (1), January (1) and May (1). Serotype O was responsible for 10 outbreaks and remaining outbreaks were caused by serotype A.

Meghalaya: Six outbreaks of FMD was recorded in the state. Serotype O virus was responsible for 3 outbreaks recorded in East Kashi, and type Asia1 virus was responsible for 3 outbreaks in Ribhoi. Maximum outbreaks were recorded in the month of February (4) followed by 1 each in April and January.

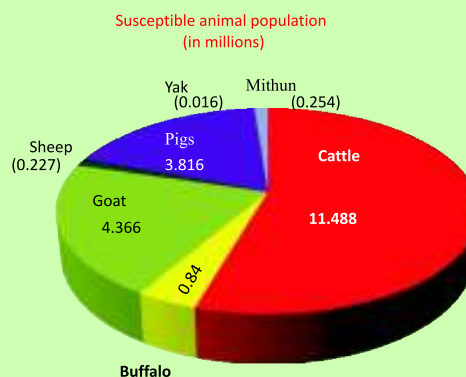
Arunachal Pradesh (Itanagar): Seven outbreaks/cases of FMD were confirmed in the state. The outbreaks were recorded in P.Pare, E.Siang, Changlang, E.Kameng and W.Kameng. Six outbreaks were caused by serotype O virus

and the remaining one was due to type Asia 1 virus. Five outbreaks were recorded in cattle and two outbreaks in Mithun.

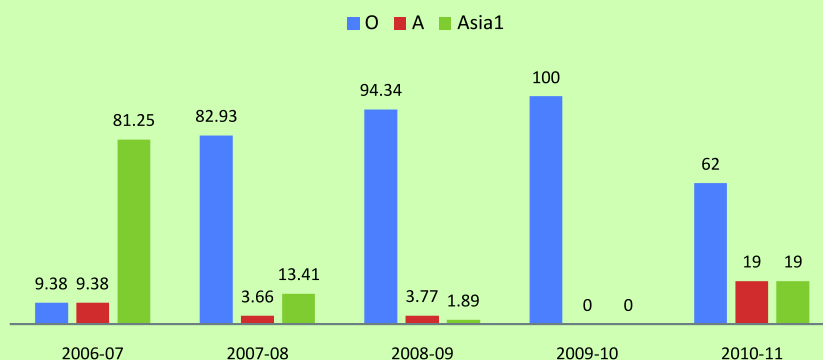
Mizoram (Aizwal): During the period under report one FMD outbreak/incidence was recorded in the district Lawnglai. The outbreak was recorded in the month November was caused by serotype O.

Tripura (Agartala): Five outbreaks/cases were recorded in the state which affected cattle. Serotype Asia1 was responsible for four outbreaks and one outbreak was caused by serotype A. Four outbreaks were recorded in West Tripura and one in South Tripura. Maximum outbreaks were recorded in the month of December (3) followed January (1) and February (1).

Scenario of FMD in North Eastern State



Serotype-wise prevalence



Manipur (Imphal): During the year, 2 outbreaks/cases of FMD in the districts Imphal-West and Imphal-East of the state were recorded. Both the outbreaks were caused by serotype O virus. The disease occurred during July and September and affected only Cattle.

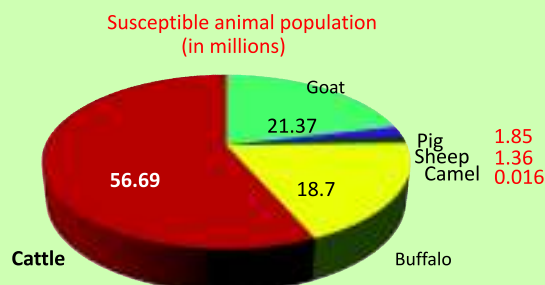
Nagaland (Kohima): Three outbreaks/cases of FMD were confirmed in the state. The outbreaks were recorded in the districts Dimapur and Phek. All the outbreaks were caused by serotype O virus.

5.6 Central Region

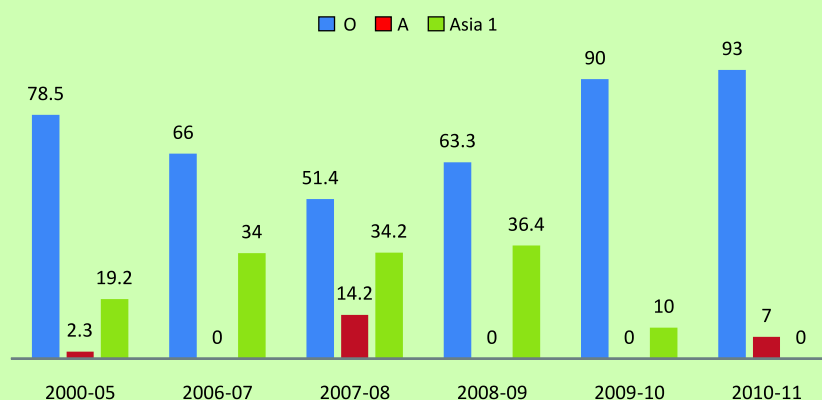
Madhya Pradesh (Bhopal): A total of 29 outbreaks/cases were reported in Madhya

Pradesh. Out of these, 2 outbreaks were reported in buffalo only, and the remaining outbreaks affected both cattle and buffaloes. Maximum (5) outbreaks were reported from Bhopal followed by 4 outbreaks each from Betul and Ujjain, 3 outbreaks each from Sagar, Seoni and Sehor, 2 each from Harda, Mandla and Narsinghpur, and 1 from Morena district. Maximum number of outbreaks were reported in the month of March (9) followed by November and December (5), September (3), August (2), and 1 each in April, July and October. Twenty seven outbreaks were caused by serotype O virus and two were due to type A virus.

Scenario of FMD in Central Region



Serotype-wise prevalence



Virology and Molecular Epidemiology

6.1 Processing of field samples and Serotyping

A total of 345 clinical materials from 126 FMD suspected outbreaks/cases were received from Regional Centres and Network Units of the project during the year 2010-11 for confirmatory diagnosis. Maximum number of clinical samples were received from Karnataka followed Gujarat and Arunachal Pradesh. A large proportion of samples were collected from Cattle followed by buffalo. Three outbreaks samples were collected from sheep and wildlife. The samples were processed using chloroform and made in to suspension in PBS. The processed materials were subjected to sandwich ELISA, and ELISA negative samples were tested using multiplex PCR for virus diagnosis. FMDV serotypes could be identified in 171 samples collected from 103 outbreaks (Table 5). FMDV type O virus caused maximum number of outbreaks (83), and serotypes A and Asia1 virus were detected in 10 outbreaks, each.

6.2 Genetic and antigenic characterization of FMD Virus field isolates

6.2.1 Serotype O FMD Virus

Antigenic analysis

During the year 2010-2011, 16 FMD virus isolates of serotype O from Assam, Karnataka, Uttar Pradesh, Arunachal Pradesh, Orissa and Manipur were subjected to antigenic analysis by 2D-MNT (Fig.2). All the 16 virus isolates were found to be antigenically (closely) related to the currently used vaccine strain (O/IND/R2/1975). Among various lineages of FMD virus serotype O, r value ranged from 0.6 to 1.0. The PanAsia 2

viruses isolated in 2010 from Arunachal Pradesh had an r-value of 1.0. The r-value of 0.76 was obtained for Branch C-II viruses isolated from Assam, whereas r value of 1.0 was obtained for viruses isolated from Orissa. The 'Ind2001' lineages of viruses were responsible for majority of the outbreaks in Uttar Pradesh, Assam, Karnataka and Manipur, and were found antigenically closer to the currently used vaccine virus (O/IND/R2/1975) as indicated by their r-value which ranged between 0.6 and 1.0. To conclude, the presently circulating field viruses of FMDV serotype O are protected by currently used vaccine virus as shown by micro-neutralization test (MNT).

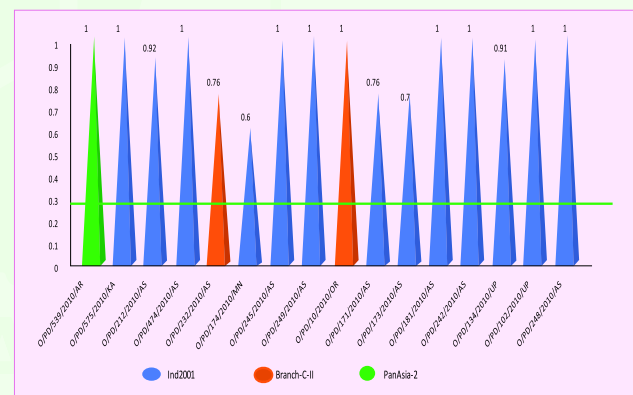


Fig.2 Antigenic analysis by 2D-MNT of FMD virus isolate of serotype O during 2010-2011. The bar diagram shows that field isolates are antigenically (closely) related to the currently vaccine strain O/IND/R2/1975 ($r \geq 0.3$), indicating protection afforded by currently used vaccine strain.

Genetic Characterization and Molecular epidemiology

Genetic characterization has been one of the fundamental objectives in the molecular epidemiology of FMD virus. This has resulted in

Table 5. Details of the field materials received at PD FMD during April 2010 to March 2011

S. No.	Centre/ State	Outbreaks and Samples Received	Virus types detected at PD on FMD			Confirmed outbreaks	Source host species				
			O	A	Asia1		Cattle	Buffalo	Cattle and buffalo	Sheep	Wild life
1.	Ahmedabad/Gujarat	9(43)	7(14)	-	1(2)	8	5	2	1	-	-
2.	Aizwal/Mizoram	1(1)	-	-	-	1	1	-	-	-	-
3.	Bangalore/Karnataka	30(75)	26(39)	-	-	26	23	-	-	3	-
4.	Bhopal/MP	15(27)	13(19)	2(2)	-	15	14	1	-	-	-
5.	CADRAD/UP	2(11)	1(2)	-	-	1	1	1	-	-	-
6.	Guwahati/Assam	11(18)	4(6)	6(10)	-	10	10	-	-	-	-
7.	Guwahati /Meghalaya	5(5)	1(1)	-	3(3)	4	4	-	-	-	-
8.	Hyderabad/AP	2(8)	-	1(4)	-	1	1	-	-	-	-
9.	Hisar/Haryana	1(2)	1(2)	-	-	1	-	-	1	-	-
10.	Itanagar/ Arunachal Pradesh	9(44)	6(11)	-	1(1)	7	5	-			2(Mithun)
11.	Jammu/J&K	1(2)	1(2)	-	-	1	-	-	-	-	1(Yak)
12.	Jaipur/Rajasthan	5(16)	1(1)	-	1(4)	2	2	-	-	-	-
13.	Jalandhar/Punjab	1(3)	1(3)	-	-	1	1	-	-	-	-
14.	Kolkata/West Bengal	12(19)	6(6)	-	3(5)	9	9	-	-	-	-
15.	Kohima/Nagaland	3(3)	1(1)	-	1(1)	2	2	-	-	-	-
16.	Mathura/UP	1(1)	1(1)	-	-	1	-	1	-	-	-
17.	Pune/Maharashtra	2(3)	2(3)	-	-	2	2	-			-
18.	Ranipet/Tamilnadu	4(41)	4(18)	-	-	4	4	-	-	-	-
19.	Thiruvananthapuram/ Kerala	12(23)	7(10)	-	-	7	7	-	-	-	-
Sub Total		126(345)	83(139)	10(16)	10(16)	103	90	5	2	3	3

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

establishing genetic identity of the virus, tracing the movement of virus across the state boundaries, reemergence of older strains (genotypes/lineages), and appearance of new variants. In the recent past including the year 2010-2011, major shift in the genetic lineage of serotype O viruses circulating in India was observed (Fig 3, 4 and 5). Viruses of 'Ind2001' lineage gained upper hand after a gap of 8 years and outcompeted PanAsia II lineage in the early part of 2008. The re-emergence of 'Ind2001' lineage has been traced back to 2008, during which it caused sporadic outbreaks in Northern India. Soon it spread to majority of states in Eastern, Central and reached Southern parts of the country by the end of 2009. Viruses of 'Ind2001' lineages recorded during the recent outbreaks in Tamil Nadu, Kerala and Karnataka were not related to those viruses of the same lineage circulated in these states during previous years especially in 2001, but were related closely to isolates from other parts of the country indicating virus incursion. PanAsian lineages of viruses namely PanAsia1 and PanAsia2 have caused only sporadic outbreaks in Punjab and West Bengal, and Maharashtra respectively. These viruses are more closely related to the PanAsian viruses previously recorded in the respective states, indicating persistence of these viruses in the region. However, disease due FMD virus serotype O lineage Branch C-II, which caused outbreaks in Tripura, Assam, West Bengal and Orissa during last year, were not recorded during 2010-2011

During the period under report, 46 type O virus isolates were subjected to 1D genomic region sequencing either directly from TE or CC supernatant. The viruses selected covered 12 out of 16 states from which type O samples were received at Central FMD Laboratory. The states covered include Kerala, Karnataka, Gujarat, West Bengal, Assam, Arunachal Pradesh, Meghalaya, Maharashtra, Madhya Pradesh, Jammu and Kashmir, Tamil Nadu and Punjab.

Results from the phylogenetic analysis shows that 'Ind2001' viruses, which re-emerged in the late part of 2008, spread to majority of states in Northern, Eastern, North-Eastern and Southern India. Ten out of 12 states where disease due to type O was experienced, were traced back to this lineage. These viruses diverged from 'Ind2001' viruses isolated in 2001 by 7.3% and 13.1% to the in-use vaccine virus (O/IND/R2/1975). Nevertheless these viruses have shown to be antigenically related to the currently used vaccine virus by 2D-MNT ($r_1=0.6-1.0$). However, even after the reemergence and dominance of 'Ind2001' lineage, disease due to other lineages was not completely absent. Next to 'Ind2001' lineage, Pan Asia II was responsible for sporadic caess/outbreaks in West Bengal, Arunachal Pradesh and Maharashtra. There were outbreaks of FMD in cattle in Punjab caused by PanAsia I

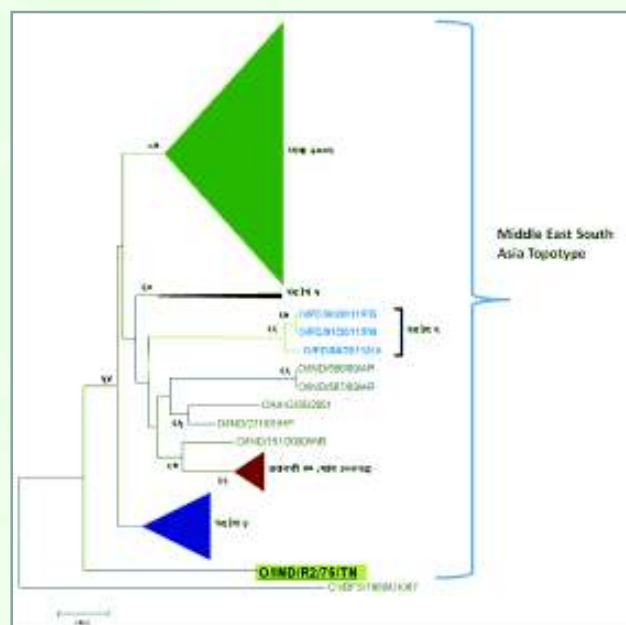


Fig. 3 Neighbour Joining Phylogenetic tree at 1D genomic region of Indian serotype O FMD virus isolates during 2010-2011. The tree shows presence of 3 sub lineages of FMD virus type O co-circulating in India during 2010-2011, namely "Ind2001", aPanAsia-1 and PanAsia-2. The PanAsia-1 isolates (highlighted) which caused sporadic cases in Punjab were more closely related to viruses recovered previously from the same region, indicating the persistence of the particular virus population in the region.



Fig. 4 Mid point rooted Neighbour Joining Phylogenetic tree at 1D genomic region of 'Ind2001' lineage of FMD virus isolates of serotype O during 2010-2011. The genetic data indicate that 'Ind2001' lineage has now entered incursion into the states of Tamil Nadu, Kerala, J & K and Karnataka.

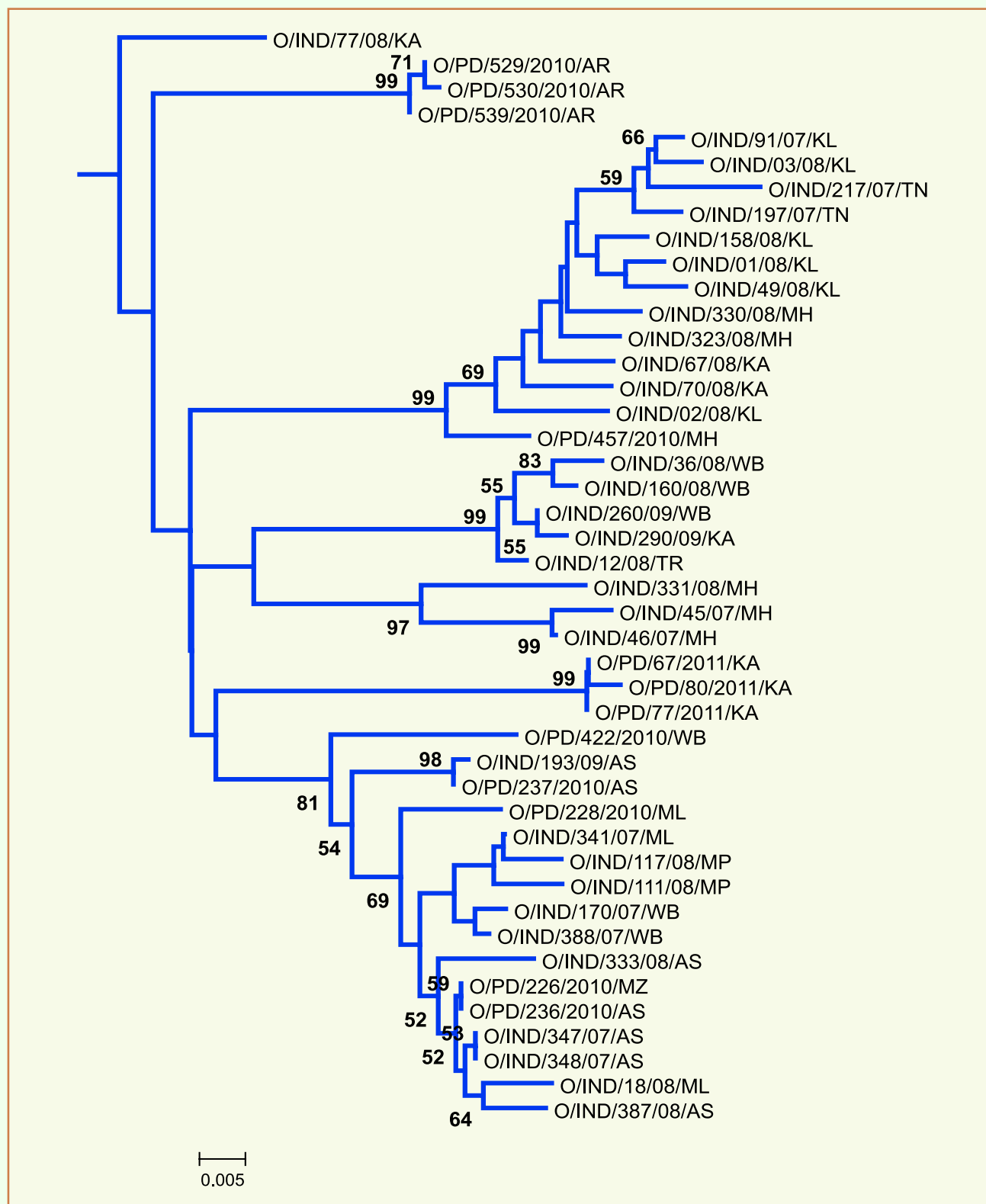


Fig. 5 Mid point rooted Neighbour Joining Phylogenetic tree at 1D genomic region of PanAsia 2 lineage of FMD virus isolates of serotype O of during 2010-2011. The virus isolates of current year from Arunachal Pradesh are different from PanAsia1 viruses isolated from outbreak during last year indicating that of different origin of these viruses that caused outbreaks.

lineage. This indicates complex epidemiological scenario of serotype O FMD virus in this country, which is not surprising because of large population of susceptible animal of different species, sparse vaccination and unrestricted movement of the animals. However, prompt surveillance system available and in place in our country, has been able to detect genetic variations in real time, and monitor appropriateness of the in-use vaccine strains.

6.2.2 Serotype A FMD Virus Antigenic Characterization

Ten(10) selected field isolates of serotype A were subjected to 2D-MNT using bovine vaccinate serum against the current vaccine strain, IND 40/2000, and one way antigenic relationship (r -value) was estimated. The ' r '-value which directly correlates with antigenic relationship between the field isolates and the vaccine strain varied widely between 0.17 and 1.0 (Fig. 6). As an ' r '-value of more than 0.3 indicates close antigenic relatedness, it can be inferred that majority (eight out of ten) of the outbreak strains showed close antigenic match with the vaccine strain without much antigenic divergence. But the field isolates IND 45/2010 from Uttar Pradesh and IND 136/2010 from Haryana revealed poor antigenic relatedness ($r < 0.2$) with the current vaccine strain. Such occasional antigenically divergent strains within the VP3⁵⁹-deletion group have been

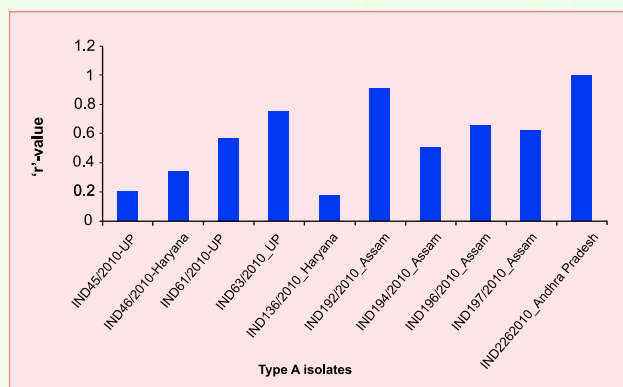


Fig. 6 Antigenic relationship of type A isolates with the vaccine strain IND 40/2000

observed earlier as well supports within the group antigenic diversity. In the scenario of 80% of the field strains exhibiting close antigenic match with the vaccine strain, it is satisfied that the current vaccine strain, IND 40/2000 offers optimum antigenic coverage over the field virus strains.

Genetic characterization and Molecular Epidemiology

Among all the three serotypes of FMD prevalent in India, type A virus population has been 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 type A so far in India. Since 2001, genotype 18 (VII) has been exclusively responsible for all the field outbreaks/cases and has outcompeted all other genotypes. Within the currently circulating genotype 18 (VII), 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 in 2002-03. Since then sporadic cases due to this sub-lineage has been observed. This 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 in sparsely vaccinated population of cattle and buffalo.

During 2010-11, eleven (11) field isolates of serotype A recovered from FMD cases/outbreaks in Andhra Pradesh (IND 226/2010), Uttar Pradesh (IND 45/2010, IND 61/2010, IND 63/2010), Haryana (IND 46/2010, IND 136/2010), Madhya Pradesh (IND 230/2010) and Assam (IND 192/2010, IND 196/2010, IND 197/2010, IND 202/2010) were sequenced at complete capsid coding (P1) region for molecular epidemiological analysis. Besides, one isolate from Maharashtra (IND 170/2010) was sequenced in the VP1 region. The determined sequences were aligned

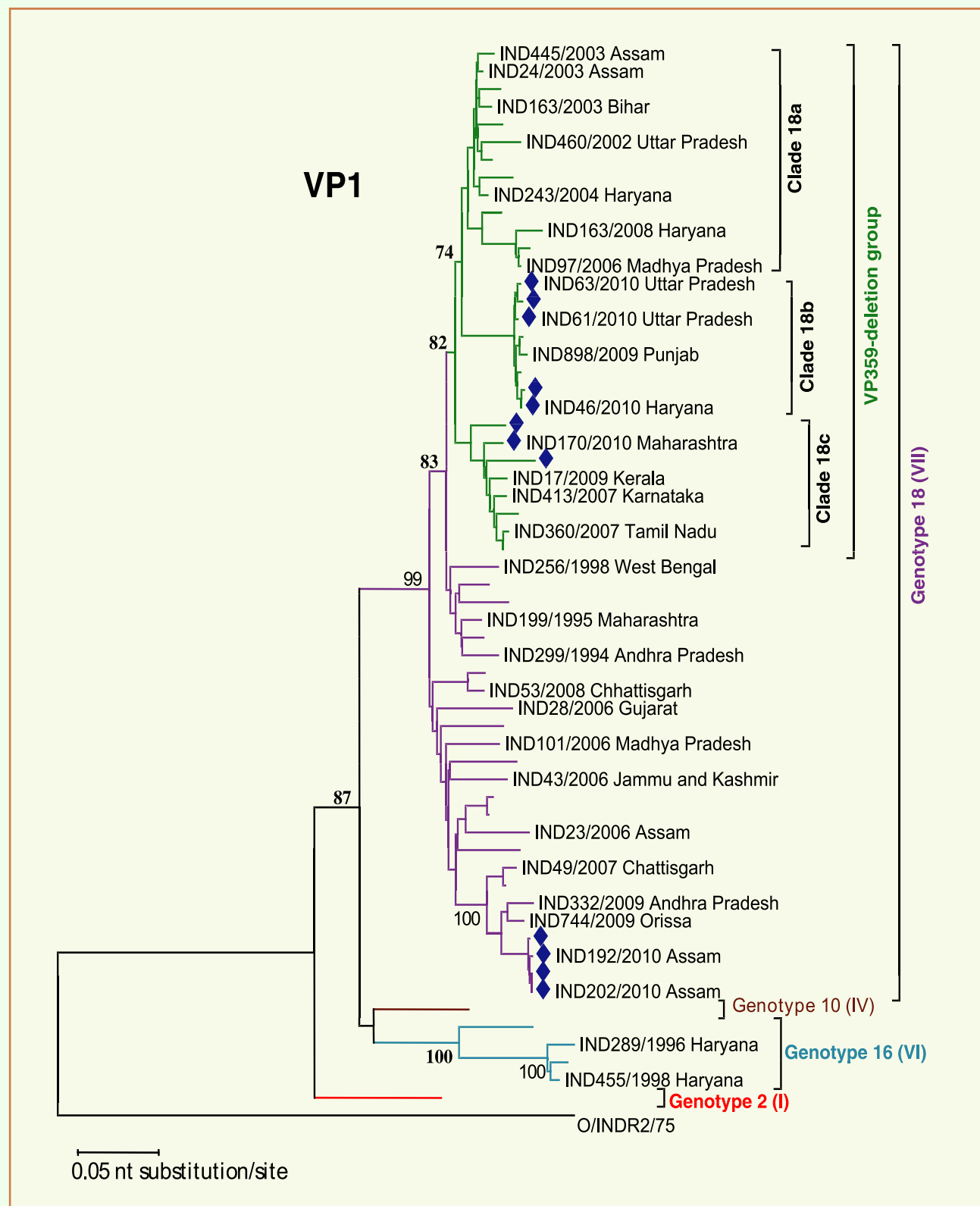


Fig. 7 Phylogenetic (N-J tree) relationship among type A FMD virus isolates at VP1 region and genotype classification. Isolates of 2010-11 are marked with blue rhombus, and the type O sequence O/IND R2/75 has been included as an outgroup

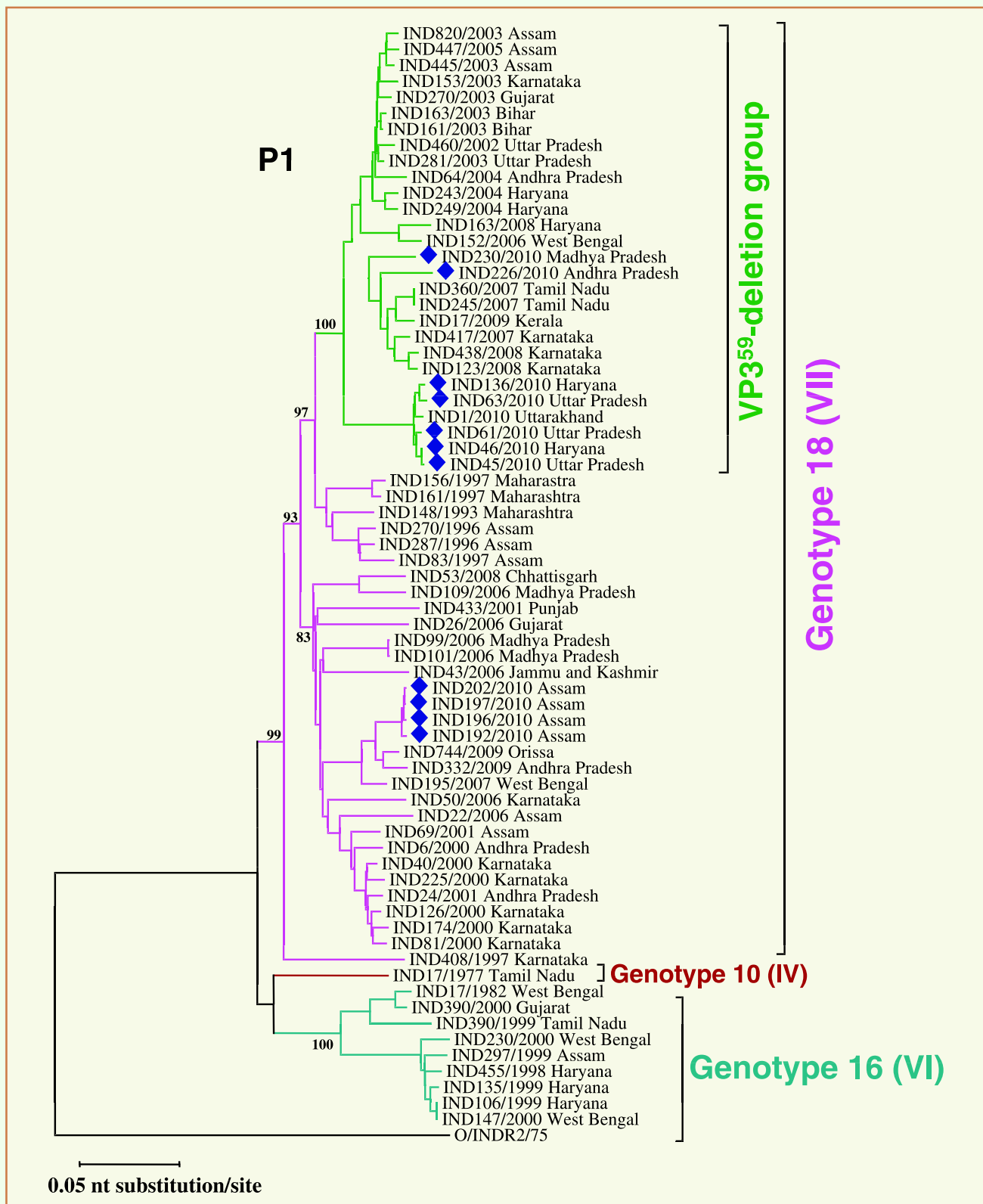


Fig.8 Phylogenetic (N-J) tree) relationship among type A FMD virus isolates at P1 region and genotype classification. Isolates of 2010-11 are marked with blue rhombus and the type O sequence O/IND R2/75 has been included as an outgroup

with other Indian sequences available in the data base of PD on FMD. During 2010-2011, all the isolates were found to cluster within genotype 18 (VII) in the N-J tree (Fig. 7 and 8), but grouped both in the non-deletion and the VP3⁵⁹-deletion sub-lineages. Precisely, the viruses from Uttar Pradesh and Haryana shared immediate common ancestry and clustered in the clade 18b of VP3⁵⁹-deletion group, where as viruses from Madhya Pradesh, Maharashtra and Andhra Pradesh grouped in the clade 18c, which was so far restricted to only Southern peninsular India. The viruses from Assam clustered together and revealed no deletion in the VP3 coding region. The Assamese strains revealed recent common ancestry with viruses from Orissa, West Bengal and Andhra Pradesh, isolated between 2007 and 2009. Hence it appears both deletion and non-deletion mutants belonging to genotype 18 (VII) are co-circulating in the field in recent times. The viruses from Assam revealed less than 1% nucleotide divergence among themselves and less than 5% nt divergence from the isolates of Orissa, West Bengal and Andhra Pradesh. Similarly, the recent isolates from Uttar Pradesh and Haryana demonstrated less than 2% nucleotide divergence among them, and with isolates from Punjab and Uttarakhand collected between 2009 and early part of 2010. Such genetic relatedness suggests epidemiological linkage and spatio-temporal relationship between the outbreak strains and movement of virus from one part of the country to the other in a sparsely vaccinated population of susceptible animals.

Phylogenetic structure of serotype A foot-and-mouth disease virus: Global diversity and the Indian perspective

Though there has been an exponential growth in the number of FMDV genomic sequences in the public domain in recent years, published epidemiological findings are mostly restricted geographically. Inadequate real-time epidemiological information and nonavailability of sequence data from most of the countries with endemic foot-and-mouth disease (FMD) has

stood in the path of understanding the global character of FMDV. So far only one complete VP1-sequence-based global genotyping study including Indian type A viruses, collected between 1977 and 2001, has been published (Tosh et al., 2002). The type A FMDV population was classified into ten major genotypes in that study with greater than 15% nucleotide divergence among the genotypes. But that analysis neither included any sequences from Africa nor those of any recent, unique genetic lineages like 'A Iran 05' from the Middle East or the 'VP3⁵⁹-deletion group' from India. To overcome this gap in our knowledge, we attempted an updated and more comprehensive global genotyping to determine the extent of genetic diversity of serotype A and to assess the relationships among the geographically segregated genetic lineages worldwide. Such epidemiological analysis holds the key to implementing sustainable progressive regional FMD control programmes.

For this purpose, complete 1D sequence of 55 Indian type A-outbreak strains collected between 2004 and 2010 as a part of national FMD-surveillance activities were resolved. The inclusion of 156 GenBank-derived sequences from 46 other countries spread across four continents (21 countries from Asia, nine from Europe, ten from Africa and six from South America) in the phylogenetic reconstruction helped in producing an integrated spatio-temporal global impression of type A FMDV.

A total of 26 regional genotypes within 3 continental topotypes, based on 15% nucleotide divergence cut-off criteria, could be identified (Fig 9). These genotypes correlated with distinct evolutionary lineages in the maximum-likelihood phylogeny. However, it should be considered that the extent of diversity detected here could be far greater than we presently realize, as surveillance and sampling might not have been foolproof in many parts of the world. We have designated the 26 genotypes by using Arabic numerals 1–26 in the order of their appearance

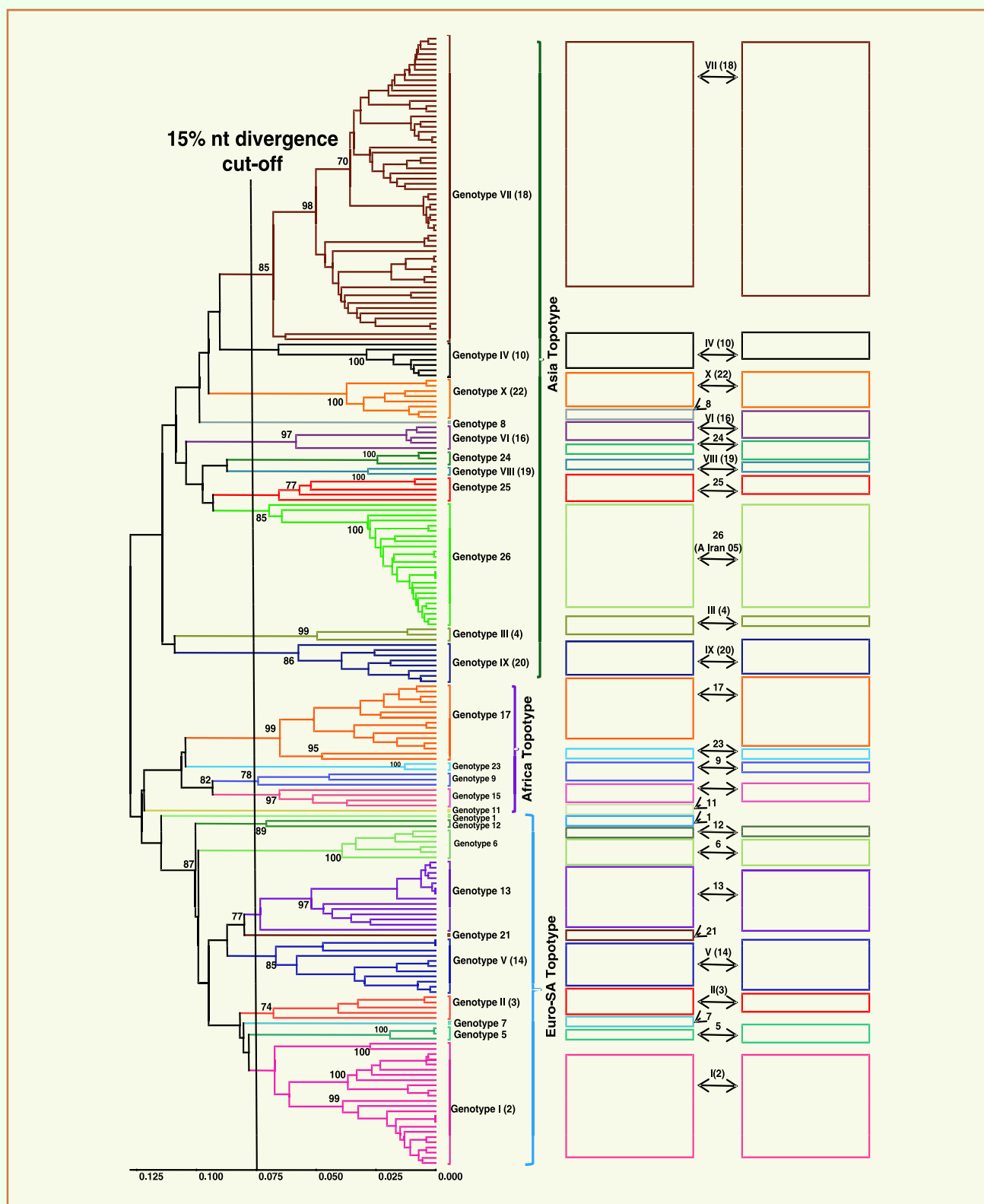


Fig. 9 UPGMA tree showing a complete VP1 sequence-based global phylogeny and toptotype/genotype distribution. Inside the boxes, accession numbers or isolate designations followed by the country of origin and year of collection are depicted serially as per their position on the branches in a top-to bottom direction.

and have also kept the Roman numeric designations (I–X) given in an earlier study for the 10 genotypes (Tosh et al., 2002) to avoid any confusion. Except for genotypes 2 and 14, all other genotypes formed monophyletic lineages in the ML tree. Though genotypes 2 and 14 formed single clusters in the UPGMA tree, strains were found to be interspersed with genotype 5 and 21, respectively, in the ML and NJ trees. This could be caused by the fact that the '15% cut-off' to delineate genotypes, though logical, is a heuristic choice. Moreover, these genotypes revealed that genotypes with just more than a 15% nucleotide difference (~16–18 %) between them are distributed in the same geographical regions. Hence, it is most likely that such clustering is the result of intermediate sequences evolving from the older genotypes, which in turn provide ancestry to the newer genotypes in a stepwise manner. Most of these genotypes (23 of 26) showed a regionally restricted geographical distribution pattern, a few even being confined

to a particular country (Fig. 10). All of these genotypes could be accommodated within the three broad continental topotypes: Asia, Europe–South America (Euro–SA) and Africa except for genotypes 2, 14 and 18 which were found to have transgressed their normal continental niches. Genotypes 2 and 14 could be traced to all four continents with endemic FMD, whereas genotype 18 could be found in Asia and Europe. More importantly, all such transcontinental movements of virus occurred before the 21st century and have been attributed to either immigration of people with their livestock to establish colonies, to the importation of livestock and livestock products or to the inadvertent release of old European strains that were extensively used in vaccines in South America during that time. Overall, the Asia, Euro–SA and Africa continental topotypes comprised 11, 10 and 5 regional genotypes, respectively. From the UPGMA tree, it is evident that a minimum of a '24% nucleotide difference cut-off' could be a

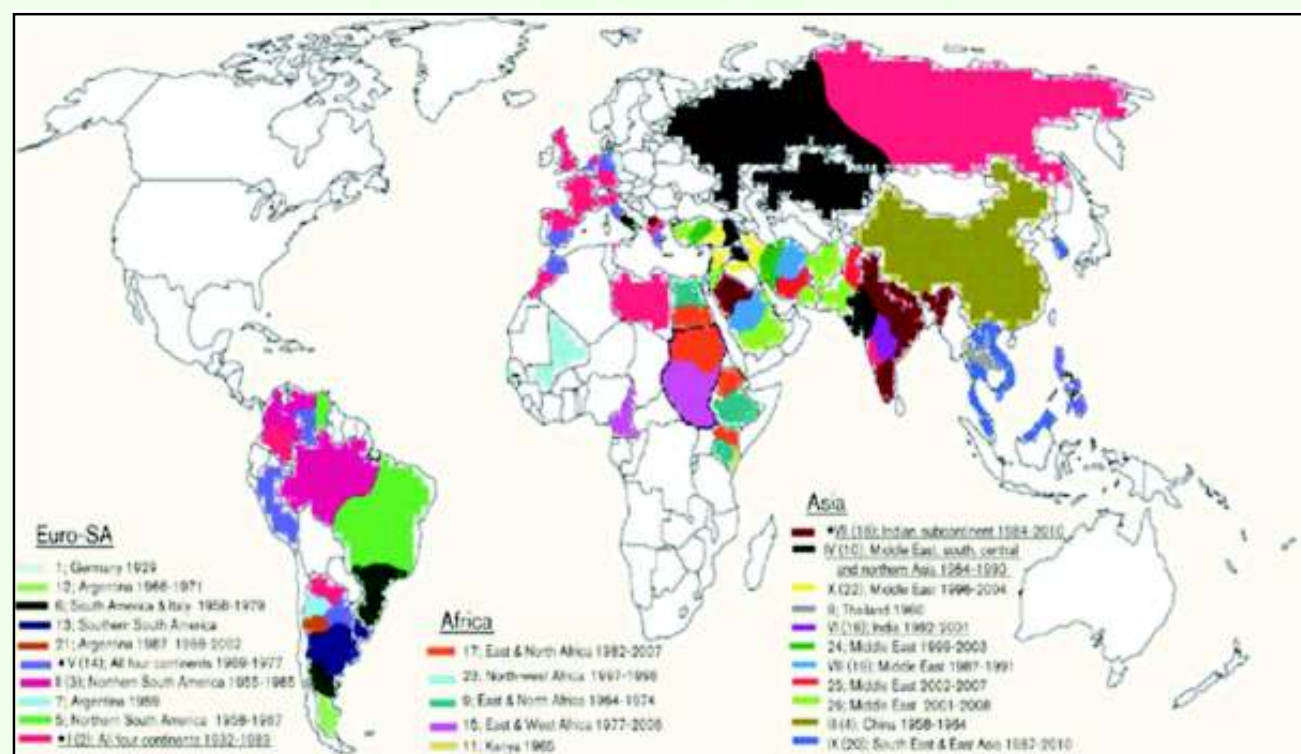


Fig. 10 Global foot-prints of serotype A FMDV genotypes. Underlined genotypes suggest grouping of Indian viruses and asterisked ones denote genotypes which have transgressed their normal continental niches

rational criterion to distinguish between the continental topotypes. Genotype 1, comprising an isolate from Germany, happens to be the oldest genotype in this study. In the ML tree, genotype 1 was placed close to the root in accordance with its genealogy. In the Asia topotype, genotype 8, recorded in Thailand during 1960, was placed close to the root and likewise, for the Africa topotype, genotype 11 from Kenya appeared to be the ancestral genotype. During the last decade, ten genotypes have been in circulation worldwide and it is apparent from the phylogram that no type A strain has jumped the continental divides during this period. Of the 11 genotypes within the Asia topotype, six genotypes could be identified only in the Middle East region. Likewise, seven of the ten genotypes within the Euro-SA topotype could be detected in Argentina only. These two regions might be considered to be hot-spots as far as genotype diversity is concerned. Strict compartmentalization within a country's boundary was less apparent for any of the genotypes indigenous to the Middle East. Hence, this whole region may be considered as an epidemio-geographical unit with respect to the spread of virus strains. Genotype 25, recorded during 2002–2007, from Iran and Pakistan appeared to be the closest neighbour of genotype 26 (A Iran 05 lineage), and two Iranian strains, collected during 2001–2002, clustered as intermediates between genotype 25 and the 'A Iran 05' lineage. Hence, with the available sequence data, it is tempting to hypothesize that these indigenous historic sequences might have provided the most recent ancestor for the 'A Iran 05' lineage. A stepwise evolution based on their order of appearance was observed for genotype 20 from South East Asia between 1987 and 2010 indicating rapid strain turnover, probably due to continuing immune selection.

In India, four genotypes [genotype I (2), IV (10), VI (16) and VII (18)] have been documented. Genotypes 2 (Euro-SA topotype) and 10 (Asia topotype) were recorded before 1990 and no longer seem to exist in India. The

epidemiological trend shows an epochal evolution of type A genotypes characterized by a continuous replacement of old genotypes with newer ones. Population dynamics studies indicate a recent genotype demographic transition from genotype 16 to genotype 18 in 2001. Apparently genotypes 16 and 18, both within the Asia topotype, evolved independently but have shared the same geo-ecology in the country as they are placed quite distantly in the ML tree, emerged from two distinct ancestral nodes in the Asia topotype and have followed different evolutionary trajectories. Each of these two genotypes appears to share their most recent common ancestor with viruses from two geographically widely separated regions. Genotypes 18 and 22 (from the Middle East) descended from a common intermediate node while genotypes 16 and 20 (from South-east Asia) showed common ancestral linkage. Strains from Nepal and Saudi Arabia collected during 1984 and 1986, respectively, clustered in genotype 18 and they appear to be intermediates between genotypes 22 and 18. Based on phylogeographical configuration, it might be suggested that the Indian viruses within genotype 18 are descended from a virus related to that from Nepal and that similar ancestral sequences have also circulated in countries of the Arabian Peninsula. The 1996 type A outbreak in Albania and Macedonia has been ascribed to the importation of on-the-bone buffalo meat from South Asia. Also the virus strains revealed close genetic relationships with the then-circulating strains from India and Saudi Arabia within genotype 18. However, in the last decade, it has become evident that a single genotype is circulating in India with neither any incursion of lineages from other countries nor any movement of type A virus out of India, except that some movement has occurred between neighbouring countries of the Indian subcontinent. The 'A Iran 05' lineage expanded its territory into Pakistan; however, further eastward dissemination into adjoining parts of India has not occurred. Intense surveillance combined with modern molecular techniques should have detected any such

incursion into India without fail. Type A viruses from India revealed more than 20% nucleotide divergence from those from South-east Asia and also clustered in separate genotypes. Hence, no epidemiological linkage could be established between contemporaneous Indian and South-east Asian viruses. As far as movement of viruses between India, Bangladesh, Nepal and Sri Lanka is concerned, the molecular phylogenetic analysis is presently handicapped because of lack of sequence data from these countries. Though the country of origin remains uncertain, phylogenetic relationships suggest that genetically similar viruses (with less than 2% nucleotide difference) belonging to clade 18a of the VP3⁵⁹-deletion group have circulated in both Bhutan (GenBank accession no. EU414525) and the neighbouring state of Assam in India (IND 24/2003) during the same time period. Although the possibility of airborne spread exists, it is difficult to exclude the possibility of their having been either some trade in live animals or the intermingling of animals from both sides of the border. In any case, such a genetic link underscores the need for rigid border surveillance.

When considering intervention strategies for the control of FMD, it is important to take account of the characteristics of different genetic clusters circulating in various ecological systems along with their routes of movement. The global genotyping and phylogeographical design presented here may serve as a platform in this regard.

Comparative complete genome analysis of Indian type A foot-and-mouth disease virus field isolates

Comparative complete genome analysis of seventeen serotype A Indian field isolates representing different genotypes and sub-lineages was carried out. Overall seventy nine percent of amino acids were invariant in the coding region. Chunk deletion of nucleotide was observed in S and L fragment of 5'UTR. More variability which is comparable to that of capsid

coding region was found in L and 3A region. Functional motifs and residues critical for virus biology were conserved most. Polyprotein cleavage sites accepted few changes. Many sites were detected to be under positive selection in L, P1, 2C, 3A, 3C and 3D region and of which some are functionally important and antigenically critical. Genotype/lineage specific signature residues could be identified which implies evolution under different selection pressure. Transmembrane domain could be predicted in 2B, 2C, 3A and 3C proteins in agreement with their membrane binding properties. Phylogenetic analysis at complete coding region placed the isolates in genotype IV, VI and VII and two broad clusters comprising VP3⁵⁹-deletion and non-deletion group within genotypes VII. The VP3⁵⁹-deletion group has diversified genetically with time giving rise to 3 lineages. Incongruence in tree topology observed for different non structural protein coding region and UTRs based phylogeny indicate suspected recombination.

6.2.3 Serotype Asia1 FMD Virus

Antigenic Characterization

Antigenic matching of field isolates with currently used vaccine strain (IND63/72) is essentially carried out to assess the protection offered and result is expressed in terms of r-value and 'r-value' range of 0.3 to 1.0 indicates close antigenic relatedness. In the current year, one FMDV Asia 1 isolate (PD508/2010) could be revived in cell culture and was subjected to antigenic analysis using anti-146S bovine serum against the vaccine strain (IND 63/72). The isolate showed an r value of more than 0.3 with in-use vaccine strain indicating its appropriate antigenic coverage

Genetic characterization and Molecular Epidemiology

Molecular phylogeny based on VP1 coding region has established circulation of three prominent lineages (lineage B, C and D) in India. The Asia 1 field isolates of India form a single

genotype with two different genetic lineages. The lineage B which includes the vaccine strain IND 63/72 has 210 amino acids in VP1 and this lineage never appeared after the year 2000. The lineage C which was prominently circulating during the period 1993 to 2001 has an extra amino acid at position 44 of VP1. A novel divergent genetic lineage (lineage D) within lineage C appeared in 2001 and it outnumbered the parent lineage in terms of field outbreaks. The isolates of lineage D was 8-13% divergent at nucleotide level from the isolates of lineage C. Lineage C has been responsible for all Asia1 outbreaks in the country since 2005. During 2010-11, outbreaks/cases due to Asia 1 serotype were recorded in Gujarat, Tripura, Arunachal Pradesh, Nagaland, Assam and Meghalaya. Seven FMDV Asia1 isolates (IND205/2010(PD508/2010), IND7/2011(PD18/2011), IND42/2011 (PD106/20011), IND43/2011(PD107/2011), IND44/2011(PD108/2011), IND45/2011(PD109/2011) and IND46/2011(PD110/2011) were sequenced at VP1 region for molecular epidemiological analysis. Phylogenetic analysis revealed grouping of all FMDV Asia1 field isolates with in lineage C reiterating the supremacy of this lineage in the field since 2005. Isolate from Gujarat (PD508/2010) grouped closely with FMDV Asia1 isolates recovered from Gujarat in previous years. Outbreaks due to FMDV Asia1 isolates are constantly reported from Gujarat. Similarly isolate (PD18/2011) from Tripura grouped closely with IND341/2008 (isolate from Assam). Isolates from Arunachal Pradesh (PD106/2011), Nagaland (PD107/2011), Assam (PD108/2011) and Meghalaya (PD109/2011 and 110/2011) were grouped closely with each other (Fig 11).

Five isolates, IND113/2006, IND96/2008, IND327/2009, IND65/2010 (PD140/2010) and IND205/2010 (PD508/2010) were subjected to capsid coding region (P1) sequence analysis. VP1

based phylogeny placed four isolates in Lineage C and one isolate (IND113_2006) in lineage D; maximum of 6.4% and 15.3% divergence at amino acid level and nucleotide level was observed respectively. Antigenic site 1 resides in GH loop (amino acid 138-154) of VP1 and more variability was observed in this site (8 of 17 positions accepted changes) compared to vaccine strain IND63/1972. The critical residue of site 1, Alanine at position 142, was conserved in all the isolates. Antigenic site II is formed by BC loop (amino acid 67-79) of VP2 and in this site one change at position 74 (Serine to alanine) was observed in all the isolates compared to IND63/1972. The isolate IND205/2010 revealed an additional change at position 72 (Asparagine to serine) in site II. Site IV formed by 58/59 amino acid of VP3 and Site V by amino acid 218 of VP3 were totally conserved in both the isolates.

A total of 11 FMDV Asia1 isolates (IND328/2004, IND34/2008, IND94/2009, IND97/2008, IND121/2009, IND137/2008, IND165/2004, IND175/2007, IND196/2006, IND206/2005 and IND79/2009) representing different lineages were characterized at 3A gene as 3A protein plays a role in virus virulence and adaptation. Four sites (112, 114, 143 and 144) were found to be under positive selection using Datamonkey webinterface. Lineage specific signature could not be identified. No discrete phylogenetic grouping as found at VP1 region was identified at 3A region. A total of 26 positions allowed amino acid replacements. Q₄₄→R mutation has been shown to be associate with pathogenic phenotype in guinea pig and none of the Indian isolates revealed this mutation. T cell epitopes were identified in positions spanning 11-25, 21-35 and 121-135. Five and three amino acid sites showed changes in the stretch 11-25 and 21-35, respectively. The region between position 125 and 135 also revealed four substitutions.

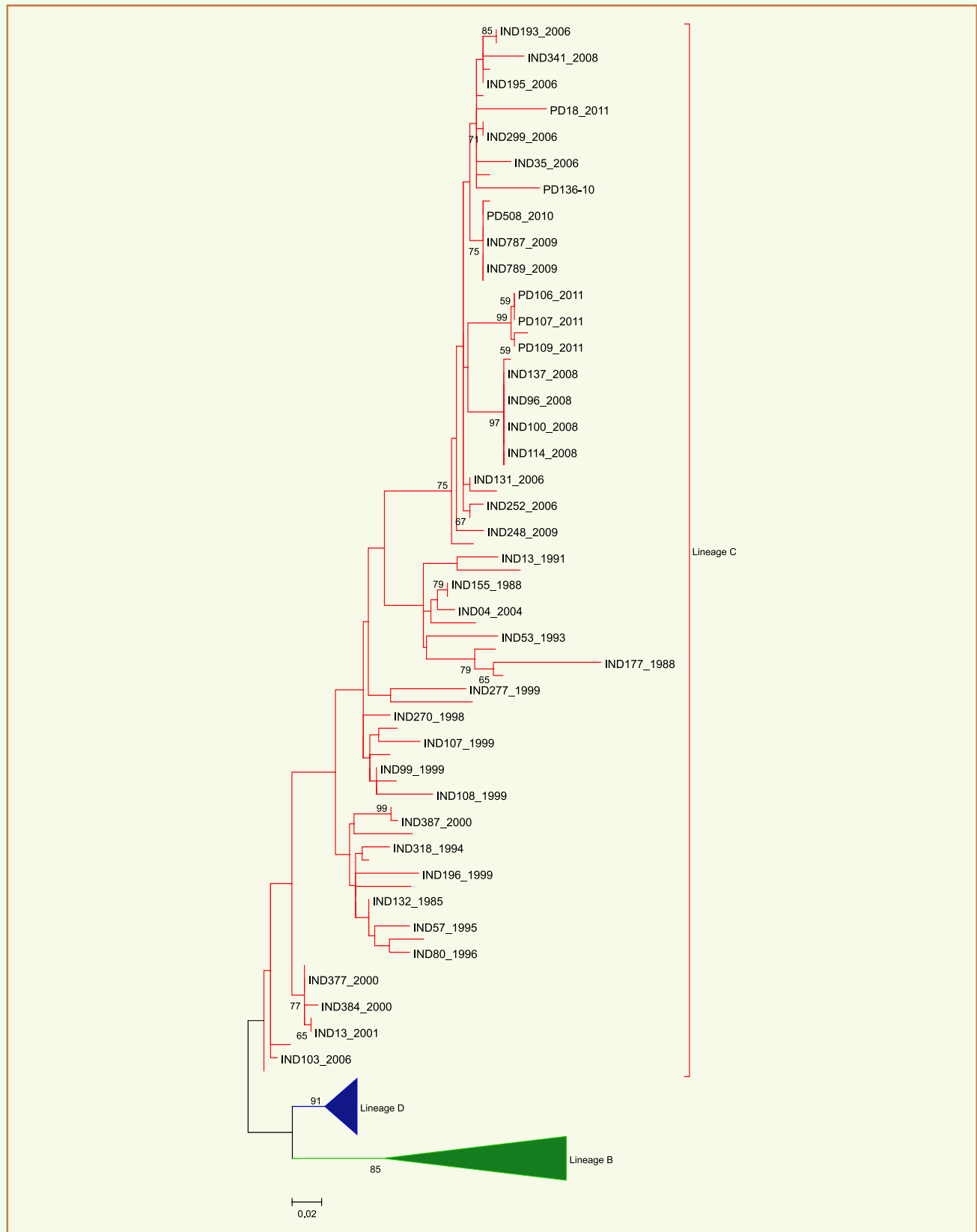


Fig.11 Phylogenetic (N-J tree) relationship among type Asia1 FMD virus isolates at 1D region

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 25 virus isolates (6 type O, 17 type A and 2 Asia 1) were added to the repository during the reported period (Table 6). At present the National FMD virus Repository holds a total of 1712 isolates (O-1102, A-276, C-15 and Asia 1-319).

Table 6. Details of isolates added to the National FMD Virus Repository during 2010-2011

Material Designation	Isolate Designation	Cell and Passage No.	Place of Origin	Host	Serotype
PD 93/2010	IND 45/2010	BHK-21, P7	Uttar Pradesh	Cattle	A
PD 131/2010	IND 61/2010	BHK-21, P5	Uttar Pradesh	Cattle	A
PD 283/2010	IND 136/2010	BHK-21, P3	Haryana	Cattle	A
PD 284/2010	IND 136/2010	BHK-21, P3	Haryana	Cattle	A
PD 285/2010	IND 136/2010	BHK-21, P4	Haryana	Cattle	A
PD 286/2010	IND 136/2010	BHK-21, P3	Haryana	Cattle	A
PD 469/2010	IND 180/2010	BHK-21, P6	Assam	Cattle	A
PD 482/2010	IND 192/2010	BHK-21, P9	Assam	Cattle	A
PD 484/2010	IND 194/2010	BHK-21, P9	Assam	Cattle	A
PD 486/2010	IND 196/2010	BHK-21, P11	Assam	Cattle	A
PD 488/2010	IND 197/2010	BHK-21, P8	Assam	Cattle	A
PD 489/2010	IND 198/2010	BHK-21, P6	Assam	Cattle	A
PD 493/2010	IND 201/2010	BHK-21, P6	Assam	Cattle	A
PD 494/2010	IND 202/2010	BHK-21, P9	Assam	Cattle	A
PD 612/2010	IND 226/2010	BHK-21, P4	Andhra Pradesh	Cattle	A
PD 613/2010	IND 226/2010	BHK-21, P3	Andhra Pradesh	Cattle	A
PD 616/2010	IND 226/2010	BHK-21, P4	Andhra Pradesh	Cattle	A
Total		17			
PD 471/2010	IND 182/2010	BHK-21, P7	Assam	Cattle	O
PD 474/2010	IND 185/2010	BHK-21, P9	Assam	Cattle	O
PD 475/2010	IND 186/2010	BHK-21, P7	Assam	Cattle	O
PD 481/2010	IND 191/2010	BHK-21, P8	Assam	Cattle	O
PD 539/2010	IND 213/2010	BHK-21, P5	Arunachal Pradesh	Cattle	O
PD 575/2010	IND 224/2010	BHK-21, P7	Karnataka	Cattle	O
Total		6			
PD 508/2010	IND 205/2010	BHK-21, P3	Gujarat	Cattle	Asia 1
PD 509/2010	IND 205/2010	BHK-21, P3	Gujarat	Cattle	Asia 1
Total		02			
Grand Total		25			

Serotype O isolates (n = 1102) preserved in the National FMD Virus Repository



Serotype A isolates (n = 276) preserved in the National FMD Virus Repository



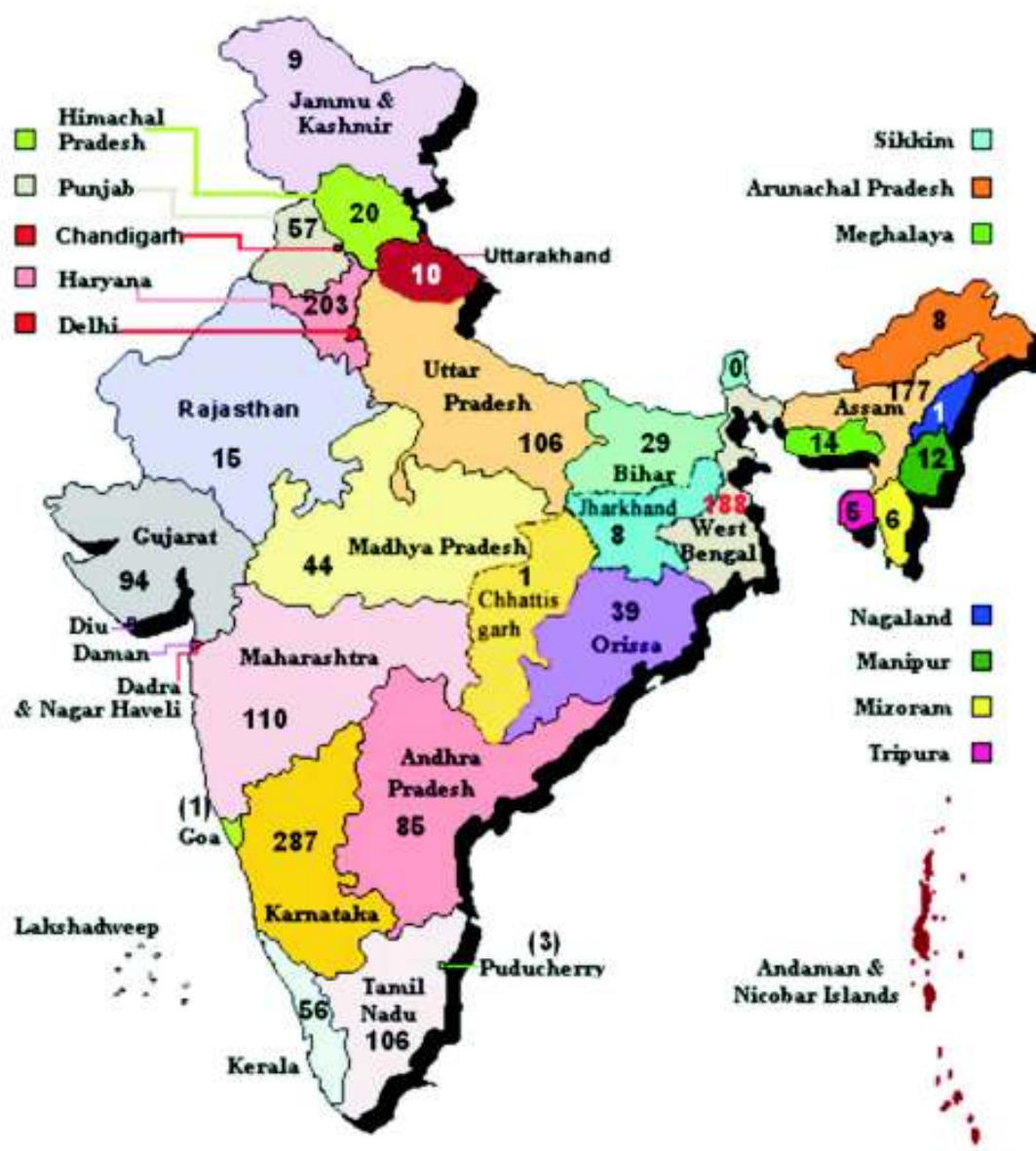
Serotype Asia 1 isolates ($n = 319$) preserved in the National FMD Virus Repository



Serotype C isolates ($n = 15$) preserved in the National FMD Virus Repository



State-wise distribution of FMD virus (serotype O, A, C and Asia 1) isolates ($n = 1712$) preserved in the National FMD Virus Repository,



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

8.1 Multiplex PCR for rapid detection of serotype A foot-and-mouth disease virus variants with amino acid deletion at position 59 of the capsid protein VP3

In India, there has been co-circulation, extinction and emergence of genotypes/lineages within serotype A foot-and-mouth disease (FMD) virus. At present an antigenically heterogeneous, unique lineage within genotype VII dominates the field outbreaks. This genetic cluster has amino acid deletion at position 59 of VP3 (VP3⁵⁹-deletion group), considered to be critical antigenically. The emergence of this group warrants rapid and accurate detection to facilitate early planning and implementation of an effective control policy. A rapid multiplex PCR assay was developed for detection of the dominating VP3⁵⁹-deletion group even before generating sequence data and confirmatory phylogenetic analysis. This method has the potential to detect also the deletion group lineages in the FMD suspected clinical tissue material.

P1 nucleotide sequences for all serotypes were aligned and an exhaustive search for conserved nt stretches exclusive to the VP3⁵⁹-deletion group was carried out. The VP3 alignment region spanning 163–180 nt, which encompasses the 59th codon deletion locus was identified for designing deletion group specific forward primer and position 20–7 in VP1 alignment was identified for designing the deletion group specific reverse primer. A set of three forward (VP3del_163F, F2 and F3) and three reverse primers (VP1del_20R, R2 and R3) were designed to cover all subtle variations observed between the three lineages (VIIb, VIIf and VIIg) of the VP3⁵⁹-deletion group in order to increase sensitivity of the assay without compromising the

binding specificity (Table 7). In addition to these designed primers, universal FMDV specific reverse primer, NK61 and serotype A specific forward primer, DHP15, which is in use for a serotype differentiating multiplex PCR were also used in this study. From the 'National FMD Virus Repository', 63 serotype confirmed field virus isolates representing all genotypes/lineages of serotype A including 30 virus isolates from the VP3⁵⁹-deletion group were taken as BHK-21 cell culture adapted viruses. The infected cell culture supernatant was used for viral RNA extraction. Original clinical material (bovine tongue vesicular epithelium) corresponding to 20 (16 deletion group and 4 nondeletion lineage virus) out of the 63 cell adapted isolates, available in the laboratory were also processed for RNA extraction. Reverse transcription and cDNA synthesis from extracted RNA was performed using FMDV specific NK61 primer.

To optimize the lineage specific multiplex PCR assay using the Maxima Hot Start TaqDNA Polymerase (Fermentas), initially cDNA of IND 40/2000 (representing type A nondeletion group virus of genotype VII), IND 818/2003, IND 1/2010 and IND 17/2009 (representing three lineages VIIb, VIIf and VIIg of type A deletion group, respectively, and corresponding to different patterns of sequence variability observed at the primer binding sites) were selected. To check the suitability of each deletion group specific primer pairs, uniplex PCR involving different permutations and combinations of forward (VP3del_163F, F2 and F3) and reverse primers (VP1del_20R, R2 and R3) on each of these four selected viruses were attempted. The desired deletion group specific amplicon at <518 base pair (bp) size and another consistent

Table 7. Details of primers used in this study

Primer designation and sense	Sequence (5'-3')	Location	Purpose
DHP15+	CAACGGGACGARCAGTACTC	1D	Serotype A specific PCR
NK61-	GACATGTCCTCTGCATCTG	2B	Reverse transcription and serotype A specific PCR
VP3del.163F+	CTTGTTCGACGGG	1C	VP3 ⁵⁹ -deletion group specific PCR
VP3del.163F2+	CTTGTTCGACGGA	1C	VP3 ⁵⁹ -deletion group specific PCR
VP3del.163F3+	CTTGTTCGACGAG	1C	VP3 ⁵⁹ -deletion group specific PCR
VP1del.20R-	GACTCCCCGGCCGA	1D	VP3 ⁵⁹ -deletion group specific PCR
VP1del.20R2-	GACTCCCCGGCAGA	1D	VP3 ⁵⁹ -deletion group specific PCR
VP1del.20R3-	GACTCCCCGGCTGA	1D	VP3 ⁵⁹ -deletion group specific PCR

amplification in the case of all deletion group virus genomes at <1264 bp size (Fig. 12) were purified from the gel using QIAquick Gel Extraction Kit (Qiagen) and were cloned into pGEM-TEasy vector (Promega) for further sequencing using vector specific primers. As expected, the 518 bp product was confirmed to be the result of lineage specific forward and reverse primer amplification (nt position 163 of 1C to position 20 of 1D) and the 1264 bp product was confirmed to be the result of amplification with lineage specific forward and universal NK61 reverse primer (nt position 163 of 1C to position

77 of 2B). Such double amplification adds to the specificity of the deletion group detection.

A particular combination of deletion group specific forward and reverse primer only could produce positive amplification in any one of the deletion group lineage representative viruses, in support of specific binding efficiency of the designed primers and the need to incorporate all six primers in a single PCR to avoid false negative results. Each PCR also included DHP15 and NK61 primers to produce a type A specific band (DHP15-NK61) of 376 bp size as observed in multiplex PCR. This was done with a two prong

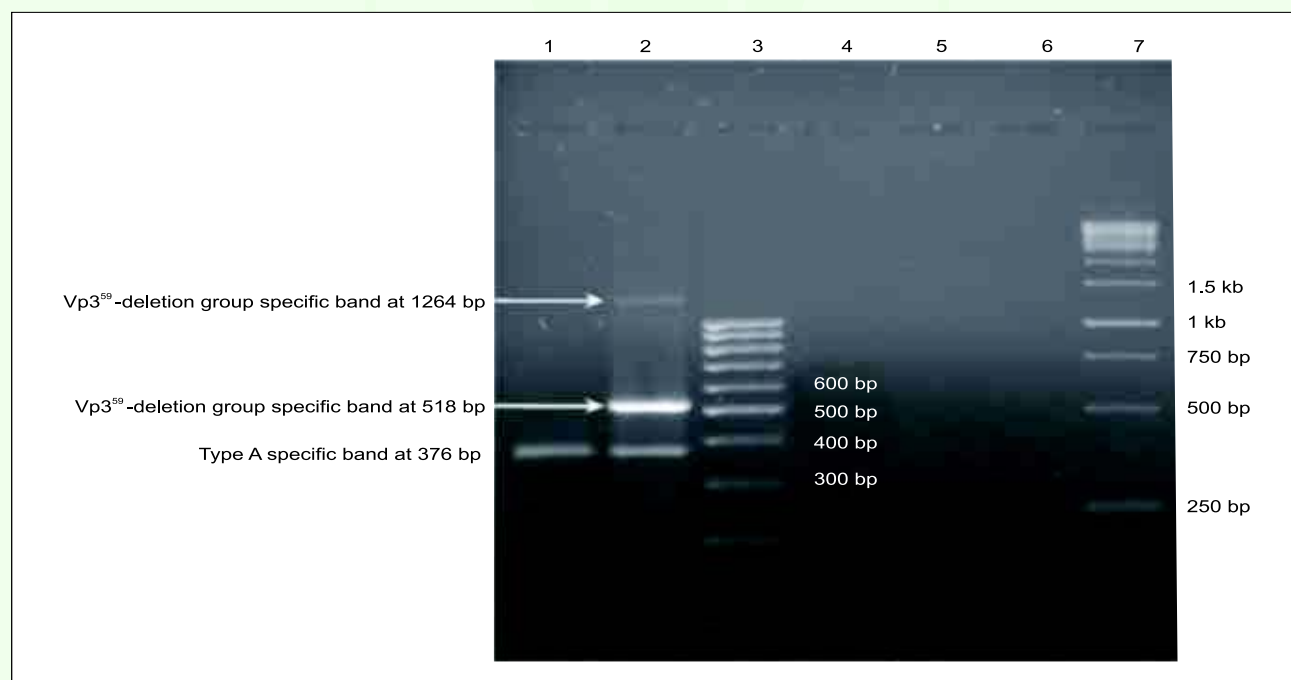


Fig. 12 VP3⁵⁹-deletion group specific RT-PCR results with representative isolates. (1) Type A FMDV without deletion, IND 40/2000, (2) type A FMDV from VP3⁵⁹-deletion group, IND 1/2010, (3) 100 bp DNA ladder (Fermentas), (4) type O FMDV, O/IND R2/75, (5) type Asia 1 FMDV, Asia 1/IND 63/72, (6) BHK-21 cell lysate as negative control, and (7) 1 kb DNA ladder (Fermentas).

objective: first, to reascertain serotype while detecting the deletion group by producing multiple amplicons at two different nonoverlapping regions on the same template (a serotype A specific 376 bp band corresponding to 3'-end of 1D and a deletion group specific 518 bp band corresponding to 3'-half of 1C). Secondly, on occasions, when the deletion group specific amplification fails, the serotype A specific amplification should occur as an intrinsic positive control, thereby indicating successful PCR but genuine absence of deletion group virus genome. Hence in a single PCR, three amplicons, a serotype A specific band at 376 bp and two deletion group specific bands at 518 bp and 1264 bp size are expected, if the virus belongs to the type A VP359-deletion group. If only a single band at 376 bp appears, it indicates presence of nondeletion lineages of type A virus. If none of these specific bands appear, there is possibility of a legitimate PCR failure or else presence of any other serotype FMDV than type A.

The optimized RT-PCR protocol was applied to 63 type A confirmed cell adapted viruses. All the thirty deletion group virus genomes could be amplified successfully producing both 518 and 1264 bp amplicons whereas, 33 nondeletion lineage viruses failed to amplify (Fig. 12). Nevertheless, in all 63 samples, type A specific amplification occurred, indicating integrity of all the synthesized cDNA templates and successful PCR and hence indisputable absence of deletion group virus genomes in the 33 samples. The test achieved 100% sensitivity on cell adapted virus samples. All the thirty isolates which produced deletion group specific amplicons were found clustered in the deletion group lineages in the 1D based phylogenetic tree, hence demonstrating the utility of this PCR assay as an alternative to phylogenetic analysis for inferring the deletion group lineage. No cross-amplification with other serotypes or nondeletion lineages of type A or with uninfected BHK-21 cell and FMD negative goat samples used as negative controls was noticed in support of its 100% specificity. But

when this PCR was applied to direct clinical material, three out of 16 samples obtained from hosts infected with deletion group viruses went undetected bringing down the diagnostic sensitivity of the test to 81%. But at the same time it can be emphasized that in none of these three samples even serotype specific amplicon could be detected, indicating PCR failure in general rather than inefficient primer binding or sensitivity issues inherent to this particular PCR. These three samples were found to be in a deteriorating state physically, which could have compromised the reaction efficiency.

In conclusion, the VP3⁵⁹-deletion group specific mPCR assay has proved to be effective for detecting the currently dominant deletion group rapidly even in the clinical tissue samples before detailed phylogenetic inference is drawn. Such first hand information on genetic lineages before proceeding with sequencing of capsid coding region for confirmation, not only saves time but also allows for rapid decision making pertaining to implementation of control policies. Although the emergence of new lineages within the existing VP3⁵⁹-deletion group with sequence diversification at the primer binding site in future may necessitate designing of suitable primers and testing of additional viruses to validate the efficiency of the assay, at present it serves the purpose and has the potential to become a relevant method for epidemiological surveillance.

8.2 FMD virus detection in cattle bull semen

Excretion of FMD virus in bull semen following clinical disease is a major threat to bio security as through Artificial Insemination the virus can travel to different place. This requires a test system to identify such semen. A PCR assay for detection of FMDV Serotypes in semen was standardized (Fig.13). Validation of assay was performed by testing 112 semen samples collected from cattle bull farms having recent history of FMD. It was established that infected cattle bull may secrete FMD virus in the semen

for more than 5 months and up to 8 months. Keeping Indian conditions in mind where maintaining cold chain during transport is difficult, a relatively thermostable kit was developed. The thermo stability testing of the kit is underway. Also the reagents required for performing reverse transcription and multiplex polymerase chain reactions are supplied as freeze dried master mix in the kit. Just before use, the contents has to be dissolved and used directly for diagnosis. This will further reduce the requirement of highly trained man power for diagnosis. The kit is equipped with self explanatory instruction manual.

FMDV was expressed in prokaryotic expression system. The expressed protein was characterized by western blotting and purified using metal affinity chromatography and passive gel elution methods. The purified protein was used for optimization of indirect DIVA ELISA for bovines. The diagnostic sensitivity and specificity were calculated, by ROC method using SAS9.2 software by testing a known panel of serum samples, as 96.77% and 100% respectively at 27.66% positive percent cut off. Further validation of assay was performed by testing 5000 random bovine serum samples obtained from different parts of country. Different reagents

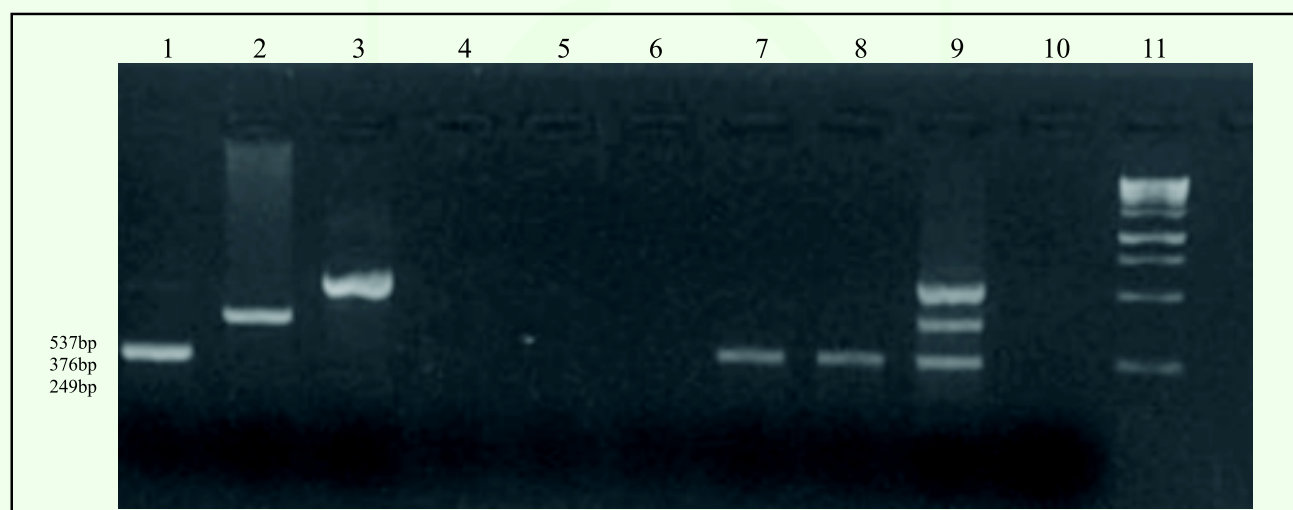


Fig. 13 Multiplex PCR for FMDV detection in cattle bull semen sample
 Lane No.1 Type O spiked semen sample (5TCID₅₀/ml)
 Lane No.2 Type A spiked semen sample (5TCID₅₀/ml)
 Lane No.3 Type Asia1 spiked semen sample (5TCID₅₀/ml)
 Lane No.7-8 Semen sample of naturally infected bull semen (Type O)
 Lane No.9 Positive control of Type O, A, Asia1 virus
 Lane No. 10 Negative control of uninfected cattle bull semen
 Lane No. 11 DNA marker of 100bp (Fermentas)

8.3 Development of recombinant 3ABC non structural protein based immunoassays for differentiation of Foot-and-Mouth Disease infected and vaccinated animals (DIVA)

Indirect ELISA (I-ELISA) for bovines

Recombinant 3ABC non structural protein of

used in 3ABC DIVA ELISA are under thermostability testing for development of a kit.

Competition ELISA (C-ELISA) using monoclonal antibodies

Recombinant 3ABC antigen was gel purified and monoclonal antibody production was outsourced. MAb clones were characterized by

measuring specificity of reaction. Monoclonal antibody clone supernatant was utilized for development of a C-ELISA using anti-mouse conjugated antibodies. All clones were repositied in PD-FMD, Mukteswar clone repository. Diagnostic sensitivity was estimated at >40%PI to 86.96% and diagnostic specificity of 97.01% by testing a panel of known bovine serum samples. More than 500 serum samples from other species like goat, sheep, pig and mithun were tested simultaneously following a common protocol. This C-ELISA enabled to use a common protocol/platform for testing serum samples from all species with increased specificity.

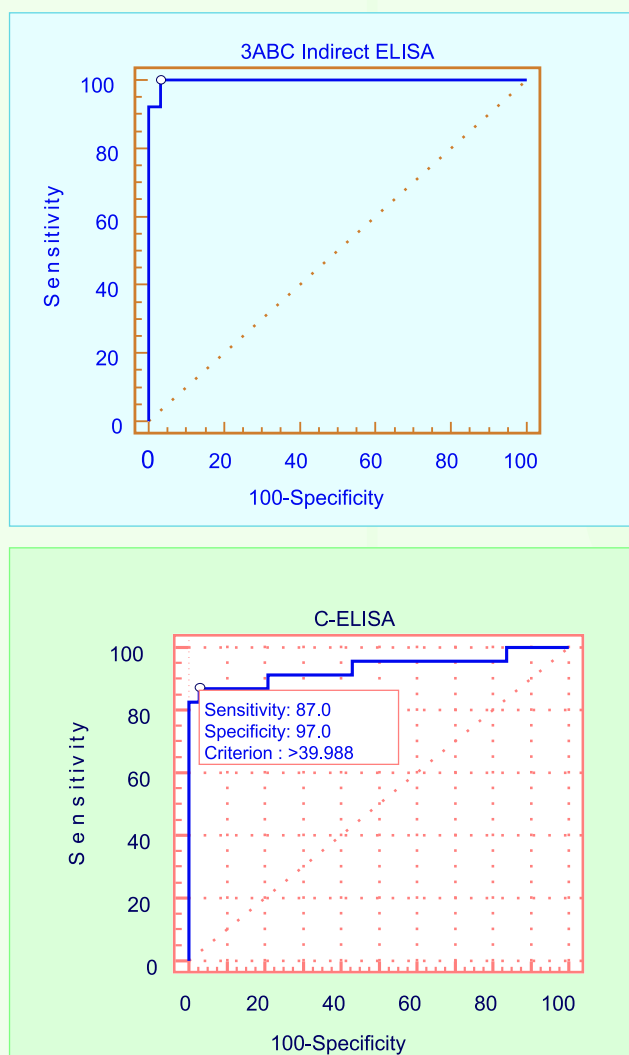
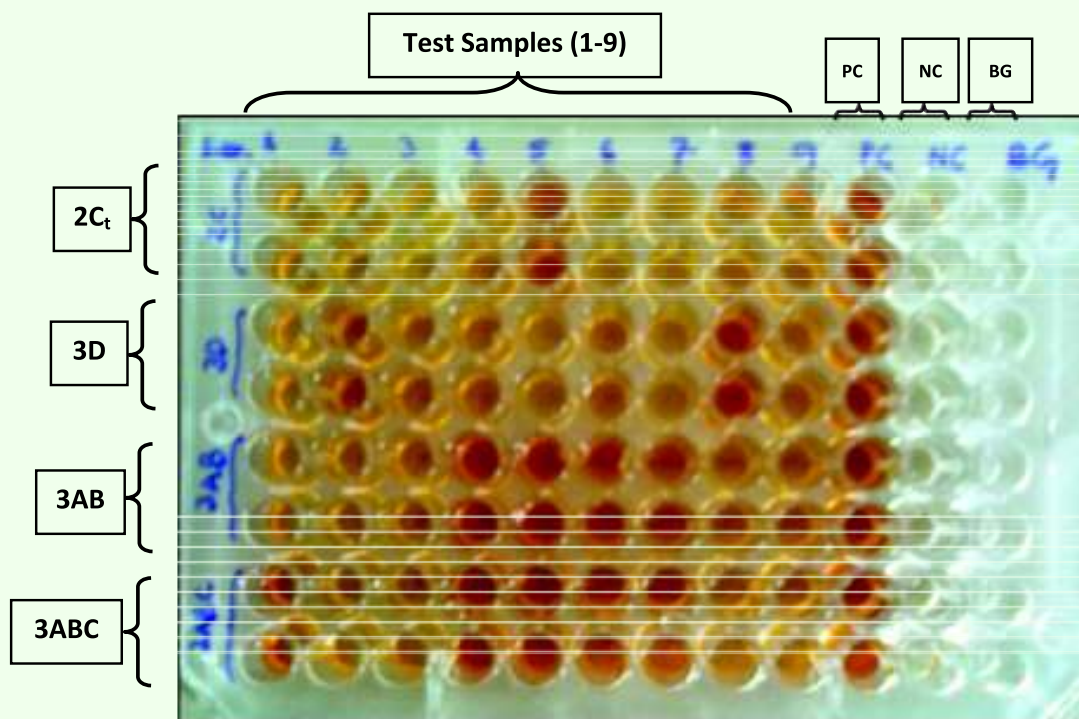


Fig. 14 Sensitivity and specificity of 3ABC based I-ELISA and C-ELISA

8.4 Development of multiple antigen ELISA

Foot and mouth disease (FMD) is a highly contagious and economically devastating viral disease of cloven-hoofed animals. India is endemic and six-monthly preventive vaccination combined with surveillance has been adopted as the major strategy for FMD control. Nonstructural proteins (NSPs) 2C and 3D of FMDV were expressed in prokaryotic host system and the performance of recombinant 2C and 3D NSPs based indirect ELISAs (I-ELISA) was evaluated for detecting FMD virus infection irrespective of the vaccination status. A profiling ELISA was developed using a panel of recombinant NSPs 2C, 3D, 3AB and 3ABC as an integrated NSP-serology system which has the potential to be employed as an easy-to-perform foolproof confirmatory assay (Fig.15).

The N-terminus truncated 2C_t protein and full length 3D protein were expressed in the prokaryotic system. Deletion of N-terminal amphipathic helix facilitated the expression of 2C_t in soluble form. Expressed proteins could be purified to near homogeneity by metal affinity chromatography and the observed sizes of the proteins on SDS-PAGE were comparable to the calculated size. Both these proteins demonstrated differential reactivity with FMD convalescent and naïve cattle sera in western blot. The optimal concentration of coating antigen (~50 ng/well) and test serum dilution (1:20) were determined by checkerboard titration for the standardization of 2C_t and 3D I-ELISAs. A uniform cut-off of 40% positivity (PP) was adopted for both 2C_t and 3D I-ELISAs on the basis of the frequency distribution of a bovine serum panel representing different expected epidemiological scenarios. Cut-off values of 40% and 30% were adopted for 3AB and 3ABC, respectively. Diagnostic sensitivity (DSn) values of 84.14%, 98.23%, 99.118% and 99.118% and diagnostic specificity (DSp) values of 97.89%, 98.42%, 100% and 97.89% were obtained for 2C_t, 3D, 3AB and 3ABC, respectively.



*PC= Positive control; NC= Negative control; BG= Background control

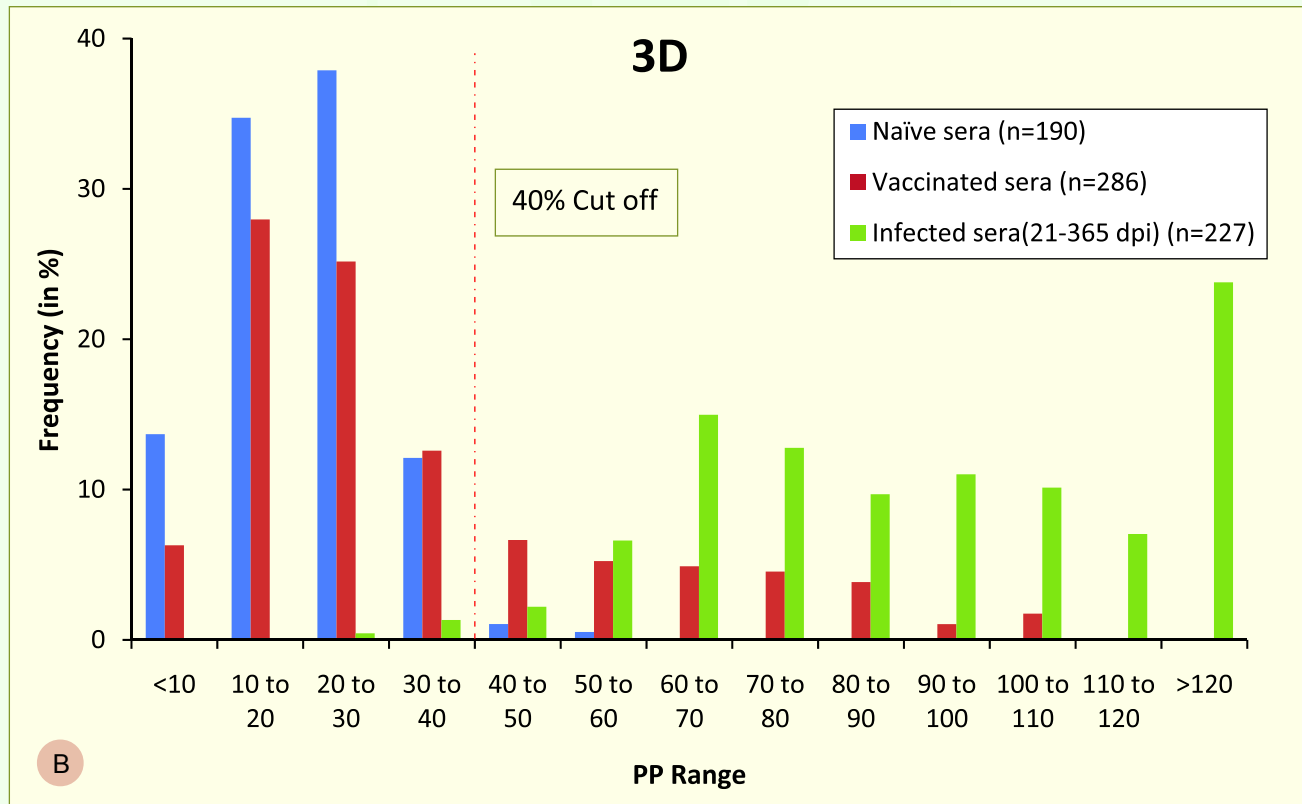
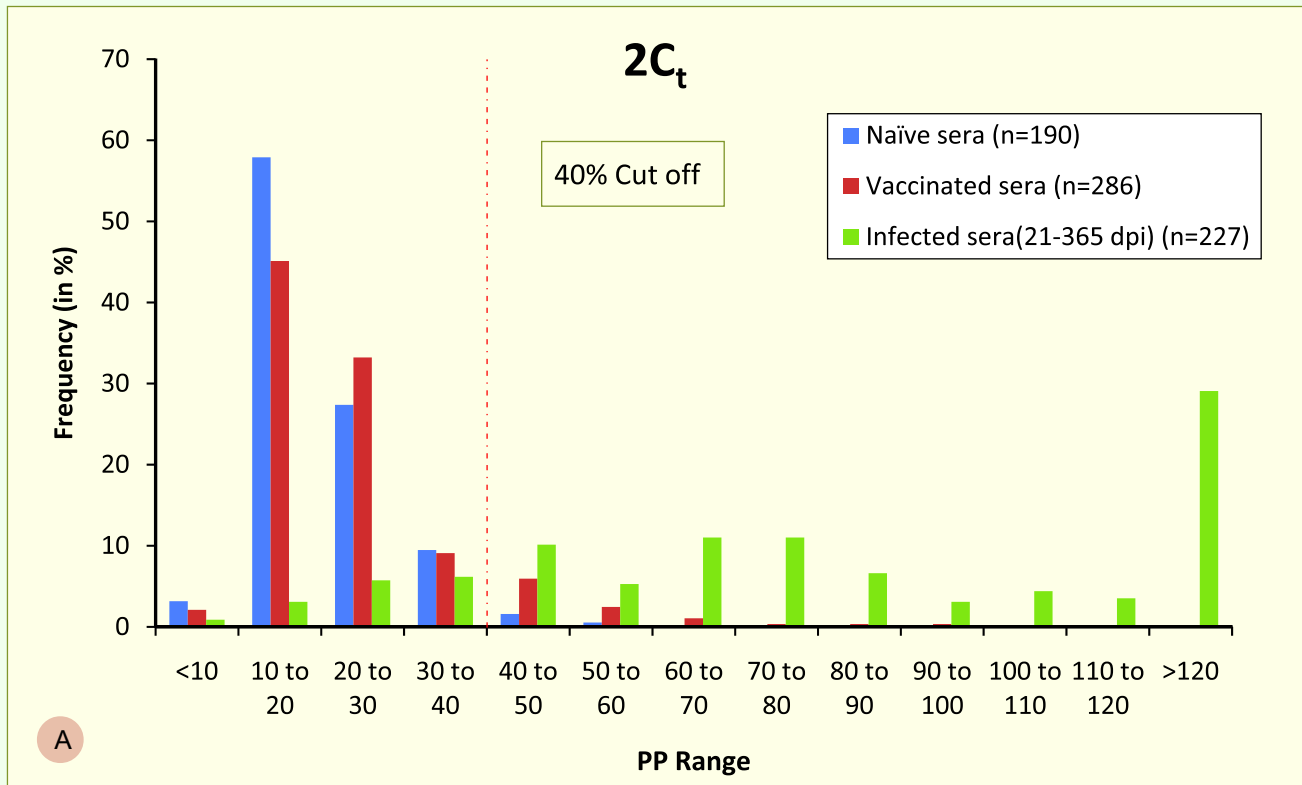
Fig.15 ELISA plate layout for testing of samples in profiling ELISA

The relatively lower DS_n for 2C_t is presumed to be most likely due to loss of reactive epitopes at the N-terminus. The rate of concordance in test results among all four NSP ELISAs was found to be 97.36%, 57.69% and 83.7% for naïve, vaccinated and infected (21-365 dpi) sera and the highest degree of concordance was observed between 3AB and 3ABC I-ELISAs. By the integrated NSP-serology system judgment criterion, a sample is confirmed for FMD virus infection if it comes positive in the preliminary 3AB screening assay along with at least any two of the other three NSP ELISAs.

A total of 8451 random sera obtained from bovines (6750), sheep (1090) and goat (611) from all over India were screened against the

NSP panel. The apparent seroprevalence of NSP-antibodies in bovine was estimated to be 23.7%, 33.4%, 32.9% and 37.6% in 2C_t, 3D, 3AB and 3ABC I-ELISA, respectively. The apparent seroprevalence of NSP-antibodies were estimated as 25.9%, 30.3% and 14.8% in sheep and 7.6%, 4.4% and 10.3% in goats as tested in 2C_t, 3D, and 3AB ELISA, respectively (Fig 16).

Considering their adequate performance attributes, it is presumed that the developed 2C_t and 3D I-ELISAs could be included in the DIVA diagnostic strategy in combination with 3AB and 3ABC I-ELISAs as an integrated confirmatory serological assay system in detecting evidence of FMDV activity at the herd level with high level of confidence.



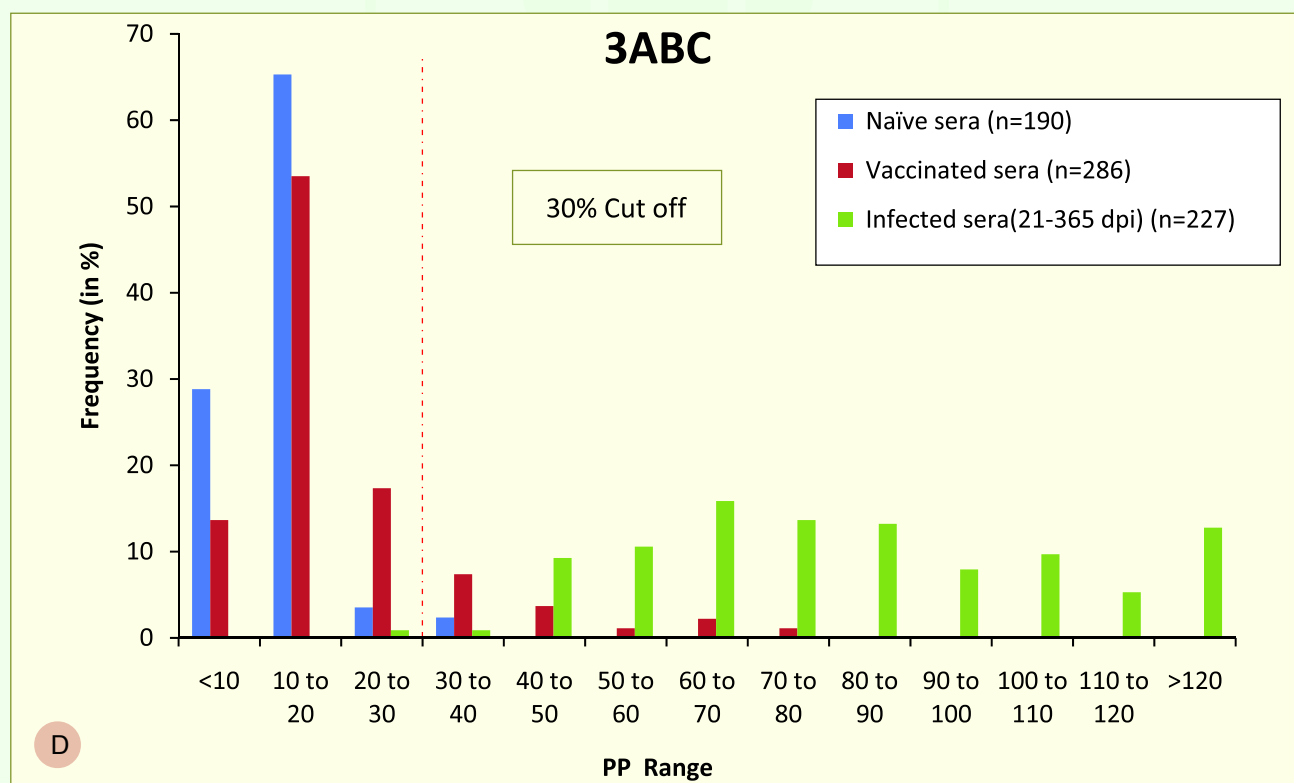
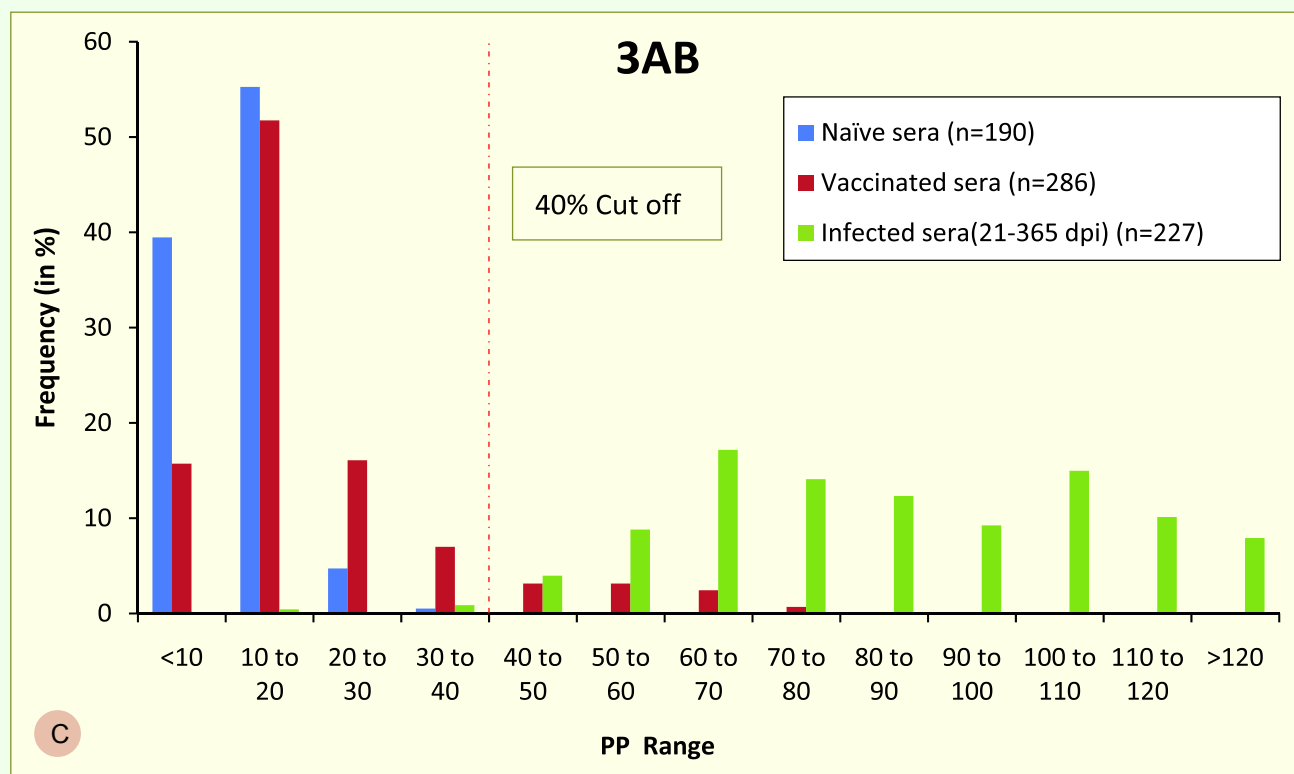


Fig. 16 Cu t-off Estimation

8.5 Evaluation of alternate assay based on Single serum dilution ELISA for estimation protective antibody titer in FMD vaccinated animals

Liquid phase blocking ELISA (LPBE) is a primary tool for sero-surveillance in India. The assay precisely determines the titers of humoral immune response against structural proteins (SPs) of FMDV in vaccinated and infected animals. The existing LPBE assay is comparatively more time-consuming as less number of serum samples could be tested on a single ELISA plate thus making it less amenable for screening of large number of serum samples within short span of time. With increase in the number of animals brought under the blanket of intensive vaccination since launch of FMD control programme in 2003, there's utmost need for the modification of the existing format so as to expedite the testing of large number of serum samples. The alternate assay utilizes only single dilution of test serum (instead of four dilutions being used in the existing format) and titer can be interpolated using the set of reference serum controls. Inhibition in the optical density of serum is linearly correlated (follow negative regression slope) with the titer or amount of protective antibodies present in the serum. This fact is utilized for the interpolation of protective titers. The handling of large number of samples throughout the country making use of this approach requires high capacity analysis software. SAS 9.2 statistical package provided by ICAR is highly efficient and capable of the interpolation of titers of the large number of samples.

The single serum dilution LPBE assay was analysed by standardizing reference serum controls for mass scale testing. Preliminary results are highly encouraging for the successful estimation of titers from the in-house serum samples. The new assay correlated well with conventional serial two fold dilution method ($r' > 0.95$).

The single serum dilution based LPBE seems to be a promising tool-in-hand to replace the conventional LPBE assay for mass scale sero-surveillance as a single ELISA plate in the new format can accommodate 66 test samples unlike the current format where only 11 samples could be tested in a 96-well ELISA plate. This considerable increase in the testing capacity would not only save time and man-power but will also result in less consumption of reagents.

The confidence level of the single serum dilution LPBE needs to be further enhanced before the assay could be successfully employed on a large scale and thus, more number of random and post vaccinated bovine serum samples is being tested in the laboratory. Inter-laboratory validation studies are also needed to assess the variations and other possible problems that can be encountered on full-fledged application of the assay. The evaluation of thermostability of the freeze dried reference serum controls is also under process to ensure that components of kit can be shipped throughout the country without any marked loss in efficiency.

National FMD Serosurveillance

9.1 DIVA (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) is assessed using purified recombinant 3AB3 (~38 kD) NSP in an indirect ELISA. The test is to be considered 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 be less than 0.1. To reduce inter-run variation due to

differences in absolute absorbance between runs, final results for each test serum needs to be expressed as the PP value $[(\text{test serum sample mean OD}/\text{positive control serum mean OD}) \times 100]$ 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 period, a total of 31,042 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 ~ 27% samples/animals (Table 8 and 9). The seroprevalence rate reached very high levels (65-81%) in areas having recent outbreaks.

Table 8. Result summary of r3AB3 NSP-ELISA

Sl. No.	Place of origin	Host	Total samples tested	Total positive	%3AB3 reactors
1	Assam	Bovine	1184	427	36.06
2	Manipur	Bovine	90	12	13.30
3	Mizoram	Bovine	800	269	33.60
4	Nagaland	Bovine	10	7	70.00
5	Tripura	Bovine	642	73	11.37
6	Haryana	Bovine	2054	245	11.92
7	Punjab	Bovine	2000	244	12.32
8	Orissa	Bovine	2140	222	10.37
9	Tamilnadu	Bovine	1390	274	19.70
10	Bihar	Bovine	3915	983	25.10
11	Andhra Pradesh	Bovine	2622	509	19.40
12	West Bengal	Bovine	1087	288	26.49
13	Himachal Pradesh	Bovine	790	77	09.74
14	MP	Bovine	98	71	72.45

Sl. No.	Place of origin	Host	Total samples tested	Total positive	%3AB3 reactors
15	Karnataka	Bovine	2795	1289	46.00
16	Gujarat	Bovine	2730	526	19.26
17	J & K	Bovine	1498	291	19.42
18	UP	Bovine	1173	329	28.00
19	MP	Bovine	285	848	29.70
20	Manipur	Bovine	900	152	16.88
21	Kerala	Bovine	540	258	47.77
22	Uttarakhand	Bovine	487	72	14.7
23	Rajasthan	Bovine	492	261	53.00
24	Arunachal Pradesh	Bovine	315	108	34.20
25	Maharashtra	Bovine	1005	506	50.34
Total			31042	8341	26.87

Table 9. Year-wise result summary of r3AB3 NSP-ELISA during 2008-09 to 2010-2011, the prevalence has been around 27%

Year	Total samples tested	States from which samples were collected	Total positive	% DIVA reactors
2008-09	18,326	Tripura, Gujarat, Mizoram, Himachal Pradesh, Nagaland, Bihar, Madhya Pradesh, West Bengal, Manipur, Maharashtra, Punjab, Kerala, Andhra Pradesh, Arunachal Pradesh, Orissa, Haryana, Jammu & Kashmir, Rajasthan, Karnataka, Tamil Nadu	5120	27.94
2009-10	29,763	Tripura, Gujarat, Mizoram, Himachal Pradesh, Nagaland, Bihar, Madhya Pradesh, West Bengal, Manipur, Maharashtra, Punjab, Kerala, Andhra Pradesh, Arunachal Pradesh, Orissa, Haryana, Jammu & Kashmir, Rajasthan, Karnataka, Tamil Nadu, Assam	8303	27.9 %
2010-11	31042	Assam, Manipur, Mizoram, Nagaland, Tripura, Haryana, Punjab, Orissa, Tamilnadu, Bihar, Andhra Pradesh, West Bengal, Himachal Pradesh, MP, Karnataka, Gujarat, J & K, UP, Manipur, Kerala, Uttarakhand, Rajasthan, Arunachal Pradesh, Maharashtra	8341	26.87

9.2 LPB-ELISA (Percent protected)

During the period under report, a total of 18953 serum samples were subjected to LPB ELISA for determination of antibody level against

serotypes O,A and Asia1. The result showed protective antibody titre in 38.5%, 35.4% and 32.3% samples/animals against types O, A and Asia1, respectively.

Table 10. Result summary of testing of Random serum samples

State	Species of Animals	Number of serum samples tested	No. of samples having antibody titre of ≥ 1.8		
			Type O	Type A	Type Asia1
Assam	Cattle+Buffalo	2075	636(30.7)	556(26.8)	405(19.5)
Meghalaya	Cattle+Buffalo	189	65(35.1)	42(22.2)	9(4.8)
Haryana	Cattle+Buffalo	1220	608(49.8)	737(60.41)	650(53.3)
Punjab	Cattle+Buffalo	739	518(70)	475(64)	518(70)
AP	Cattle+Buffalo	2245	991(44.1)	1050(46.7)	942(41.9)

State	Species of Animals	Number of serum samples tested	No. of samples having antibody titre of ≥ 1.8		
			Type O	Type A	Type Asia1
West Bengal	Cattle+Buffalo	626	157(25.07)	125(19.96)	68(10.86)
HP	Cattle+Buffalo	1238	520(42)	468(37.8)	486(39.2)
Karnataka	Cattle+Buffalo	3035	1282(42.2)	1121(36.9)	1112(36.6)
J & K	Cattle+Buffalo	943	197(20.89)	194(20.57)	161(17.07)
UP	Cattle+Buffalo	1707	875(51.2)	641(37.5)	657(38.4)
Manipur	Cattle+Buffalo	792	391(49.37)	336(42.42)	293(37.63)
MP	Cattle+Buffalo	1784	288(16.14)	267(14.96)	244(13.67)
Tripura	Cattle+Buffalo	194	47(24.2)	60(30.9)	50(25.7)
Uttarakhand	Cattle+Buffalo	405	93(22.9)	91(22.4)	65(16)
Gujarat	Cattle+Buffalo	72	46(63.8)	30(41.6)	39(54)
Maharashtra	Cattle+Buffalo	178	27(15.1)	42(23.6)	35(19.6)
Arunachal Pradesh	Cattle+Buffalo	315	38(12)	46(14.6)	56(17.7)
Rajasthan	Cattle+Buffalo	918	288(31.7)	368(40)	287(31.3)
Tamilnadu	Cattle+Buffalo	278	231(83)	70(25)	56(20)
Total		18953	7301(38.5)	6719(35.4)	6133(32.3)

9.3 Surveillance and Seromonitoring of FMD in ovine, caprine and porcine species in India

The data regarding the prevalence of FMD virus infection in sheep, goat and pigs of our country is not sharply known. Whether these species are routinely vaccinated against the disease is also not actively surveyed. When we aim to eradicate FMD from the country, then our target should also be focused on these species of animals, along with bovines. Because, these animals always don't show the overt clinical symptoms of the disease and act as symptomless carriers. Thus, they can be reservoirs of infection/

virus and virus shedders in the environment. Hence, focused study of FMD in small ruminants and pigs initiated.

Serum samples were collected from sheep, goat and pigs in Uttarakhand, Gujarat, MP, J & K, Arunachal Pradesh, Orissa, Assam, UP, Punjab, Andaman and HP. The collected serum samples were subjected to LPB ELISA and DIVA.

Sheep: Out of 271 Ovine serum samples tested in LPBE, 38 (14.02%) samples showed log₁₀ titer of 1.5, 49 (18.08%) samples showed log₁₀ titer of 1.8 and 43 (15.86%) samples showed log₁₀ titer of >2.1 for any prevalent serotypes (O, A

Table 11. Result summary of LPB ELISA performed on ovine serum samples

States	Serotype wise LPBE titre (Log ₁₀)								
	1.5			1.8			>2.1		
	O	A	Asia1	O	A	Asia1	O	A	Asia1
UK	1	1	0	0	0	0	0	0	0
Gujarat	0	1	3	0	0	0	0	0	0
MP	5	3	2	11	5	9	14	8	3
J&K	3	6	8	4	7	8	16	13	15
Arunachal Pradesh	1	0	0	0	0	0	0	0	0
Orissa	5	5	5	6	4	3	5	2	2
Punjab	0	0	0	0	3	0	4	2	0
HP	0	0	0	0	0	0	0	0	0
Total	15 (5.53%)	16 (5.90%)	18 (6.64%)	21 (7.74%)	19 (7.01%)	20 (7.38%)	39 (14.39%)	25 (9.22%)	20 (7.38%)

and Asia 1) of FMDV. Out of 1253 ovine serum samples tested in DIVA-E, 178 (14.20%) samples were found to be 3AB-NSP reactors (Table 11 & 14).

Goat: Out of 655 caprine serum samples tested in LPBE, 155 (23.66%) samples showed log₁₀ titer of 1.5, 119 (18.16%) samples showed log₁₀ titer of 1.8 and 224 (34.19%) samples showed log₁₀ titer of >2.1 for any prevalent serotypes (O, A and Asia 1) of FMDV. Out of 1272 caprine

serum samples tested in DIVA-E, 132 (10.37 %) samples were found to be 3AB-NSP reactors (Table 12 & 14).

Pig: Out of 175 porcine serum samples tested in LPBE, 6 (3.42%) samples showed log₁₀ titer of 1.5, 6 (3.42%) samples showed log₁₀ titer of 1.8 and 4 (2.28%) samples showed log₁₀ titer of >2.1 for any prevalent serotypes (O, A and Asia 1) of FMDV (Table 13 & 14).

Table 12. Result summary of LPB ELISA performed on caprine serum samples

States	Serotype wise LPBE titre (Log ₁₀)								
	1.5			1.8			>2.1		
	O	A	Asia1	O	A	Asia1	O	A	Asia1
UK	13	6	11	5	4	3	2	0	0
Gujarat	2	4	6	3	0	1	10	1	1
MP	14	16	18	15	18	20	39	20	20
J & K	2	0	4	1	5	4	3	1	4
Orissa	11	9	11	9	4	3	5	3	1
Assam	1	3	8	8	10	7	34	30	2
Punjab	10	19	29	19	40	43	120	84	60
HP	0	0	0	0	0	0	0	0	0
Total	53 (8.09%)	57 (8.70%)	87 (13.28%)	58 (8.85%)	81 (12.36%)	81 (12.36%)	213 (32.51%)	139 (21.22%)	88 (13.43%)

Table 13. Result summary of LPB ELISA performed on porcine serum samples

States	Serotype wise LPBE titre (Log ₁₀)								
	1.5			1.8			>2.1		
	O	A	Asia1	O	A	Asia1	O	A	Asia1
UK	0	0	0	0	0	0	0	0	0
Arunachal Pradesh	0	0	0	0	0	0	0	0	0
Orissa	3	0	3	2	3	2	0	2	0
Punjab	0	0	0	0	1	0	2	0	0
Total	3 (1.71%)	0	3 (1.71%)	2 (1.14%)	4 (2.28%)	2 (1.14%)	2 (1.14%)	2 (1.14%)	0

Table 14. Result summary of r3AB3 NSP-ELISA (DIVA) performed on ovine and caprine serum samples

states	Total No. of serum samples	Serum samples collected from Sheep	DIVA reactivity	Serum samples collected from Goat	DIVA reactivity
UK	171	5	0	135	10
Gujarat	454	133	12	321	50
MP	159	31	2	128	30
J&K	82	39	26	43	7
Arunachal Pradesh	58	29	9	Nil	-
Orissa	299	93	3	104	2
UP	192	29	4	163	13
Punjab	174	4	1	157	14
Andhra Pradesh	1100	887	121	203	6
HP	21	3	0	18	0

9.3.1 Serological Evidence of Foot-and-Mouth Disease Virus Infection in Randomly Surveyed Goat Population of Orissa, India

The reason why sheep and goats have generally been ignored with regard to their role in the epidemiology of FMD is partly attributable to the often inapparent nature of the disease in these hosts and partly attributable to some evidence that FMD in sheep and goats is self-limiting. Because of the less florid and subdued clinical picture of FMD in small ruminants, on many occasions, the disease goes undiagnosed in these species. However, the role of small ruminants in maintaining FMD epidemics has, over time, attracted more attention, and it has been established that sheep and goats can also become carriers for up to 9 and 4 months, respectively. Nevertheless, their ability to become subclinically infected represents a reservoir for further rounds of infection, inter-epidemic survival of the virus and spread of disease. There are many examples of FMD being carried into countries previously disease free by the movement of infected sheep and goats.

India is endemic for FMD, and goats constitute the second largest susceptible population of domestic livestock. Although a mixed farming and communal grazing system is practised in India, only in a few outbreaks, the disease in small ruminants is documented and diagnosed. The FMD Control Programme running in India at present includes vaccination and sero-surveillance in only bovines, overlooking caprine and ovine species. In order for India to implement effective FMD control strategies, it is imperative that the epidemiology of the disease is completely and clearly understood. This cannot be realized if the role of small ruminants in the overall epidemiology of the disease is ignored, in spite of representing such a large part of the susceptible domestic livestock in India. Despite the availability of much circumstantial evidence to suggest the importance of small ruminants in the transmission of FMD virus, studies on these

species have been very limited in comparison to other agriculturally important species.

Serological surveillance provides retrospective analysis of FMD outbreaks from the prevalence estimation of anti-FMDV antibodies. Here, serological investigations were carried out to generate estimates of antibody prevalence in goats of Orissa state to both non-structural (NSP-Ab) and structural proteins (SP-Ab) of FMD. A total of 544 serum samples from goats (kids <1 year old, $n = 176$, and adults, $n = 368$) were collected from 12 villages in six districts of southern Orissa, India (Fig. 17). Seventy-three serum samples of adult goats from abattoirs located at the state capital Bhubaneswar were also collected for the study. An indirect ELISA was performed using r3AB3 DIVA ELISA kit (PDFMD, Mukteswar), recently validated on goat serum samples, to assess antibodies against 3AB NSP of FMDV.

The husbandry practice in Orissa reflects both cattle and goats being reared in close proximity and at many places even co-housed in a single shed. Communal grazing is practised in most of the areas, and both small and large ruminants are allowed to use the same pasture land and water sources. The state experiences FMD outbreaks throughout the year, and the seroprevalence rate for NSPAb in bovines has been estimated to be 40% (Annual Report 2008–2009, PDFMD). The apparent overall NSP-Ab and SP-Ab seroprevalences were 38% and 20.7%, respectively, which signifies a very high level of FMD virus circulation in the goat population despite the lack of clinical signs in this species. The percentage of seropositive (NSP reactors) animals varied widely from 5% in Koraput district to 62.5% each in Nuapada and Ganjam districts. This corroborates with the outbreak history obtained for the survey areas. Such a large variation of antibody prevalence values among the villages surveyed most likely reflects variation in the extent of FMDV exposure rather than any difference in the agro-climatic environment or in



Fig: 17 Overall district-wise percentage apparent prevalence estimates of non-structural-Ab (up) and structural-Ab (down) are depicted on Orissa state map

husbandry practices. For instance, in Dandabadi village, the apparent seroprevalence was found to be 0%, while in Petafulla village, which has experienced a recent FMD outbreak, the NSP-Ab prevalence reached 70%.

The apparent prevalence of NSP-Ab and SP-Ab was positively correlated in the sampling areas. Interestingly, the values found for NSP-Ab prevalence were almost consistently higher than those found for SP-Ab prevalence. This could have been attributable to either issues related to sensitivity and specificity of the test systems employed or differences in the post-infection kinetics of NSP and SP-Ab. One more consideration could be that ≥ 32 titre cut-off taken in LPB ELISA for judging seroconversion in goats might have underestimated the SP-Ab prevalence status in the context of declining titre post-infection. Hence, the cut-off for seroconversion, specifically in goats, needs to be addressed further. The pattern that emerged from SP-Ab analysis indicated goats being infected with all three prevalent serotypes (O, A

and Asia 1) and reinforces the concept that non-vaccinated goats can be exploited as tracer animals for detecting serotypes involved in outbreaks.

When an age-wise analysis of seropositive animals was made, 40.4% adult goats and 32.3% kids were found 3AB reactors, while 15.4% adult goats and 14.7% kids were found seropositive for SP-Ab. Such comparable level of seropositivity demonstrated in kids could be attributable to either active infection or passive transfer of maternal antibodies, but in either case, it would indicate the presence of FMDV in the flocks. It is likely that FMD in small ruminants under natural conditions resembles an 'iceberg infection' where the greater part of the weight of the infection is hidden below the morbidity level. When goat serum samples were investigated from an outbreak area (Jharigaon), a high proportion of samples were found seropositive (70% for NSP-Ab and 37.5% for SP-Ab) in comparison to the overall average values. During this outbreak, the majority of the in-contact goats remained

symptomless excepting a few, where mild signs such as anorexia and salivation were noticed. This confirms that silent involvement of goats in FMD epidemics is taking place in a mixed farming situation. When 39 goats were investigated from a village (Kandili, Patangi) where no outbreaks have been reported in the past 3 years, only three samples (two female adults and one kid) revealed seroconversion against 3AB NSP, while only one seropositive adult female showed low titre in LPBE. Hence, we suspect that either the observed level of seropositivity is attributable to a non-specific reaction or these animals have been procured from another infected village, and by the time they reached the village, they were virologically negative and unable to further transmit the virus in the flock while remaining seropositive. However, the first possibility looks unlikely as samples have scored positive for both NSP- and SP-Ab. The chance of infection of only a few goats with a strain having limited adaptability and potential to spread that has died out spontaneously without being noticed could not be totally ruled out. Out of 73 samples collected from the abattoirs, 52% and 31.5% samples were found to be positive in NSP and LPB ELISA, respectively. These figures are significantly higher than the overall average values. Although the specific history for these animals was not available, all the animals were observed to be of higher age group and were

procured from various parts of Orissa. So, it is expected that these goats might have been exposed to FMDV infection resulting in the observed seroconversion.

Although here only 617 goat serum samples were tested in both NSP and LPB ELISA, this preliminary work has gathered serological evidence of a high overall level of viral activity in goat population of Orissa, concurrent FMD virus infection and asymptomatic goats in outbreak areas, all of which underscores the fact that unrecognized FMDV-infected goats could pose a potential risk of virus dissemination. Further investigations into the dynamics of anti-FMDV antibodies in the country-wide goat population using a larger sample size supplemented with virological examination data should be carried out to better understand the role of goats in the disease epidemiology. Vaccination has been shown to reduce infection and the probability of establishing the carrier state in the ovine species. Hence, a requirement exists to bring such species under the umbrella of ongoing control measures including vaccination coupled with zoosanitary measures, at least in regions with high small ruminant density and wherever they are in close association with other agriculturally important susceptible livestock to reduce silent amplification, excretion and transmission of the virus, and gain freedom from FMD. Similar study will be initiated in other state of the country.

Post Vaccinal Seroconversion Studies

10.1 Sero-monitoring under FMD Control Programme (FMD-CP)

A major vaccination programme has been initiated by the Government of India since August 2003 for Control of FMD (FMD-CP) covering 54 specified districts in the country. This involves 6 monthly vaccinations (trivalent; O, A and Asia1) 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 type specific neutralizing antibodies by Liquid Phase

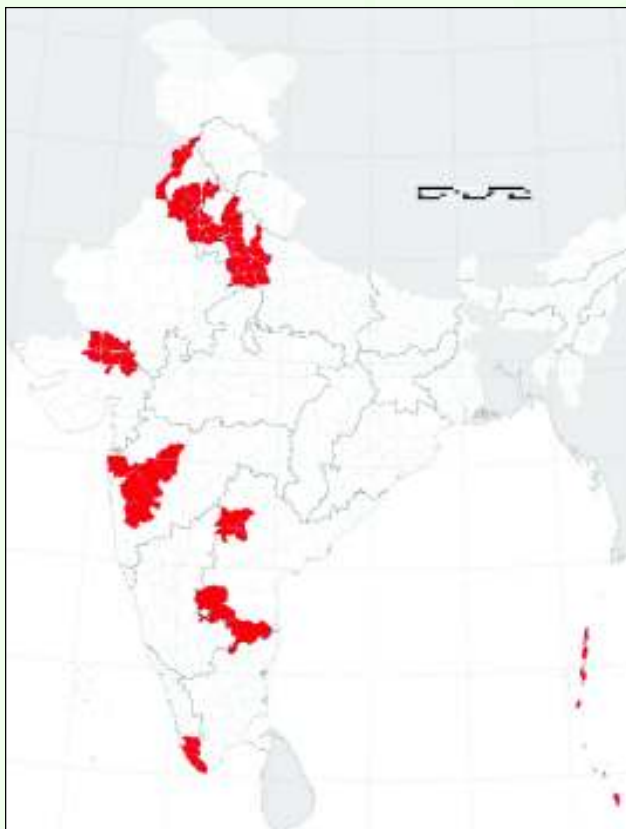
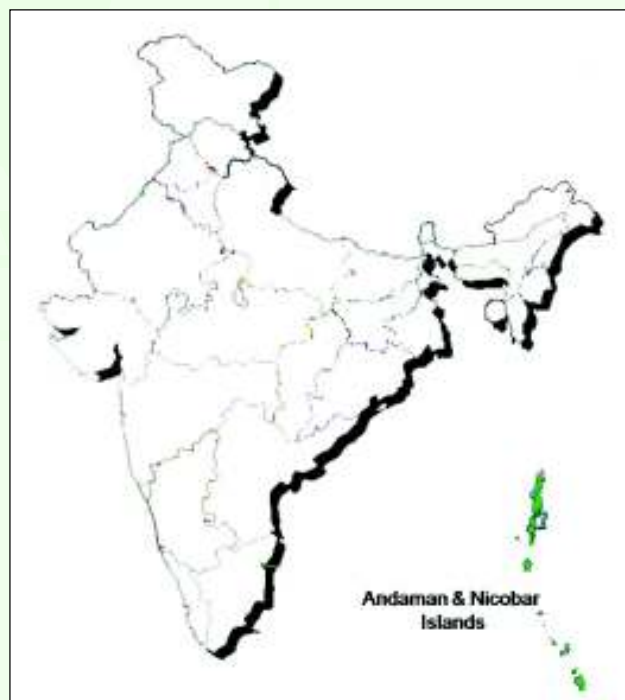


Fig.18 Fifty four districts in states covered under FMD Control Programme (Gov. of India).

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. All reagent and training to conduct LPB ELISA were provided by the institute. The test was compared with SNT, and it is recommended that LPB ELISA titer (in serum) of $e'' \log_{10} 1.8$ indicates protection against FMD.

10.1.1 Sero-surveillance in Andaman & Nicobar Island under FMDCP

Eight villages of Andaman & Nicobar are covered under FMDCP namely, Junglighat, Rangachang, Portmout, Garacharama, Wimberligunj, Monglutan, Elephant Point and Dollygunj. Serum samples were tested by Kolkata Regional Center.



- No serum samples were received for phases I and II.
- In phase III, 154 pre and 162 post-vac serum samples were tested. Percent serum sample having protective antibody titer of 1.8 and above for pre-vac samples was 25.9 for type 'O', 2.8 for type 'A' and 34.0 for type 'Asia-1'. The same for post-vac samples was 60.0 for type 'O', 20.3 for type 'A' and 73.6 for type 'Asia-1'.
- In phase IV, 149 pre and 146 post-vac serum samples were tested. Percent serum samples having protective antibody titer of 1.8 and above for pre-vac samples was 33.5 for type 'O', 33.5 for type 'A' and 23.4 for type 'Asia-1'. The same for post-vac samples were 64.6 for type 'O', 65.9 for type 'A' and 67.6 for type 'Asia-1'.
- In phase V, 126 pre and 122 post-vac serum samples were tested. Percent serum samples having protective antibody titer of 1.8 and above for pre-vac samples were 57.2 for type 'O', 50.8 for type 'A' and 44.3 for type 'Asia-1'. The same for post-vac samples were 55.8 for type 'O', 52.5 for type 'A' and 50.8 for type 'Asia-1'.
- In phase VI, 270 pre and 270 post-vac serum samples were tested. Percent serum samples having protective antibody titer of 1.8 and above for pre-vac samples were 18.5 for type 'O', 24.4 for type 'A' and 10.2 for type 'Asia-1'. The same for post-vac samples were 29.6 for type 'O', 38.4 for type 'A' and 13.2 for type 'Asia-1'.
- In phase VII, 265 pre and 265 post-vac serum samples were tested. Percent serum samples having protective antibody titer of 1.8 and above for pre-vac samples were 42.3 for type 'O', 30.9 for type 'A' and 21.1 for type 'Asia-1'. The same for post-vac samples were 65.7 for type 'O', 41.5 for type 'A' and 24.9 for type 'Asia-1'.
- In phase VIII, 251 pre and post-vac serum samples were tested. Percent serum samples having protective antibody titer of 1.8 and above for pre-vac samples were 21.11 for type 'O', 7.17 for type 'A' and 18.72 for type 'Asia-1'. The same for post-vac samples were 40.63 for type 'O', 19.52 for type 'A' and 33.86 for type 'Asia-1'.
- In phase IX, 228 pre and post-vac serum samples were tested. Percent serum samples having protective antibody titer of 1.8 and above for pre-vac samples were 32.01 for type 'O', 13.59 for type 'A' and 24.56 for type 'Asia-1'. The same for post-vac samples were 30.26 for type 'O', 15.35 for type 'A' and 18.82 for type 'Asia-1'.

Table 15. Result of seroconversion in Andaman & Nicobar Islands

Phase	Species	Number & % animals showing titres $\geq 1.8 \log_{10}$ against FMDV					
		Type O		Type A		Type Asia 1	
		Pre-vac	Post-vac	Pre-vac	Post-vac	Pre-vac	Post-vac
I	Cattle+Buffalo	Serum samples not available					
II	Cattle+Buffalo	Serum samples not available					
III	Cattle+Buffalo	40(25.9)	97(60.0)	5(2.8)	37(20.3)	52(34.0)	118(73.6)
IV	Cattle+Buffalo	50(33.5)	94(64.6)	50(33.5)	96(65.9)	35(23.4)	101(67.6)
V	Cattle+Buffalo	72(57.2)	68(55.8)	62(50.8)	64(52.5)	54(44.3)	62(50.8)
VI	Cattle+Buffalo	50 (18.5)	80 (29.6)	66 (24.4)	104 (38.4)	28 (10.2)	36 (13.2)
VIIa	Cattle+Buffalo	112 (42.3)	174 (65.7)	82 (30.9)	110 (41.5)	56 (21.1)	66 (24.9)
VIII	Cattle+Buffalo	53(21.11)	102(40.63)	18(7.17)	49(19.52)	47(18.72)	85(33.86)
IX	Cattle+Buffalo	73(32.01)	69(30.26)	31(13.59)	35(15.35)	56(24.56)	42(18.42)

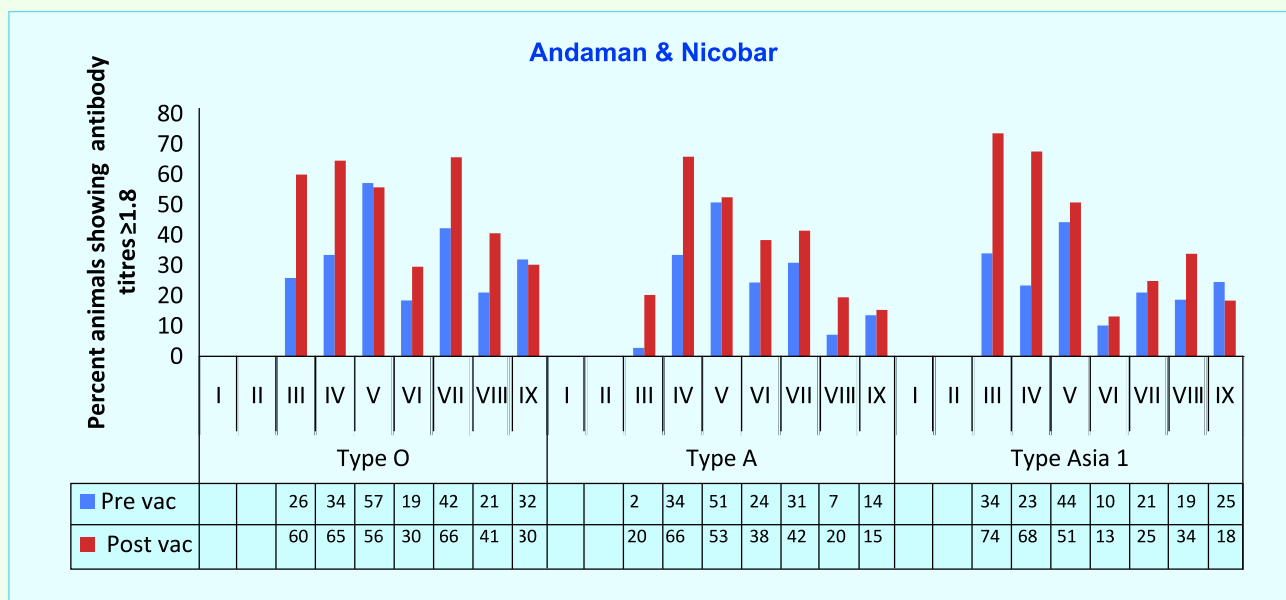


Fig. 19 Seroconversion in Andaman & Nicobar Islands

10.1.2 Sero-surveillance in Andhra Pradesh under FMDCP

Four districts of Andhra Pradesh namely, Ananthapur, Chittoor, Medak and Rangareddy are covered under FMDCP. The serum samples were tested by Hyderabad Regional Center.

- In phase I, 800 each of pre and post-vac serum samples were tested. Percent serum sample having protective antibody titer of 1.8 and above for pre-vac samples was 10.3 for type 'O', 5.3 for type 'A' and 11.5 for type 'Asia-1'. The same for post-vac samples was



Fig. 20 Districts covered under FMD Control Programme in Andhra Pradesh

42.5 for type 'O', 30.5 for type 'A' and 42.5 for type 'Asia-1'. It shows boosting of antibody level following vaccination.

- In phase II, 800 post-vac serum samples were tested. Pre-vac serum samples were not available. Percent serum sample having protective antibody titer of 1.8 and above was 54.2 for type 'O', 62.3 for type 'A' and 54.7 for type 'Asia-1'.
- In phase III, 800 each of pre and post-vac serum samples were tested. Percent serum sample having protective antibody titer of 1.8 and above for pre-vac samples was 26.2 for type 'O', 49.3 for type 'A' and 38.2 for type 'Asia-1'. The same for post-vac samples was 35.7 for type 'O', 66.5 for type 'A' and 52.7 for type 'Asia-1'.
- In phase IV, 800 each of pre and post-vac serum samples were tested. Percent serum sample having protective antibody titer of 1.8 and above for pre-vac samples was 35.1 for type 'O', 58.1 for type 'A' and 41.1 for type 'Asia-1'. The same for post-vac samples was 46.8 for type 'O', 77.1 for type 'A' and 64.8 for type 'Asia-1'.
- In phase V, 800 each of pre and post-vac serum samples were tested. Percent serum sample having protective antibody titer of 1.8 and above for pre-vac samples was 30.8 for type 'O', 58.2 for type 'A' and 42.8 for type 'Asia-1'. The same for post-vac samples

was 55.0 for type 'O', 71.8 for type 'A' and 56.3 for type 'Asia-1'.

- In phase VI, 800 each of pre and post-vac serum samples were tested. Percent serum sample having protective antibody titer of 1.8 and above for pre-vac samples was 34.3 for type 'O', 69.2 for type 'A' and 55.7 for type 'Asia-1'. The same for post-vac samples was 61.3 for type 'O', 86.3 for type 'A' and 79.3 for type 'Asia-1'.
- In phase VII, 800 each of pre and post-vac serum samples were tested. Percent serum sample having protective antibody titer of 1.8 and above for pre-vac samples was 34.0 for type 'O', 44.0 for type 'A' and 48.8 for type 'Asia-1'. The same for post-vac samples was 60.3 for type 'O', 67.5 for type 'A' and 64.7 for type 'Asia-1'.
- In phase VIII, 800 each of pre and post-vac serum samples were tested. Percent serum sample having protective antibody titer of 1.8 and above for pre-vac samples was 44.5 for type 'O', 51.8 for type 'A' and 41.6 for type 'Asia-1'. The same for post-vac samples was 74.0 for type 'O', 78.0 for type 'A' and 65.8 for type 'Asia-1'.
- In phase IX, 800 each of pre and post-vac serum samples were tested. Percent serum sample having protective antibody titer of 1.8 and above for pre-vac samples was 52.8 for type 'O', 41.1 for type 'A' and 35.9 for

Table 16. Result of seroconversion in Andhra Pradesh

Phase	Species	Number & % animals showing titres $\geq 1.8 \log_{10}$ against FMDV					
		Type O		Type A		Type Asia 1	
		Pre-vac	Post-vac	Pre-vac	Post-vac	Pre-vac	Post-vac
I	Cattle+Buffalo	83 (10.3)	340 (42.5)	43 (5.3)	244 (30.5)	92 (11.5)	340 (42.5)
II	Cattle+Buffalo	N.A.	434 (54.2)	N.A.	498 (62.3)	N.A.	438 (54.7)
III	Cattle+Buffalo	210 (26.2)	286 (35.7)	395 (49.3)	532 (66.5)	306 (38.2)	422 (52.7)
IV	Cattle+Buffalo	281 (35.1)	374 (46.8)	465 (58.1)	617 (77.1)	329 (41.1)	518 (64.8)
V	Cattle+Buffalo	247 (30.8)	440 (55)	466 (58.2)	574 (71.8)	343 (42.8)	450 (56.3)
VI	Cattle+Buffalo	275 (34.3)	490 (61.3)	554 (69.2)	690 (86.3)	446 (55.7)	634 (79.3)
VII	Cattle+Buffalo	274 (34.0)	483 (60.3)	349 (44.0)	540 (67.5)	391 (48.8)	518 (64.7)
VIII	Cattle+Buffalo	356 (44.5)	594 (74.0)	415 (51.8)	624 (78.0)	333 (41.6)	527 (65.8)
IX	Cattle+Buffalo	422 (52.8)	673 (84.1)	329 (41.1)	534 (66.8)	287 (35.9)	534 (66.8)
X	Cattle+Buffalo	502(62.7)	635(79.3)	368(46)	575(71.8)	411(51.3)	602(75.2)

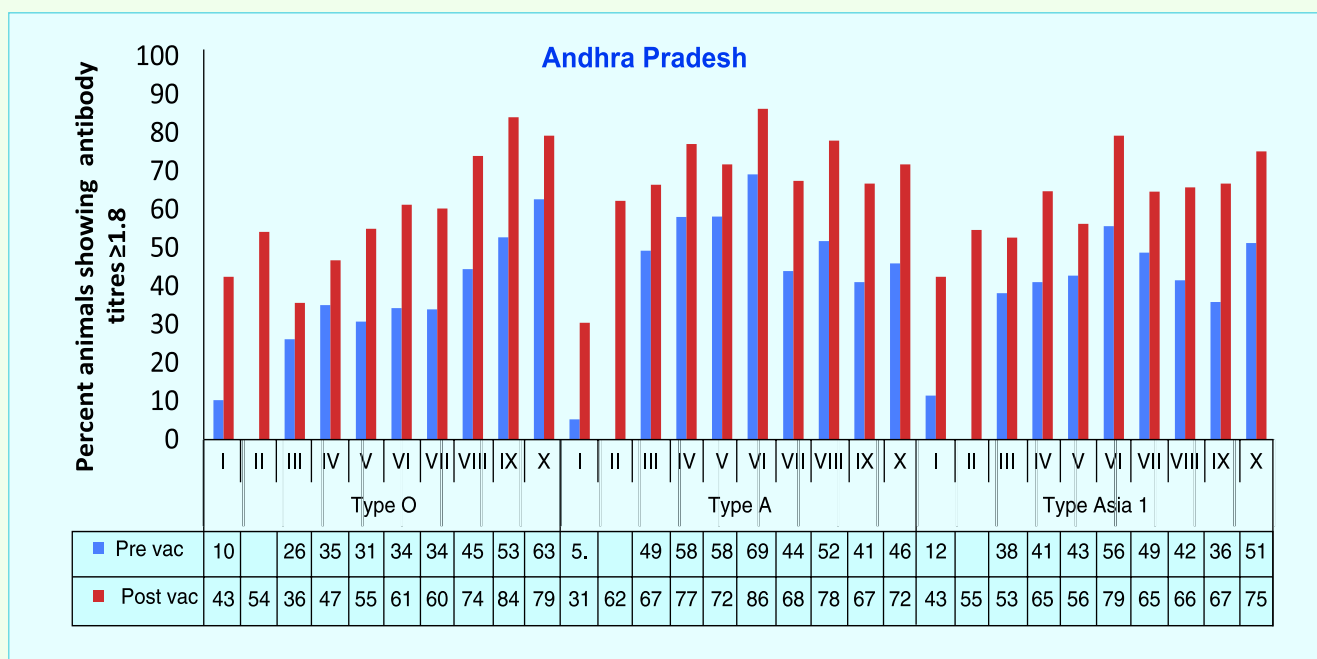


Fig. 21 Seroconversion in Andhra Pradesh

type 'Asia-1'. The same for post-vac samples was 84.1 for type 'O', 66.8 for type 'A' and 66.8 for type 'Asia-1'.

In phase X, 800 each of pre and post-vac serum samples were tested. Percent serum sample having protective antibody titer of 1.8 and above for pre-vac samples was 62.7 for type 'O', 46 for type 'A' and 51.3 for type 'Asia-1'. The same for post-vac samples was 79.3 for type 'O', 71.8 for type 'A' and 75.2 for type 'Asia-1'.

10.1.3 Sero-surveillance in Delhi under FMDCP

Serum samples collected under FMDCP from the state of Delhi were tested by Hissar Regional Center.

- In phase I, 50 each of pre and post-vac serum samples from buffaloes were tested. Percent serum sample having protective antibody titer of 1.8 and above for pre-vac samples was 53 for type 'O', 26 for type 'A' and 34 for type 'Asia-1'. The same for post-vac samples was 100 for type 'O', 94 for type 'A' and 96 for type 'Asia-1'.



Fig. 22 Districts covered in Delhi under FMD Control Programme

- In phase II, 24 each of pre-vac and post-vac serum samples from buffaloes were tested. Pre-vac serum samples were not available. Percent serum sample having protective antibody titer of 1.8 and above was 91 for type 'O', 40 for type 'A' and 95 for type 'Asia-1'. The same for post-vac samples was 96 for type 'O', 62 for type 'A' and 86 for type 'Asia-1'.

- In phase III, 50 each of pre and post-vac serum samples were tested. Percent serum sample having protective antibody titer of 1.8 and above for pre-vac samples was 94 for type 'O', 60 for type 'A' and 86 for type 'Asia-1'. The same for post-vac samples was 98 for type 'O', 80 for type 'A' and 92 for type 'Asia-1'.
- In phase IV, 50 pre and 46 post-vac serum samples were tested. Percent serum sample having protective antibody titer of 1.8 and above for pre-vac samples was 76 for type 'O', 28 for type 'A' and 54 for type 'Asia-1'. The same for post-vac samples was 82.6 for type 'O', 86.9 for type 'A' and 89.1 for type 'Asia-1'.
- In phase V, 44 pre and 53 post-vac serum samples were tested. Percent serum sample having protective antibody titer of 1.8 and above for pre-vac samples was 59 for type 'O', 52.2 for type 'A' and 72.7 for type 'Asia-1'. The same for post-vac samples was 88.6 for type 'O', 69.8 for type 'A' and 77.3 for type 'Asia-1'.
- In phase VI, 98 each of pre and post-vac serum samples were tested. Percent serum sample having protective antibody titer of 1.8 and above for pre-vac samples was 77.5 for type 'O', 61.2 for type 'A' and 72.4 for type 'Asia-1'. The same for post-vac samples was 98.9 for type 'O', 94.9 for type 'A' and 98.9 for type 'Asia-1'.
- In phase VII, 50 each of pre and post-vac serum samples were tested. Percent serum sample having protective antibody titer of 1.8 and above for pre-vac samples was 78 for type 'O', 66 for type 'A' and 50 for type 'Asia-1'. The same for post-vac samples was 88 for type 'O', 86 for type 'A' and 82 for type 'Asia-1'.
- In phase VIII, 100 each of pre and post-vac serum samples were tested. Percent serum sample having protective antibody titer of 1.8 and above for pre-vac samples was 92 for type 'O', 66 for type 'A' and 83 for type 'Asia-1'. The same for post-vac samples was 100 for type 'O', 86 for type 'A' and 98 for type 'Asia-1'.
- In phase IX, 100 pre-vac serum samples were tested. Percent serum sample having protective antibody titer of 1.8 and above for pre-vac samples was 57 for type 'O', 65 for type 'A' and 33 for type 'Asia-1'. Post-vac serum samples were not available.
- In phase X, serum samples were not available.
- In phase XI, 200 pre-vac serum samples were tested. Percent serum sample having

Table 17. Result of seroconversion in Delhi

Phase	Species	Number & % animals showing titres $\geq 1.8 \log_{10}$ against FMDV					
		Type O		Type A		Type Asia 1	
		Pre-vac	Post-vac	Pre-vac	Post-vac	Pre-vac	Post-vac
I	Cattle+Buffalo	83 (10.3)	340 (42.5)	43 (5.3)	244 (30.5)	92 (11.5)	340 (42.5)
I	Buffalo	26 (53)	50 (100)	13 (26)	47 (94)	17 (34)	48 (96)
II	Buffalo	22 (91)	23 (96)	12 (40)	15 (62)	23 (95)	22 (86)
III	Cattle+Buffalo	47 (94)	49 (98)	30 (60)	40 (80)	43 (86)	46 (92)
IV	Cattle+Buffalo	38 (76)	38 (82.6)	14 (28)	40 (86.9)	27 (54)	41 (89.1)
V	Cattle+Buffalo	26 (59)	47 (88.6)	23 (52.2)	37 (69.8)	32 (72.7)	41 (77.3)
VI	Cattle+Buffalo	76 (77.5)	97 (98.9)	60 (61.2)	93 (94.9)	71 (72.4)	97 (98.9)
VII	Cattle+Buffalo	39(78)	44(88)	33(66)	43(86)	25(50)	41(82)
VIII	Cattle+Buffalo	92 (92)	100 (100)	66 (66)	86 (86)	83 (83)	98 (98)
IX	Cattle+Buffalo	57(57)	NA	65(65)	NA	33(33)	NA
X	Cattle+Buffalo	Samples were not available					
XI	Buffalo	172(86)	NA	100(50)	NA	91(45.5)	NA

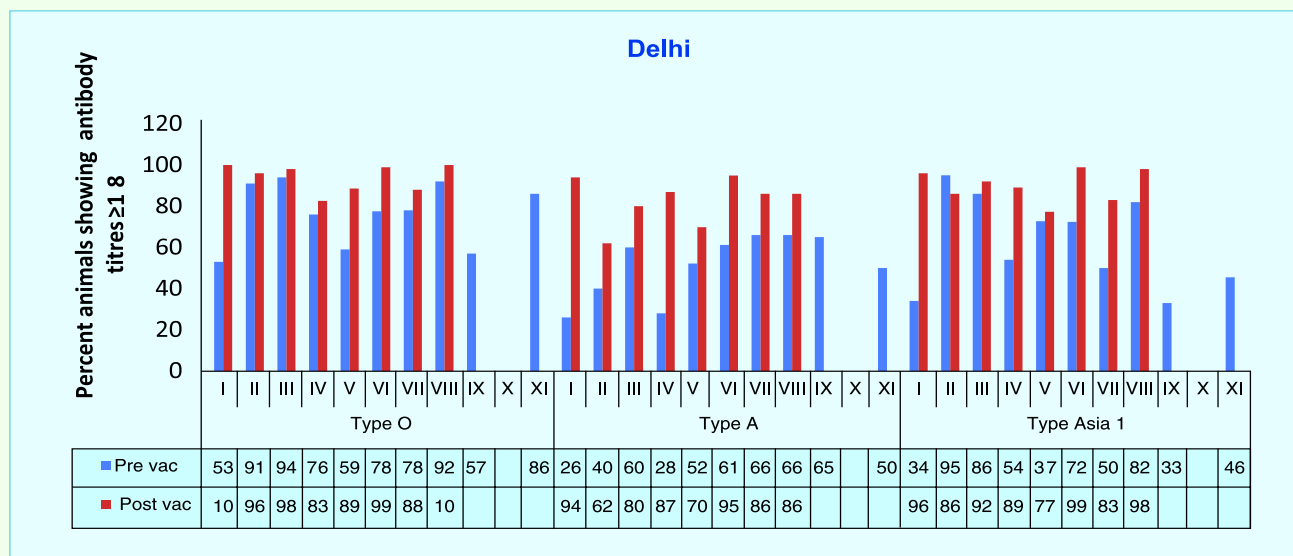


Fig. 23 Seroconversion in Delhi

protective antibody titer of 1.8 and above for pre-vac samples was 86 for type 'O', 50 for type 'A' and 45.5 for type 'Asia-1'.

10.1.4 Sero-surveillance in Gujarat under FMDCP

Under FMDCP four districts of Gujarat are covered namely, Banaskantha, Sabarkantha, Mehsana and Patan.

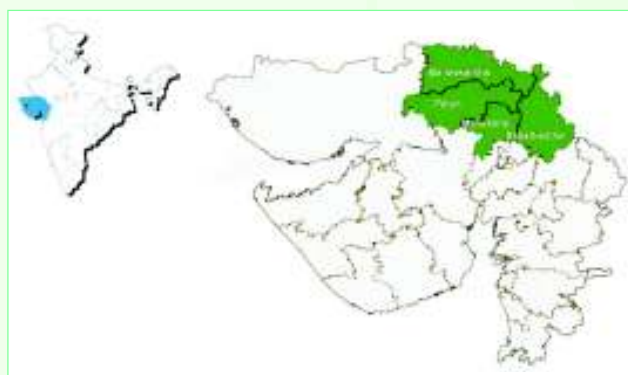


Fig. 24 Districts covered in Gujarat under FMD Control Programme

- Till fifth phase serum samples were tested at Pune Regional Center and sixth phase onwards the samples were tested at Ahmadabad Network Unit.
- In phase I, 382 pre and 259 post-vac serum

samples were tested. Percent serum sample having protective antibody titer of 1.8 and above for pre-vac samples was 19.1 for type 'O', 24.5 for type 'A' and 16.1 for type 'Asia-1'. The same for post-vac samples was 44.7 for type 'O', 48.7 for type 'A' and 43.5 for type 'Asia-1'.

- Serum samples were not available for Phase II.
- In phase III, 442 pre and 357 post-vac serum samples were tested. Percent serum sample having protective antibody titer of 1.8 and above for pre-vac samples was 27.8 for type 'O', 39.2 for type 'A' and 12.4 for type 'Asia-1'. The same for post-vac samples was 47.9 for type 'O', 58.3 for type 'A' and 35.4 for type 'Asia-1'.
- In phase IV, 497 and 456 post-vac serum samples were tested. Percent serum sample having protective antibody titer of 1.8 and above for pre-vac samples was 22.7 for type 'O', 40.7 for type 'A' and 14.6 for type 'Asia-1'. The same for post-vac samples was 60.7 for type 'O', 81.2 for type 'A' and 46.8 for type 'Asia-1'.
- In phase V, 195 pre and 202 post-vac serum samples were tested. Percent serum sample

having protective antibody titer of 1.8 and above for pre-vac samples was 23.6 for type 'O', 66.1 for type 'A' and 26.5 for type 'Asia-1'. The same for post-vac samples was 49 for type 'O', 91.6 for type 'A' and 51.3 for type 'Asia-1'.

- In phase VI, 395 each of pre and post-vac serum samples were tested. Percent serum sample having protective antibody titer of 1.8 and above for pre-vac samples was 30.1 for type 'O', 63.0 for type 'A' and 49.3 for type 'Asia-1'. The same for post-vac samples was 56.4 for type 'O', 80.2 for type 'A' and 60.7 for type 'Asia-1'.
- In phase VII, 800 each of pre and post-vac serum samples were tested. Percent serum

sample having protective antibody titer of 1.8 and above for pre-vac samples was 54.3 for type 'O', 48.1 for type 'A' and 43 for type 'Asia-1'. The same for post-vac samples was 78.8 for type 'O', 69.9 for type 'A' and 69.5 for type 'Asia-1'.

- In phase VIII, 800 each of pre and post-vac serum samples were tested. Percent serum sample having protective antibody titer of 1.8 and above for pre-vac samples was 23.9 for type 'O', 24.6 for type 'A' and 33 for type 'Asia-1'. The same for post-vac samples was 49.3 for type 'O', 44.6 for type 'A' and 50.4 for type 'Asia-1'.
- In phase IX, 800 each of pre and post-vac serum samples were tested. Percent serum

Table 18. Result of seroconversion in Gujarat

Phase	Species	Number & % animals showing titres $\geq 1.8 \log_{10}$ against FMDV					
		Type O		Type A		Type Asia 1	
		Pre-vac	Post-vac	Pre-vac	Post-vac	Pre-vac	Post-vac
I	Cattle+Buffalo	50 (19.1)	116 (44.7)	59 (24.5)	128 (48.7)	42 (16.1)	114 (43.5)
II	Cattle+Buffalo	Serum samples not available					
III	Cattle+Buffalo	123 (27.8)	171 (47.9)	171 (39.2)	268 (58.3)	51 (12.4)	149 (35.4)
IV	Cattle+Buffalo	113 (22.7)	277 (60.7)	184 (40.7)	355 (81.2)	73 (14.6)	218 (46.8)
V	Cattle+Buffalo	46 (23.6)	99 (49.0)	126 (66.1)	179 (91.6)	44 (26.5)	92 (51.3)
VI	Cattle+Buffalo	119 (30.1)	223 (56.4)	249 (63.0)	317(80.2)	195 (49.3)	240 (60.7)
VII	Cattle+Buffalo	434 (54.3)	630 (78.8)	385 (48.1)	559 (69.9)	344 (43.0)	556 (69.5)
VIII	Cattle+Buffalo	191 (23.9)	394 (49.3)	197 (24.6)	357 (44.6)	264 (33.0)	403 (50.4)
IX	Cattle+Buffalo	230(28.7)	618(77.2)	284(35.5)	572(71.5)	326(40.7)	595(74.4)
X	Cattle+Buffalo	356(44.5)	620(77.5)	286(35.7)	525(65.6)	276(34.5)	535(66.9)

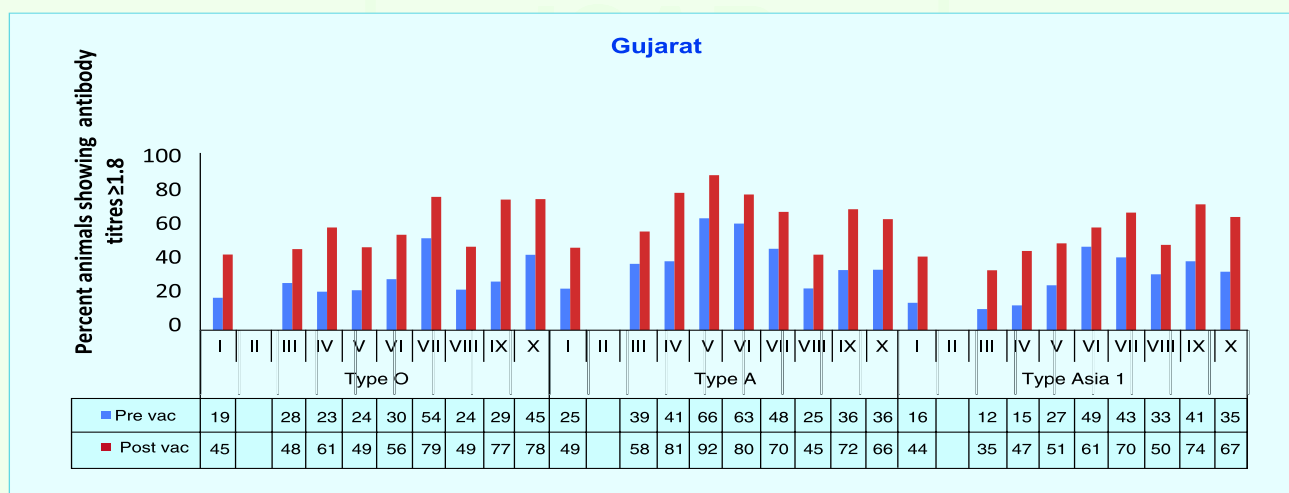


Fig . 25 Seroconversion in Gujarat

sample having protective antibody titer of 1.8 and above for pre-vac samples was 28.7 for type 'O', 35.5 for type 'A' and 40.7 for type 'Asia-1'. The same for post-vac samples was 77.2 for type 'O', 71.5 for type 'A' and 74.4 for type 'Asia-1'.

In phase X, 800 each of pre and post-vac serum samples were tested. Percent serum sample having protective antibody titer of 1.8 and above for pre-vac samples was 44.5 for type 'O', 35.7 for type 'A' and 34.5 for type 'Asia-1'. The same for post-vac samples was 77.5 for type 'O', 65.6 for type 'A' and 66.9 for type 'Asia-1'.

10.1.5 Sero-surveillance Haryana under FMDCP

Under FMDCP, eight districts of Haryana are covered namely, Bhiwani, Fatehabad, Hisar, Jhajjar, Jind, Rohtak, Sirsa and Sonapat.



Fig . 26 Districts covered in Haryana under FMD Control Programme

- Serum samples were tested by Hissar Regional Center.
- Serum samples were not available for Phase I.
- In phase II, 1558 post-vac serum samples were tested. Pre-vac serum samples were not available. Percent serum sample having protective antibody titer of 1.8 and above was 68.3 for type 'O', 55.1 for type 'A' and 53.3 for type 'Asia-1'.
- In phase III, 1585 post-vac serum samples were tested. Pre-vac serum samples were not available. Percent serum sample having protective antibody titer of 1.8 and above was 72.3 for type 'O', 63.6 for type 'A' and 63.4 for type 'Asia-1'.
- In phase IV, 1589 pre and 1552 post-vac serum samples were tested. Percent serum sample having protective antibody titer of 1.8 and above for pre-vac samples was 60.1 for type 'O', 42.1 for type 'A' and 53.2 for type 'Asia-1'. The same for post-vac samples was 78.7 for type 'O', 57.1 for type 'A' and 75.3 for type 'Asia-1'.
- In phase V, 1600 pre and 1599 post-vac serum samples were tested. Percent serum sample having protective antibody titer of 1.8 and above for pre-vac samples was 59.7 for type 'O', 50.8 for type 'A' and 58.8 for type 'Asia-1'. The same for post-vac samples was 84.5 for type 'O', 79.6 for type 'A' and 84.5 for type 'Asia-1'.
- In phase VI, 1496 pre and 1499 post-vac serum samples were tested. Percent serum sample having protective antibody titer of 1.8 and above for pre-vac samples was 66.5 for type 'O', 59.8 for type 'A' and 56.4 for type 'Asia-1'. The same for post-vac samples was 87.1 for type 'O', 82 for type 'A' and 74.6 for type 'Asia-1'.
- In phase VII, 1562 pre and 1574 post-vac serum samples were tested. Percent serum sample having protective antibody titer of 1.8 and above for pre-vac samples was 54.8 for type 'O', 65.3 for type 'A' and 56.8 for type 'Asia-1'. The same for post-vac samples was 82.3 for type 'O', 87.6 for type 'A' and 83.6 for type 'Asia-1'.
- In phase VIII, 1547 pre and 1540 post-vac serum samples were tested. Percent serum sample having protective antibody titer of 1.8 and above for pre-vac samples was 61.3 for type 'O', 56.6 for type 'A' and 49.4 for type 'Asia-1'. The same for post-vac samples

Table 19. Result of seroconversion in Haryana

Phase	Species	Number & % animals showing titres $\geq 1.8 \log_{10}$ against FMDV					
		Type O		Type A		Type Asia 1	
		Pre-vac	Post-vac	Pre-vac	Post-vac	Pre-vac	Post-vac
I	Cattle+Buffalo	Serum samples not available					
II	Cattle+Buffalo	NA	1065(68.3)	NA	859 (55.1)	NA	831 (53.3)
III	Cattle+Buffalo	NA	1146(72.3)	NA	1007(63.6)	NA	1005(63.4)
IV	Cattle+Buffalo	953 (60.1)	1222(78.7)	668 (42.1)	887 (57.1)	844(53.2)	1170(75.3)
V	Cattle+Buffalo	955 (59.7)	1352(84.5)	813 (50.8)	1274(79.6)	941(58.8)	1353(84.5)
VI	Cattle+Buffalo	995 (66.5)	1306(87.1)	895 (59.8)	1229(82.0)	844(56.4)	1118(74.6)
VII	Cattle+Buffalo	856(54.8)	1296 (82.3)	1021 (65.3)	1380 (87.6)	888 (56.8)	1317 (83.6)
VIII	Cattle+Buffalo	949(61.3)	1289 (83.7)	877 (56.6)	992 (64.4)	765 (49.4)	1101 (71.4)
IX	Cattle+Buffalo	647(43.2)	1140(77.2)	590(39.4)	1022(69.2)	410(27.4)	879(59.6)
X	Cattle+Buffalo	851(59.9)	1350(93.8)	615(43.3)	1003(69.7)	587(41.3)	1145(79.5)

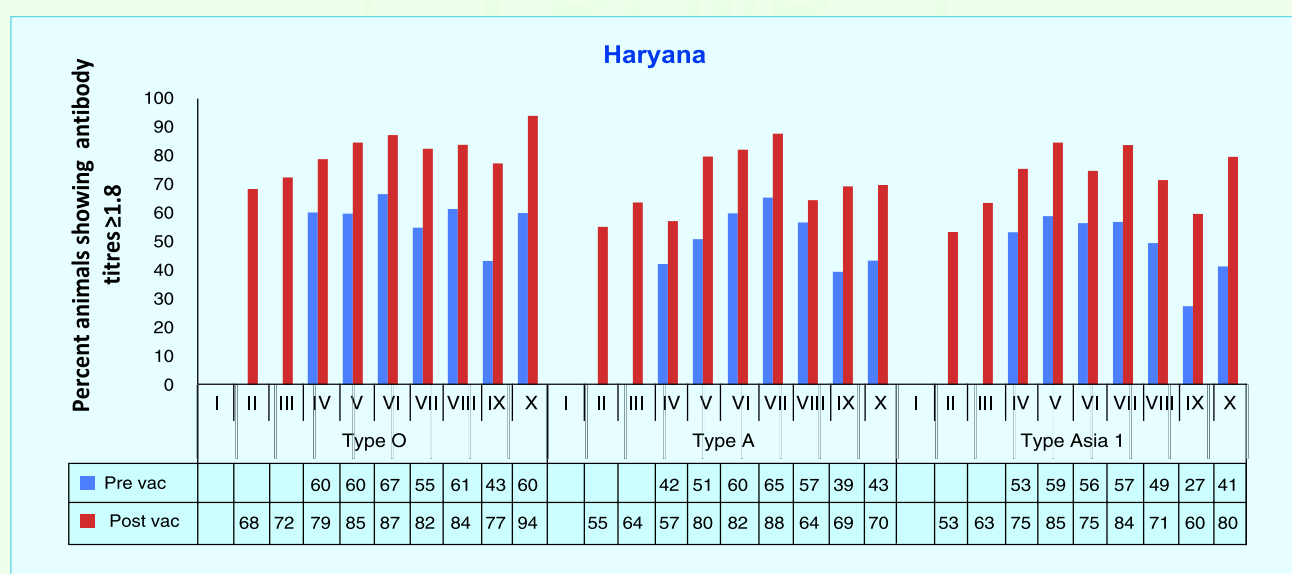


Fig. 27 Seroconversion in Haryana

was 83.7 for type 'O', 64.4 for type 'A' and 71.4 for type 'Asia-1'.

- In phase IX, 1497 pre and 1476 post-vac serum samples were tested. Percent serum sample having protective antibody titer of 1.8 and above for pre-vac samples was 43.2 for type 'O', 39.4 for type 'A' and 27.4 for type 'Asia-1'. The same for post-vac samples was 77.2 for type 'O', 69.2 for type 'A' and 59.6 for type 'Asia-1'.

In phase X, 1420 pre and 1439 post-vac serum samples were tested. Percent serum sample having protective antibody titer of 1.8 and above for pre-vac samples was 59.9

for type 'O', 43.3 for type 'A' and 41.3 for type 'Asia-1'. The same for post-vac samples was 93.8 for type 'O', 69.7 for type 'A' and 79.5 for type 'Asia-1'.

10.1.6 Sero-surveillance in Kerala under FMDCP

Three districts of Kerala namely, Trivandrum, Kollam and Pathanamthitta are covered under FMDCP.

- Serum samples were tested by Ranipet Reginal center and Thiruvananthapuram network unit from Phase X.
- In phase I, II & IV 483 pre and 496 post-vac

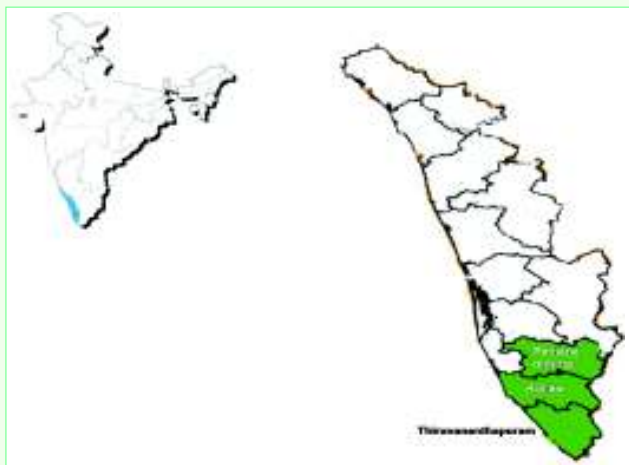


Fig. 28 Districts of Kerala covered under FMD Control Programme

serum samples were tested. Percent serum sample having protective antibody titer of 1.8 and above for pre-vac samples was 32.7 for type 'O', 29 for type 'A' and 34.2 for type 'Asia-1'. The same for post-vac samples was 51.4 for type 'O', 47.5 for type 'A' and 56.4 for type 'Asia-1'.

- For phase III, serum samples were not available.
- In phase V, each of 290 pre and post-vac serum samples were tested. Percent serum sample having protective antibody titer of 1.8 and above for pre-vac samples was 23.1 for type 'O', 17.9 for type 'A' and 21 for type 'Asia-1'. The same for post-vac samples was 67.9 for type 'O', 58.9 for type 'A' and 72.7 for type 'Asia-1'.

- In phase VI, each of 70 pre and post-vac serum samples were tested. Percent serum sample having protective antibody titer of 1.8 and above for pre-vac samples was 20.4 for type 'O', 17.1 for type 'A' and 15.8 for type 'Asia-1'. The same for post-vac samples was 77.1 for type 'O', 70.4 for type 'A' and 71.3 for type 'Asia-1'.
- In phase VII, each of 300 pre and post-vac serum samples were tested. Percent serum sample having protective antibody titer of 1.8 and above for pre-vac samples was 16.0 for type 'O', 14.3 for type 'A' and 17.3 for type 'Asia-1'. The same for post-vac samples was 69.3 for type 'O', 71.0 for type 'A' and 70.0 for type 'Asia-1'.
- In phase VIII & IX 600 pre and 600 post-vac serum samples were tested. Percent serum sample having protective antibody titer of 1.8 and above for pre-vac samples was 37.6 for type 'O', 44.16 for type 'A' and 43.3 for type 'Asia-1'. The same for post-vac samples was 65.8 for type 'O', 56.8 for type 'A' and 66.2 for type 'Asia-1'.
- In phase X, each of 400 pre and 100 post-vac serum samples were tested. Percent serum sample having protective antibody titer of 1.8 and above for pre-vac samples was 40.0 for type 'O', 36.25 for type 'A' and 37.5 for type 'Asia-1'. The same for post-vac samples was 59 for type 'O', 66 for type 'A' and 53 for type 'Asia-1'.

Table 20. Result of seroconversion in Kerala

Phase	Species	Number & % animals showing titres $\geq 1.8 \log_{10}$ against FMDV					
		Type O		Type A		Type Asia 1	
		Pre-vac	Post-vac	Pre-vac	Post-vac	Pre-vac	Post-vac
I, II & IV	Cattle+Buffalo	158(32.7)	255(51.4)	140(29.0)	236(47.5)	165(34.2)	280(56.4)
V	Cattle+Buffalo	67(23.1)	197(67.9)	52(17.9)	171(58.9)	61(21.0)	211(72.7)
VI	Cattle+Buffalo	49 (20.4)	185(77.1)	41(17.1)	169(70.4)	38(15.8)	171(71.3)
VII	Cattle+Buffalo	48 (16.0)	208 (69.3)	43 (14.3)	213 (71.0)	52 (17.3)	210 (70.0)
VIII & IX	Cattle+Buffalo	226(37.6)	395(65.8)	265(44.16)	341(56.8)	260(43.3)	397(66.2)
X	Cattle+Buffalo	160(40)	59(59)	145(36.25)	66(66)	150(37.5)	53(53)

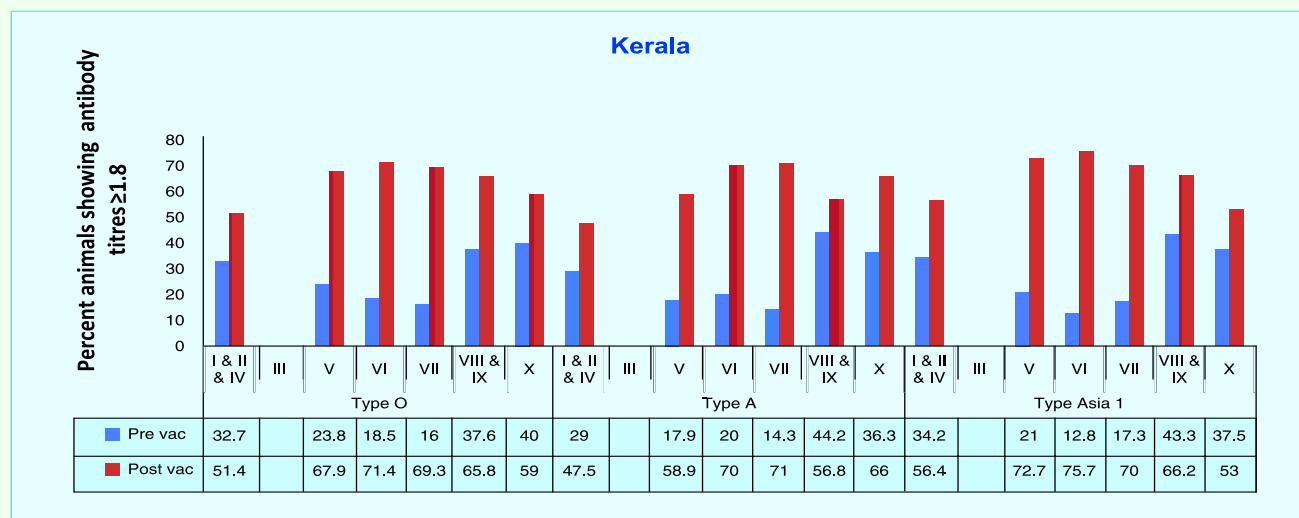


Fig. 29 Seroconversion in Kerala

10.1.7 Sero-surveillance in Maharashtra under FMDCP

Under FMDCP six districts are covered namely, Ahmadnagar, Aurangabad, Pune, Satara, Mumbai and Thane.



Fig. 30 Districts covered under FMD Control Programme in Maharashtra

- In phase II, 834 post-vac serum samples were tested. Pre-vac serum samples were not available. Percent serum sample having protective antibody titer of 1.8 and above was 60.9 for type 'O', 58.6 for type 'A' and 66.2 for type 'Asia-1'.
- In phase III, 753 pre and 799 post-vac serum samples were tested. Percent serum sample having protective antibody titer of 1.8 and above for pre-vac samples was 34.4 for type 'O', 46.8 for type 'A' and 34.7 for type 'Asia-1'. The same for post-vac samples was 54.8 for type 'O', 72.7 for type 'A' and 66.9 for type 'Asia-1'.
- In phase IV, 789 and 797 post-vac serum samples were tested. Percent serum sample having protective antibody titer of 1.8 and above for pre-vac samples was 24.2 for type 'O', 65.6 for type 'A' and 35.2 for type 'Asia-1'. The same for post-vac samples was 52.3 for type 'O', 85.3 for type 'A' and 63.9 for type 'Asia-1'.
- In phase V, 802 pre and 772 post-vac serum samples were tested. Percent serum sample having protective antibody titer of 1.8 and above for pre-vac samples was 17.7 for type 'O', 44.2 for type 'A' and 15 for type 'Asia-1'. The same for post-vac samples was 35.1 for

type 'O', 62.3 for type 'A' and 31.8 for type 'Asia-1'.

- In phase VI, 901 pre and 928 post-vac serum samples were tested. Percent serum sample having protective antibody titer of 1.8 and above for pre-vac samples was 44.9 for type 'O', 69 for type 'A' and 27.2 for type 'Asia-1'. The same for post-vac samples was 71.4 for type 'O', 91.9 for type 'A' and 48.1 for type 'Asia-1'.
- In phase VII, 1000 pre and 1000 post-vac serum samples were tested. Percent serum sample having protective antibody titer of 1.8 and above for pre-vac samples was 44.6

for type 'O', 70.1 for type 'A' and 43.1 for type 'Asia-1'. The same for post-vac samples was 69.2 for type 'O', 89.3 for type 'A' and 66.7 for type 'Asia-1'.

- In phase VIII, 1000 pre and 1000 post-vac serum samples were tested. Percent serum sample having protective antibody titer of 1.8 and above for pre-vac samples was 64.6 for type 'O', 57.4 for type 'A' and 19.8 for type 'Asia-1'. The same for post-vac samples was 90.4 for type 'O', 84.8 for type 'A' and 45.2 for type 'Asia-1'.
- In phase IX, 1000 pre and 1000 post-vac serum samples were tested. Percent serum

Table 21. Result of seroconversion in Maharashtra

Phase	Species	Number & % animals showing titres $\geq 1.8 \log_{10}$ against FMDV					
		Type O		Type A		Type Asia 1	
		Pre-vac	Post-vac	Pre-vac	Post-vac	Pre-vac	Post-vac
I	Cattle+Buffalo	173 (20.5)	456 (59.9)	151(17.9)	437 (57.4)	192 (22.8)	466 (61.2)
II	Cattle+Buffalo	N.A.	508 (60.9)	N.A.	490 (58.6)	N.A.	553 (66.2)
III	Cattle+Buffalo	184 (24.4)	438 (54.8)	351 (46.8)	580 (72.7)	262 (34.7)	534 (66.9)
IV	Cattle+Buffalo	191 (24.2)	417 (52.3)	517 (65.6)	679 (85.3)	278 (35.2)	509 (63.9)
V	Cattle+Buffalo	142 (17.7)	271 (35.1)	353 (44.2)	477 (62.3)	121 (15.0)	245 (31.8)
VI	Cattle+Buffalo	404 (44.9)	663 (71.4)	622 (69)	853 (91.9)	245 (27.2)	446 (48.1)
VII	Cattle+Buffalo	446 (44.6)	692 (69.2)	701 (70.1)	893 (89.3)	431 (43.1)	667 (66.7)
VIII	Cattle+Buffalo	646 (64.6)	904 (90.4)	574 (57.4)	848 (84.8)	198 (19.8)	452 (45.2)
IX	Cattle+Buffalo	730(73)	951(95.1)	524(52.4)	817(81.7)	324(32.4)	695(69.5)
X	Cattle+Buffalo	785(78.5)	978(97.8)	686(68.6)	935(93.5)	607(60.7)	846(84.6)

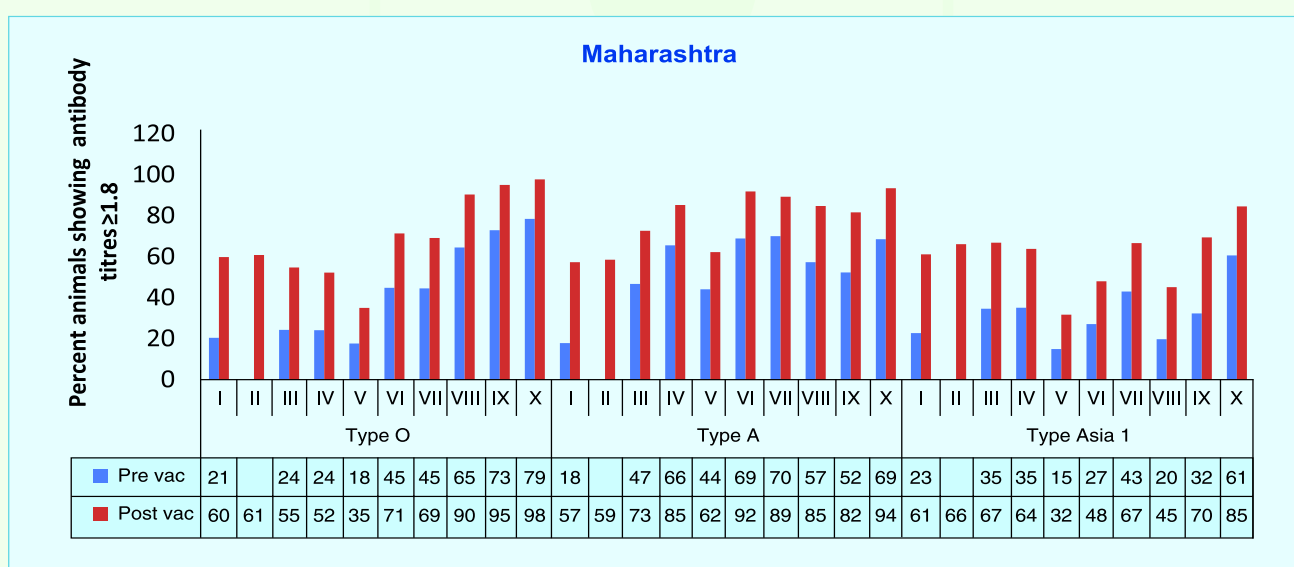


Fig. 31 Seroconversion in Maharashtra

sample having protective antibody titer of 1.8 and above for pre-vac samples was 73 for type 'O', 52.4 for type 'A' and 32.4 for type 'Asia-1'. The same for post-vac samples was 95.1 for type 'O', 51.7 for type 'A' and 69.5 for type 'Asia-1'.

- In phase X, 1000 pre and 1000 post-vac serum samples were tested. Percent serum sample having protective antibody titer of 1.8 and above for pre-vac samples was 78.5 for type 'O', 68.6 for type 'A' and 60.7 for type 'Asia-1'. The same for post-vac samples was 97.8 for type 'O', 93.5 for type 'A' and 84.6 for type 'Asia-1'.

10.1.8 Sero-surveillance in Punjab under FMDCP

Under FMDCP eight districts of Punjab are covered namely, Amritsar, Bhatinda, Fatehgarh Sahib, Ferozpur, Mansa, Sangrur, Patiala and Gurdaspur.



Fig. 32 Districts of Punjab covered under FMD Control Programme

- In phase I, 742 post-vac serum samples were tested. Pre-vac serum samples were not available. Percent serum sample having protective antibody titer of 1.8 and above was 25.2 for type 'O', 11.5 for type 'A' and 49.5 for type 'Asia-1'.
- In phase II, 500 post-vac serum samples were tested. Pre-vac serum samples were

not available. Percent serum sample having protective antibody titer of 1.8 and above was 43.8 for type 'O', 20.9 for type 'A' and 58.1 for type 'Asia-1'.

- In Phase III, 1084 pre and 1365 post-vac serum samples were tested. Percent serum sample having protective antibody titer of 1.8 and above for pre-vac samples was 84.4 for type 'O', 75.3 for type 'A' and 40.2 for type 'Asia-1'. The same for post-vac samples was 86.1 for type 'O', 73.8 for type 'A' and 42.0 for type 'Asia-1'.
- In phase IV, 1291 pre and 978 post-vac serum samples were tested. Percent serum sample having protective antibody titer of 1.8 and above for pre-vac samples was 76.5 for type 'O', 61.5 for type 'A' and 53.8 for type 'Asia-1'. The same for post-vac samples was 81.0 for type 'O', 64.1 for type 'A' and 36.4 for type 'Asia-1'.
- In phase V, 1370 pre and 1139 post-vac serum samples were tested. Percent serum sample having protective antibody titer of 1.8 and above for pre-vac samples was 34.8 for type 'O', 32.8 for type 'A' and 38.5 for type 'Asia-1'. The same for post-vac samples was 54.5 for type 'O', 53.7 for type 'A' and 60.1 for type 'Asia-1'.
- In phase VI, 1509 pre and 1568 post-vac serum samples were tested. Percent serum sample having protective antibody titer of 1.8 and above for pre-vac samples was 43.3 for type 'O', 43.3 for type 'A' and 32.9 for type 'Asia-1'. The same for post-vac samples was 60.2 for type 'O', 58.7 for type 'A' and 47.4 for type 'Asia-1'.
- In phase VII, 1265 pre and 1432 post-vac serum samples were tested. Percent serum sample having protective antibody titer of 1.8 and above for pre-vac samples was 36.3 for type 'O', 22.8 for type 'A' and 33.0 for type 'Asia-1'. The same for post-vac samples was 57.8 for type 'O', 42.0 for type 'A' and 46.4 for type 'Asia-1'.

Table 22. Result of seroconversion in Punjab

Phase	Species	Number & % animals showing titres $\geq 1.8 \log_{10}$ against FMDV					
		Type O		Type A		Type Asia 1	
		Pre-vac	Post-vac	Pre-vac	Post-vac	Pre-vac	Post-vac
I	Cattle+Buffalo	83 (10.3)	340 (42.5)	43 (5.3)	244 (30.5)	92 (11.5)	340 (42.5)
I	Cattle+Buffalo	N.A.	187(25.2)	N.A.	90(11.5)	N.A.	273(49.5)
II	Cattle+Buffalo	N.A.	219(43.8)	N.A.	113(20.9)	N.A.	279(58.1)
III	Cattle+Buffalo	915(84.4)	1175(86.1)	816(75.3)	1007(73.8)	437(40.2)	573(42.0)
IV	Cattle+Buffalo	988(76.5)	792 (81.0)	794(61.5)	627 (64.1)	694 (53.8)	356(36.4)
V	Cattle+Buffalo	477(34.8)	621(54.5)	445(32.8)	630(53.7)	513(38.5)	690(60.1)
VI	Cattle+Buffalo	653 (43.3)	944 (60.2)	654 (43.3)	921 (58.7)	496 (32.9)	743 (47.4)
VII	Cattle+Buffalo	520 (41.1)	898 (62.7)	356 (28.1)	639 (44.6)	448 (35.4)	696 (48.6)
VIII	Cattle+Buffalo	580(58.94)	825(73.33)	410(41.66)	643(57.15)	452(45.93)	741(65.86)
IX	Cattle+Buffalo	1035(66.43)	1193(77.16)	831(53.33)	978(63.26)	926(59.43)	1132(73.22)

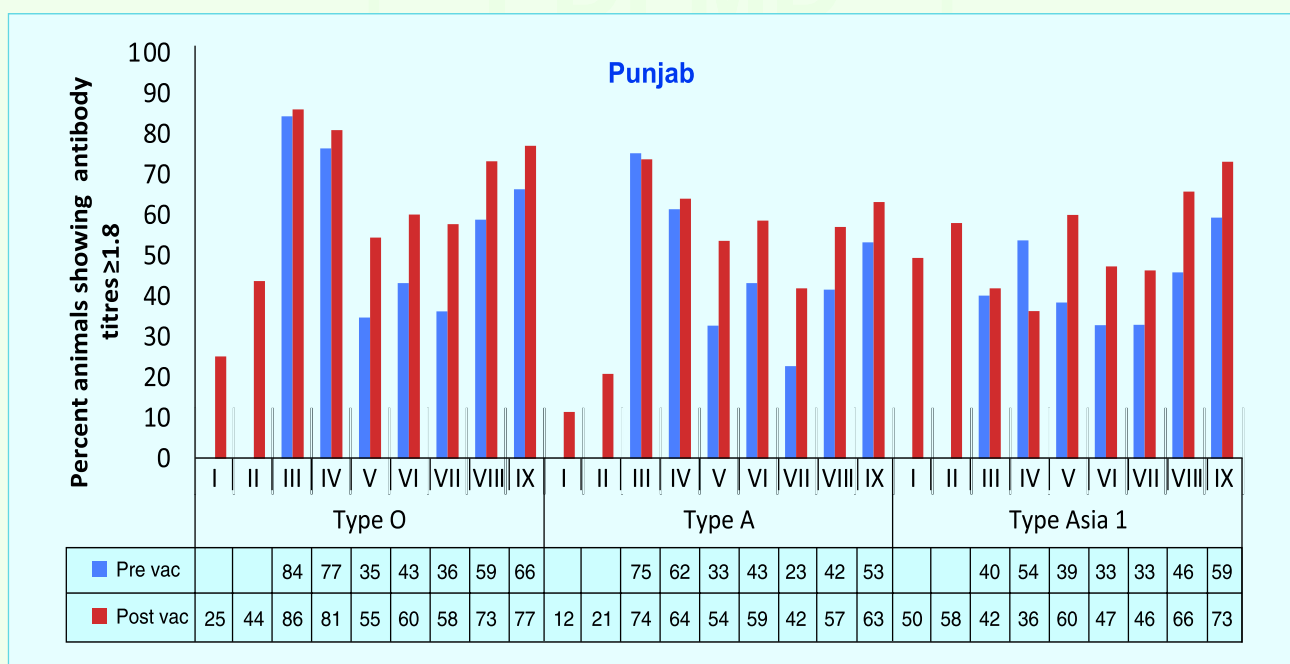


Fig. 33 Seroconversion in Punjab

- In phase VIII, 984 pre and 1125 post-vac serum samples were tested. Percent serum sample having protective antibody titer of 1.8 and above for pre-vac samples was 58.94 for type 'O', 41.66 for type 'A' and 45.93 for type 'Asia-1'. The same for post-vac samples was 73.33 for type 'O', 57.15 for type 'A' and 65.86 for type 'Asia-1'.
- In phase IX, 1558 pre and 1546 post-vac serum samples were tested. Percent serum sample having protective antibody titer of 1.8 and above for pre-vac samples was 66.43

for type 'O', 53.33 for type 'A' and 59.43 for type 'Asia-1'. The same for post-vac samples was 77.16 for type 'O', 63.26 for type 'A' and 73.22 for type 'Asia-1'.

10.1.9 Sero-surveillance in Tamil Nadu under FMDCP

Kanyakumari district is covered under FMDCP. This is the only district of Tamil Nadu under FMD-CP.

- Serum samples were tested by Ranipet Regional centre.



Fig . 34 Districts covered under FMD Control Programme in Tamil Nadu

- In phase I, each of 100 pre and post-vac serum samples were tested. Percent serum sample having protective antibody titer of 1.8 and above for pre-vac samples was 28 for type 'O', 29 for type 'A' and 24 for type 'Asia-1'. The same for post-vac samples was 51 for type 'O', 57 for type 'A' and 54 for type 'Asia-1'.
- In phase II, each of 100 pre and post-vac serum samples were tested. Percent serum sample having protective antibody titer of 1.8 and above for pre-vac samples was 23 for type 'O', 24 for type 'A' and 18 for type 'Asia-1'. The same for post-vac samples was 63 for type 'O', 40 for type 'A' and 61 for type 'Asia-1'.
- In phase III & IV, 180 pre and 330 post-vac serum samples were tested. Percent serum sample having protective antibody titer of 1.8 and above for pre-vac samples was 32.7 for type 'O', 33.8 for type 'A' and 25 for type 'Asia-1'. The same for post-vac samples was 74.5 for type 'O', 60.9 for type 'A' and 65.4 for type 'Asia-1'.
- For phase V, serum samples were not available.
- In phase VI, 160 pre and 130 post-vac serum samples were tested. Percent serum sample having protective antibody titer of 1.8 and above for pre-vac samples was 18.7 for type 'O', 23.8 for type 'A' and 21.5 for type 'Asia-1'. The same for post-vac samples was 76.1 for type 'O', 83.8 for type 'A' and 79.2 for type 'Asia-1'.
- In phase VII, 300 pre and 300 post-vac serum samples were tested. Percent serum sample having protective antibody titer of 1.8 and above for pre-vac samples was 11.7 for type 'O', 11.3 for type 'A' and 12.0 for type 'Asia-1'. The same for post-vac samples was 70.0 for type 'O', 77.0 for type 'A' and 75.3 for type 'Asia-1'.
- In phase VIII, 100 pre and 100 post-vac serum samples were tested. Percent serum sample having protective antibody titer of 1.8 and above for pre-vac samples was 34 for type 'O', 40 for type 'A' and 25 for type 'Asia-1'. The same for post-vac samples was 74 for type 'O', 60 for type 'A' and 78 for type 'Asia-1'.

Table 23. Result of seroconversion in Tamil Nadu

Phase	Species	Number & % animals showing titres $\geq 1.8 \log_{10}$ against FMDV					
		Type O		Type A		Type Asia 1	
		Pre-vac	Post-vac	Pre-vac	Post-vac	Pre-vac	Post-vac
I	Cattle+Buffalo	28(28)	51(51)	29(29)	57(57)	24(24)	54(54)
II	Cattle+Buffalo	23(23.0)	63(63.0)	24(24.0)	40(40.0)	18(18.0)	61(61.0)
III & IV	Cattle+Buffalo	59(32.7)	246(74.5)	61(33.8)	201(60.9)	45(25.0)	216(65.4)
VI	Cattle+Buffalo	30(18.7)	99(76.1)	31(23.8)	109(83.8)	28(21.5)	103(79.2)
VII	Cattle+Buffalo	35(11.7)	210(70)	34(11.3)	231(77)	36(12)	226(75.3)
VIII	Cattle+Buffalo	34(34)	74(74)	40(40)	60(60)	25(25)	78(78)
IX	Cattle+Buffalo	40(40)	58(58)	45(45)	64(64)	33(33)	74(74)
X	Cattle+Buffalo	32(32)	62(62)	45(45)	63(63)	41(41)	70(70)

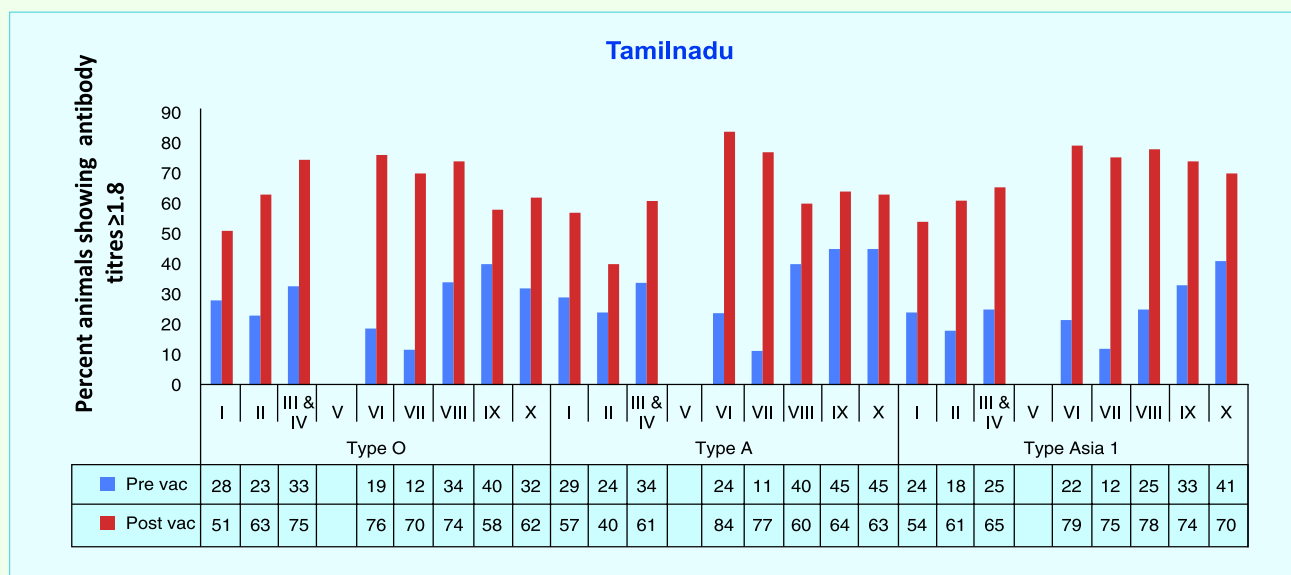


Fig. 35 Seroconversion in Tamilnadu

- In phase IX, 100 pre and 100 post-vac serum samples were tested. Percent serum sample having protective antibody titer of 1.8 and above for pre-vac samples was 40 for type 'O', 45 for type 'A' and 33 for type 'Asia-1'. The same for post-vac samples was 58 for type 'O', 64 for type 'A' and 74 for type 'Asia-1'.
- In phase X, 100 pre and 100 post-vac serum samples were tested. Percent serum sample having protective antibody titer of 1.8 and above for pre-vac samples was 32 for type 'O', 45 for type 'A' and 41 for type 'Asia-1'. The same for post-vac samples was 62 for type 'O', 63 for type 'A' and 70 for type 'Asia-1'.

10.1.10 Sero-surveillance in Uttar Pradesh under FMDCP

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.

- Mathura Regional center received and tested serum samples collected in 12 districts of UP (Agra, Aligarh, Budaun Bulandsahar, Etah,

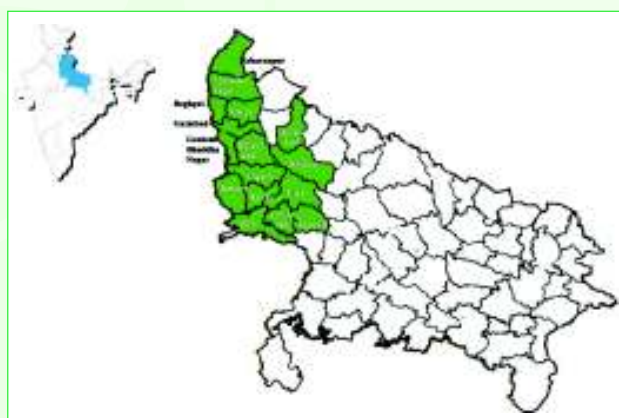


Fig. 36 Districts of Uttar Pradesh covered under FMD Control Programme

Ferozabad, Gautam Bhuddha Nagar, Gaziabad, Hatras, J.P.Nagar Mathura and Muradabad) during phases II to VI.

- Bangalore center received and tested serum samples collected from 4 districts of UP (Meerut, Baghpat, Saharanpur and Muzaffarnagar) for phases III to VI.
- Since Phase III, Mathura Regional Center is testing the serum samples of all the 16 districts under FMD-CP.
- No serum samples were received for phase I.

- In phase II, 139 and 407 post-vac serum samples were tested. Percent serum sample having protective antibody titer of 1.8 and above for pre-vac samples was nil for type 'O', 'A' and 'Asia-1'. The same for post-vac samples was 44.2 for type 'O', 38.1 for type 'A' and 72.0 for type 'Asia-1'.
- In phase III, 1155 and 1584 post-vac serum samples were tested. Percent serum sample having protective antibody titer of 1.8 and above for pre-vac samples was 34.5 for type 'O', 42.7 for type 'A' and 42.4 for type 'Asia-1'. The same for post-vac samples was 49.2 for type 'O', 57.4 for type 'A' and 71.8 for type 'Asia-1'.
- In phase IV, 1910 and 1770 post-vac serum samples were tested. Percent serum sample having protective antibody titer of 1.8 and above for pre-vac samples was 18 for type 'O', 31.9 for type 'A' and 27.2 for type 'Asia-1'. The same for post-vac samples was 30.3 for type 'O', 48.9 for type 'A' and 45.6 for type 'Asia-1'.
- In phase V, 1440 pre and 1591 post-vac serum samples were tested. Percent serum sample having protective antibody titer of 1.8 and above for pre-vac samples was 35.8 for type 'O', 43.4 for type 'A' and 47.5 for type 'Asia-1'. The same for post-vac samples was 44.9 for type 'O', 50.4 for type 'A' and 49.4 for type 'Asia-1'.

Table 24. Result of seroconversion in Uttar Pradesh

Phase	Species	Number & % animals showing titres $\geq 1.8 \log_{10}$ against FMDV					
		Type O		Type A		Type Asia 1	
		Pre-vac	Post-vac	Pre-vac	Post-vac	Pre-vac	Post-vac
I	Cattle+Buffalo	Serum samples not available					
II	Cattle+Buffalo	0(0)	180(44.2)	0(0)	155(38.1)	0(0)	293(72.0)
III	Cattle+Buffalo	399(34.5)	780(49.2)	494(42.7)	910(57.4)	490(42.4)	1138(71.8)
IV	Cattle+Buffalo	344(18.0)	537(30.3)	610(31.9)	866(48.9)	519(27.2)	808(45.6)
V	Cattle+Buffalo	516(35.8)	715(44.9)	625(43.4)	802(50.4)	684(47.5)	786(49.4)
VI	Cattle+Buffalo	514 (34.5)	968 (61.3)	520 (34.9)	826 (52.3)	400 (26.9)	838 (53.1)
VII	Cattle+Buffalo	706 (24.9)	911 (43.9)	597 (21.1)	808 (38.9)	740 (26.1)	930 (44.8)
VIII	Cattle+Buffalo	707(37.1)	1550(56.5)	502(26.4)	1310(47.7)	617(32.41)	1288(46.9)
IX	Cattle+Buffalo	334(45.9)		134(18.4)		177(24.31)	

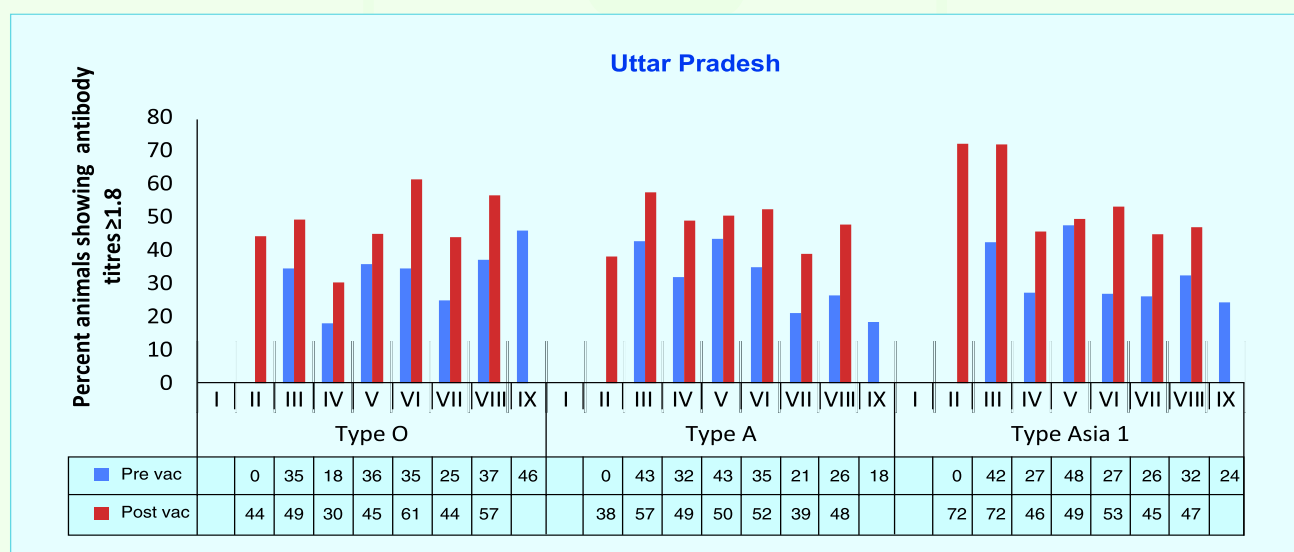


Fig. 37 Seroconversion in Uttar Pradesh

- In phase VI, 1488 pre and 1579 post vac serum samples out of total 2182 pre and 1986 post vac serum samples collected were tested. Percent serum sample having protective antibody titer of 1.8 and above for pre-vac samples was 34.5 for type 'O', 34.9 for type 'A' and 26.9 for type 'Asia-1'. The same for post-vac samples was 61.3 for type 'O', 52.3 for type 'A' and 53.1 for type 'Asia-1'.
- In phase VII, 2833 pre and 2075 post vac serum samples were tested. Percent serum sample having protective antibody titer of 1.8 and above for a pre-vac sample was 23.4 for type 'O', 18.6 for type 'A' and 19.3 for type 'Asia-1'. The same for post-vac samples was 43.9 for type 'O', 38.9 for type 'A' and 44.8 for type 'Asia1'.
- In phase VIII at present 1904 pre and 2744 post vac serum samples were tested. Percent serum sample having protective antibody titer of 1.8 and above for a pre-vac sample was 37.1 for type 'O', 26.4 for type 'A' and 32.41 for type 'Asia-1'. The same for post-vac samples was 56.5 for type 'O', 47.7 for type 'A' and 46.9 for type 'Asia1'.
- In phase IX at present 728 pre vac serum samples were tested. Percent serum sample having protective antibody titer of 1.8 and above for a pre-vac sample was 45.9 for type 'O', 18.4 for type 'A' and 24.1 for type 'Asia-1'.



10.2 Phase wise number and percent of animals showing antibody titer $\geq 1.8 \log_{10}$ against FMD virus from phase I to X (all 54 districts)

Table 25. Phase I

Phase	Species	Number & % animals showing titres $\geq 1.8 \log_{10}$ against FMDV					
		Type O		Type A		Type Asia 1	
		Pre-vac	Post-vac	Pre-vac	Post-vac	Pre-vac	Post-vac
Andaman& Nicobar		Serum samples not available					
Andhra Pradesh	Cattle+Buff	83 (10.3)	340 (42.5)	43 (5.3)	244 (30.5)	92 (11.5)	340 (42.5)
Delhi	Buffalo	26 (53)	50 (100)	13 (26)	47 (94)	17 (34)	48 (96)
Gujarat	Cattle+Buff	50 (19.1)	116 (44.7)	59 (24.5)	128 (48.7)	42 (16.1)	114 (43.5)
Haryana		Serum samples not available					
Kerala*	Cattle+Buff	158(32.7)	255(51.4)	140(29.0)	236(47.5)	165(34.2)	280(56.4)
Maharashtra	Cattle+Buff	173 (20.5)	456 (59.9)	151(17.9)	437 (57.4)	192 (22.8)	466 (61.2)
Punjab	Cattle+Buff	N.A.	187(25.2)	N.A.	90(11.5)	N.A.	273(49.5)
Tamil Nadu	Cattle+Buff	28(28)	51(51)	29(29)	57(57)	24(24)	54(54)
Uttar Pradesh		Serum samples not available					

* Kerala Phase I, II & IV data is combined.

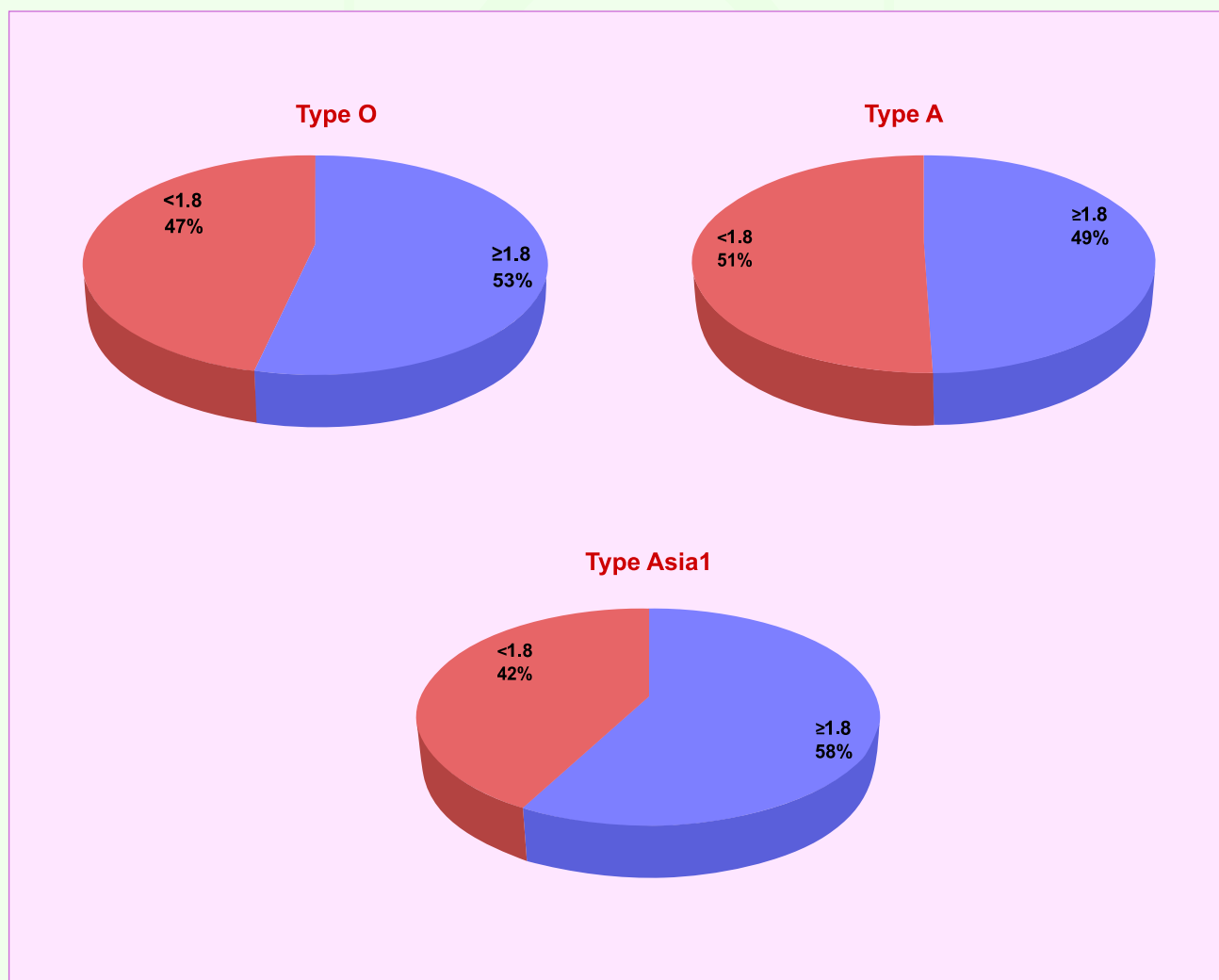


Fig. 38 Average post vaccinal seroconversion in Phase I

Table 26. Phase II

Phase	Species	Number & % animals showing titres $\geq 1.8 \log_{10}$ against FMDV					
		Type O		Type A		Type Asia 1	
		Pre-vac	Post-vac	Pre-vac	Post-vac	Pre-vac	Post-vac
Andaman& Nicobar		Serum samples not available					
Andhra Pradesh	Cattle+Buff	N.A.	434 (54.2)	N.A.	498 (62.3)	N.A.	438 (54.7)
Delhi	Buffalo	22 (91)	23 (96)	12 (40)	15 (62)	23 (95)	22 (86)
Gujarat		Serum samples not available					
Haryana	Cattle+Buff	NA	1065(68.3)	NA	859 (55.1)	NA	831 (53.3)
Kerala*	Cattle+Buff	158(32.7)	255(51.4)	140(29.0)	236(47.5)	165(34.2)	280(56.4)
Maharashtra	Cattle+Buff	N.A.	508 (60.9)	N.A.	490 (58.6)	N.A.	553 (66.2)
Punjab	Cattle+Buff	N.A.	219(43.8)	N.A.	113(20.9)	N.A.	279(58.1)
Tamil Nadu	Cattle+Buff	23(23.0)	63(63.0)	24(24.0)	40(40.0)	18(18.0)	61(61.0)
Uttar Pradesh	Cattle+Buff	0(0)	180(44.2)	0(0)	155(38.1)	0(0)	293(72.0)

* Kerala Phase I, II & IV data is combined

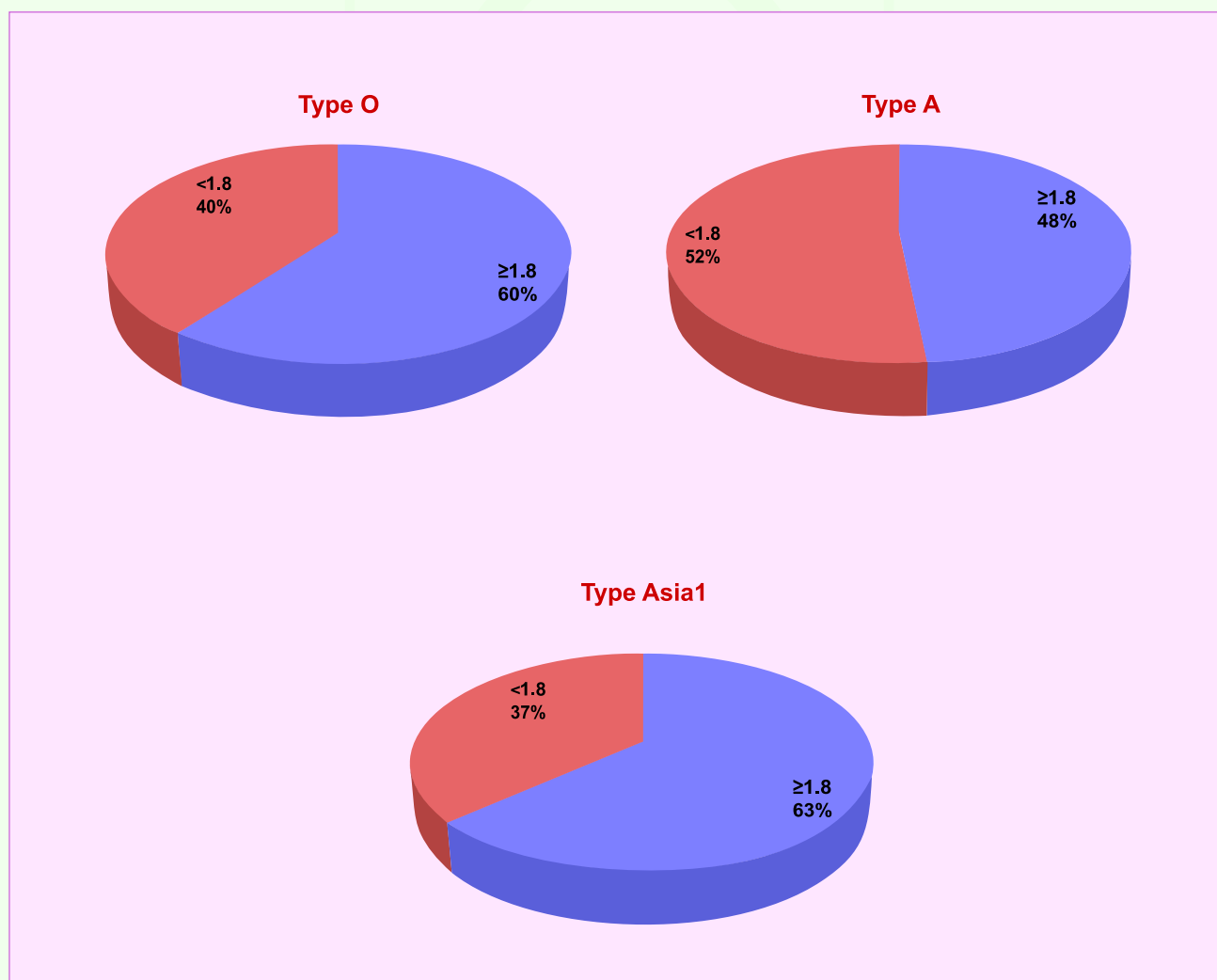


Fig. 39 Average post vaccinal seroconversion in Phase II

Table 27. Phase III

Phase	Species	Number & % animals showing titres $\geq 1.8 \log_{10}$ against FMDV					
		Type O		Type A		Type Asia 1	
		Pre-vac	Post-vac	Pre-vac	Post-vac	Pre-vac	Post-vac
Andaman& Nicobar	Cattle+Buff	40(25.9)	97(60.0)	5(2.8)	37(20.3)	52(34.0)	118(73.6)
Andhra Pradesh	Cattle+Buff	210 (26.2)	286 (35.7)	395 (49.3)	532 (66.5)	306 (38.2)	422 (52.7)
Delhi	Cattle+Buff	47 (94)	49 (98)	30 (60)	40 (80)	43 (86)	46 (92)
Gujarat	Cattle+Buff	123 (27.8)	171 (47.9)	171 (39.2)	268 (58.3)	51 (12.4)	149 (35.4)
Haryana	Cattle+Buff	NA	1146(72.3)	NA	1007(63.6)	NA	005(63.4)
Kerala		Serum samples not available					
Maharashtra	Cattle+Buff	184 (24.4)	438 (54.8)	351 (46.8)	580 (72.7)	262 (34.7)	534 (66.9)
Punjab	Cattle+Buff	915(84.4)	1175(86.1)	816(75.3)	1007(73.8)	437(40.2)	573(42.0)
Tamil Nadu**	Cattle+Buff	59(32.7)	246(74.5)	61(33.8)	201(60.9)	45(25.0)	216(65.4)
Uttar Pradesh	Cattle+Buff	399(34.5)	780(49.2)	494(42.7)	910(57.4)	490(42.4)	138(71.8)

** Tamil Nadu Phase III & IV data is combined

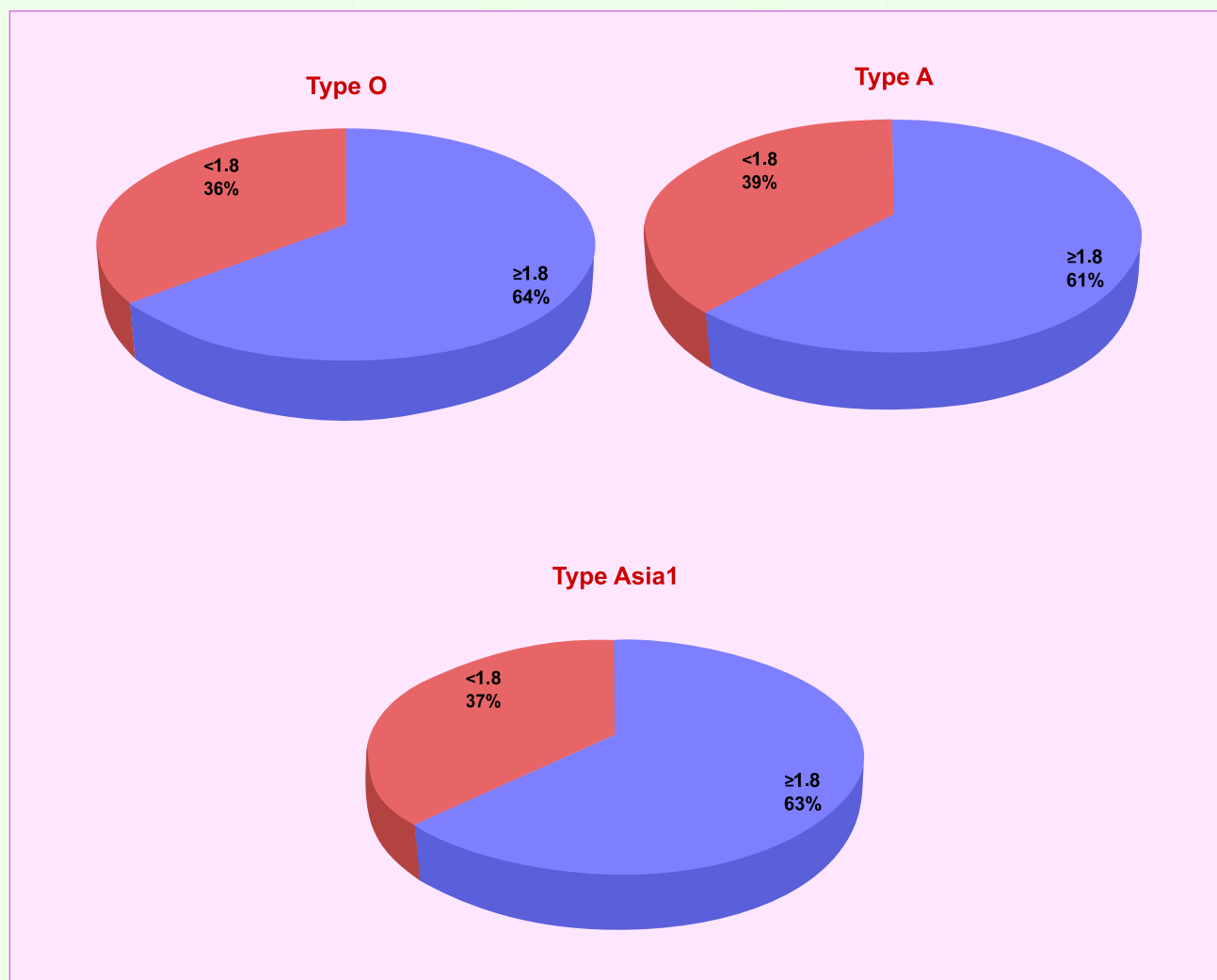


Fig. 40 Average post vaccinal seroconversion in Phase III

Table 28. Phase IV

Phase	Species	Number & % animals showing titres $\geq 1.8 \log_{10}$ against FMDV					
		Type O		Type A		Type Asia 1	
		Pre-vac	Post-vac	Pre-vac	Post-vac	Pre-vac	Post-vac
Andaman&Nicobar	Cattle+Buff	50(33.5)	94(64.6)	50(33.5)	96(65.9)	35(23.4)	101(67.6)
Andhra Pradesh	Cattle+Buff	281 (35.1)	374 (46.8)	465 (58.1)	617 (77.1)	329 (41.1)	518 (64.8)
Delhi	Cattle+Buff	38 (76)	38 (82.6)	14 (28)	40 (86.9)	27 (54)	41 (89.1)
Gujarat	Cattle+Buff	113 (22.7)	277 (60.7)	184 (40.7)	355 (81.2)	73 (14.6)	218 (46.8)
Haryana	Cattle+Buff.	953 (60.1)	1222(78.7)	668 (42.1)	887 (57.1)	844 (53.2)	170(75.3)
Kerala*	Cattle+Buff	158(32.7)	255(51.4)	140(29.0)	236(47.5)	165(34.2)	280(56.4)
Maharashtra	Cattle+Buff	191 (24.2)	417 (52.3)	517 (65.6)	679 (85.3)	278 (35.2)	509 (63.9)
Punjab	Cattle+Buff	988(76.5)	792 (81.0)	794(61.5)	627 (64.1)	694 (53.8)	356(36.4)
Tamil Nadu**	Cattle+Buff	59(32.7)	246(74.5)	61(33.8)	201(60.9)	45(25.0)	216(65.4)
Uttar Pradesh	Cattle+ Buff	344(18.0)	537(30.3)	610(31.9)	866(48.9)	519(27.2)	808(45.6)

* Kerala Phase I, II & IV data is combined;

* **Tamil Nadu Phase III & IV data is combined

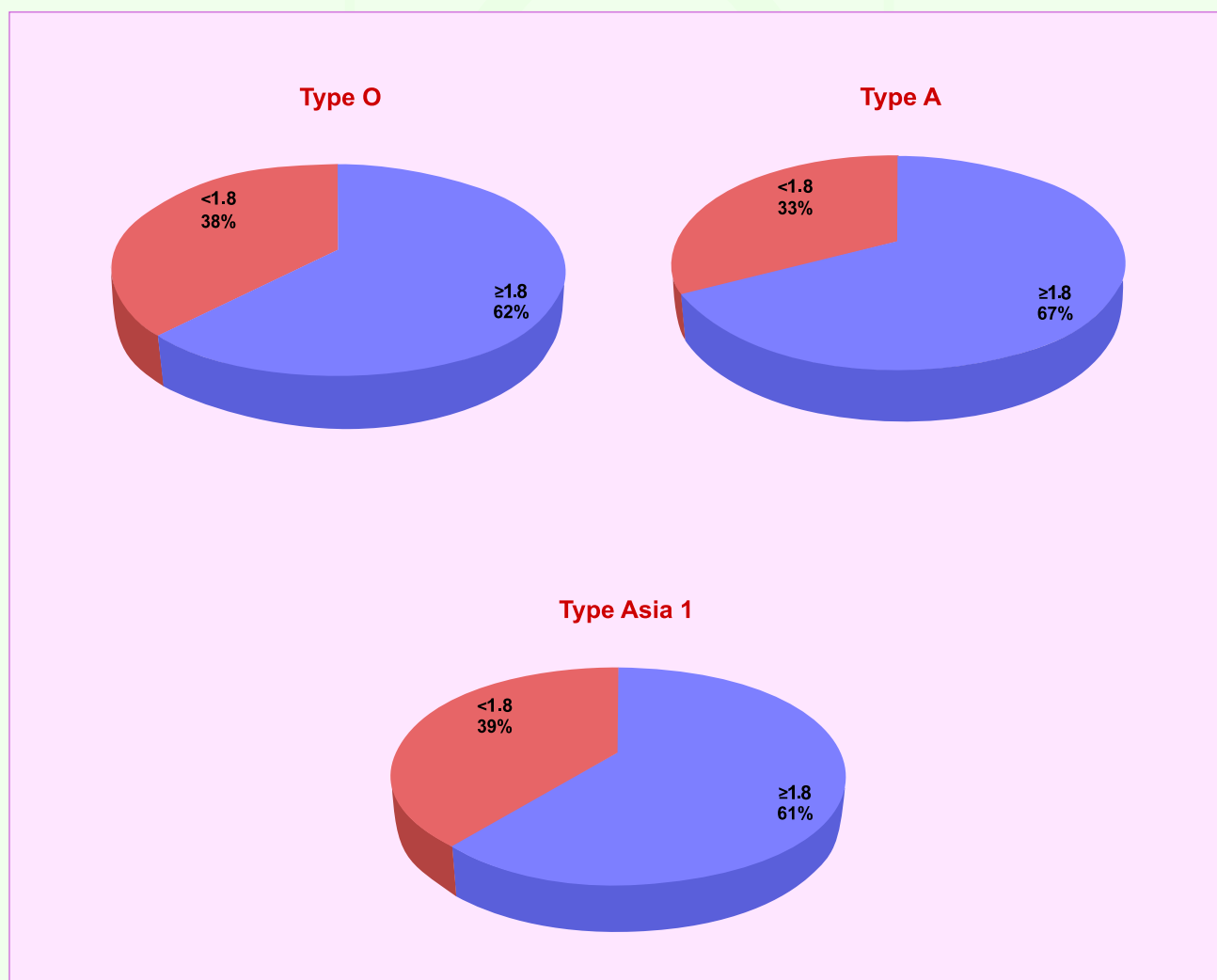


Fig. 41 Average post vaccinal seroconversion in Phase IV

Table 29. Phase V

Phase	Species	Number & % animals showing titres $\geq 1.8 \log_{10}$ against FMDV					
		Type O		Type A		Type Asia 1	
		Pre-vac	Post-vac	Pre-vac	Post-vac	Pre-vac	Post-vac
Andaman& Nicobar	Cattle+Buff	72(57.2)	68(55.8)	62(50.8)	64(52.5)	54(44.3)	62(50.8)
Andhra Pradesh	Cattle+Buff.	247 (30.8)	440 (55)	466 (58.2)	574 (71.8)	343 (42.8)	450 (56.3)
Delhi	Cattle+Buff	26 (59)	47 (88.6)	23 (52.2)	37 (69.8)	32 (72.7)	41 (77.3)
Gujarat	Cattle+ Buff	46 (23.6)	99 (49.0)	126 (66.1)	179 (91.6)	44 (26.5)	92 (51.3)
Haryana	Cattle+Buff.	955 (59.7)	1352(84.5)	813 (50.8)	1274(79.6)	941 (58.8)	353(84.5)
Kerala	Cattle+Buff	67(23.1)	197(67.9)	52(17.9)	171(58.9)	61(21.0)	211(72.7)
Maharashtra	Cattle+Buff	142 (17.7)	271 (35.1)	353 (44.2)	477 (62.3)	121 (15.0)	245 (31.8)
Punjab	Cattle	477(34.8)	621(54.5)	445(32.8)	630(53.7)	513(38.5)	690(60.1)
Tamil Nadu		Serum samples not available					
Uttar Pradesh	Cattle+ Buff	516(35.8)	715(44.9)	625(43.4)	802(50.4)	684(47.5)	786(49.4)

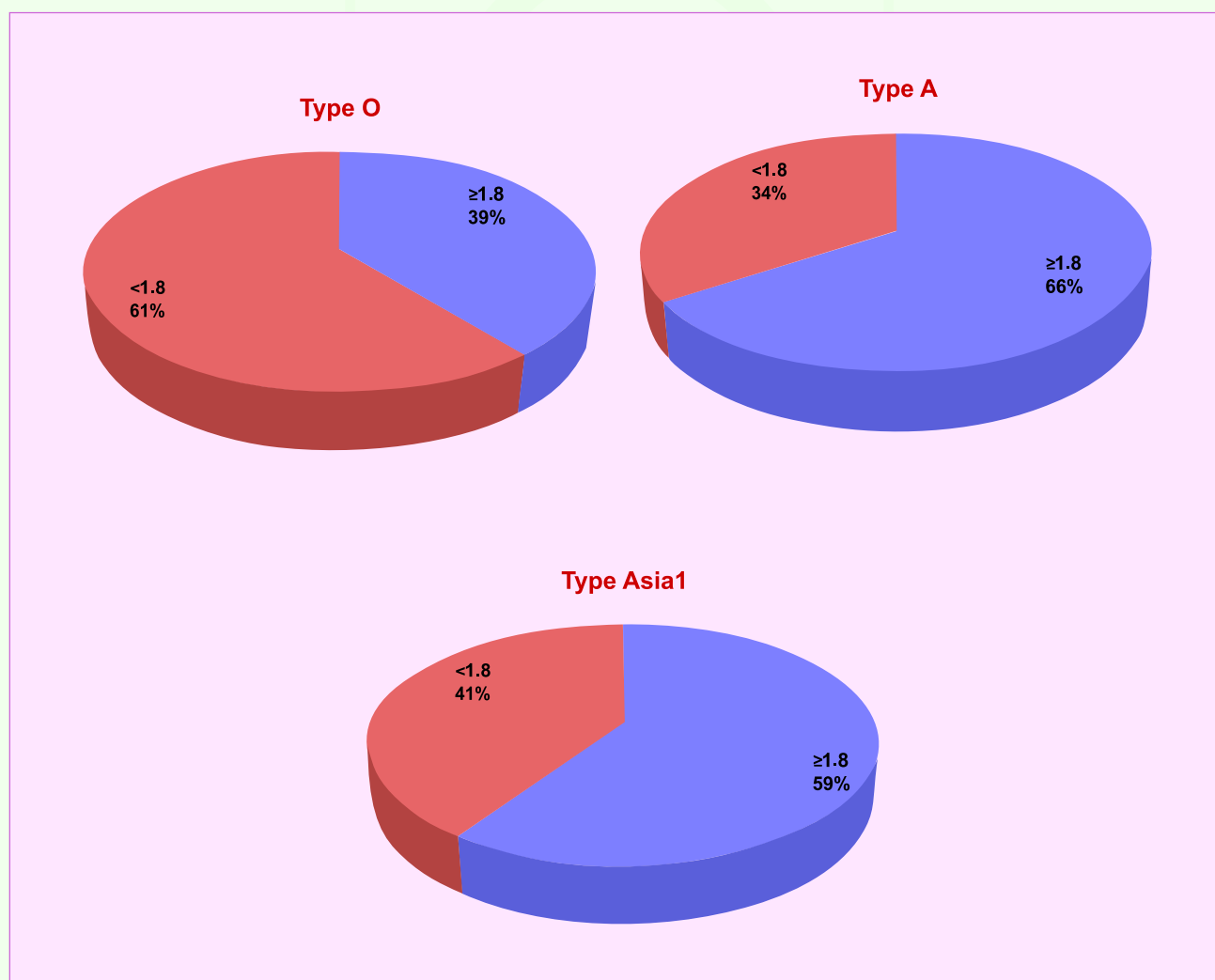


Fig. 42 Average post vaccinal seroconversion in Phase V

Table 30. Phase VI

Phase	Species	Number & % animals showing titres $\geq 1.8 \log_{10}$ against FMDV					
		Type O		Type A		Type Asia 1	
		Pre-vac	Post-vac	Pre-vac	Post-vac	Pre-vac	Post-vac
Andaman& Nicobar		50 (18.5)	80 (29.6)	66 (24.4)	104 (38.4)	28 (10.2)	36 (13.2)
Andhra Pradesh	Cattle+Buff.	275 (34.3)	490 (61.3)	554 (69.2)	690 (86.3)	446 (55.7)	634 (79.3)
Delhi	Cattle+Buff	76 (77.5)	97 (98.9)	60 (61.2)	93 (94.9)	71 (72.4)	97 (98.9)
Gujarat	Cattle+Buff	119 (30.1)	223 (56.4)	249 (63.0)	317(80.2)	195 (49.3)	240 (60.7)
Haryana	Cattle+Buff.	995 (66.5)	1306(87.1)	895 (59.8)	1229(82.0)	844 (56.4)	118(74.6)
Kerala	Cattle+Buff	49 (20.4)	185(77.1)	41(17.1)	169(70.4)	38(15.8)	171(71.3)
Maharashtra	Cattle+Buff	404 (44.9)	663 (71.4)	622 (69)	853 (91.9)	245 (27.2)	446 (48.1)
Punjab	Cattle+ Buff	653 (43.3)	944 (60.2)	654 (43.3)	921 (58.7)	496 (32.9)	743 (47.4)
Tamil Nadu	Cattle+Buff.	30(18.7)	99(76.1)	31(23.8)	109(83.8)	28(21.5)	103(79.2)
Uttar Pradesh	Cattle+Buff.	514 (34.5)	968 (61.3)	520 (34.9)	826 (52.3)	400 (26.9)	838 (53.1)

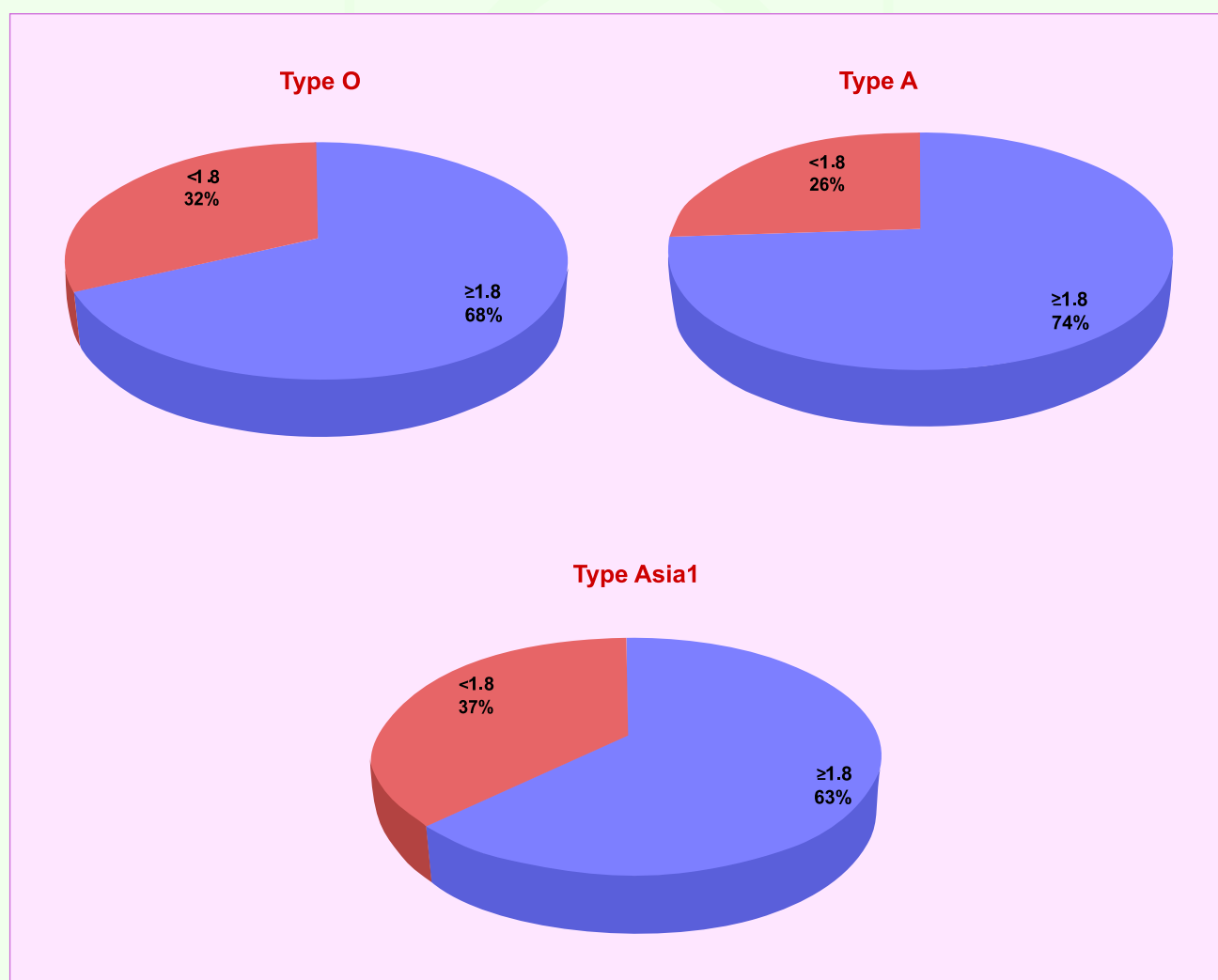


Fig. 43 Average post vaccinal seroconversion in Phase VI

Table 31. Phase VII

Phase	Species	Number & % animals showing titres $\geq 1.8 \log_{10}$ against FMDV					
		Type O		Type A		Type Asia 1	
		Pre-vac	Post-vac	Pre-vac	Post-vac	Pre-vac	Post-vac
Andaman& Nicobar	Cattle+Buff.	112 (42.3)	174 (65.7)	82 (30.9)	110 (41.5)	56 (21.1)	66 (24.9)
Andhra Pradesh	Cattle+Buff.	274 (34.0)	483 (60.3)	349 (44.0)	540 (67.5)	391 (48.8)	518 (64.7)
Delhi	Cattle+Buff.	39(78)	44(88)	33(66)	43(86)	25(50)	41(82)
Gujarat	Cattle+Buff.	434 (54.3)	630 (78.8)	385 (48.1)	559 (69.9)	344 (43.0)	556 (69.5)
Haryana	Cattle+Buff.	856(54.8)	1296(82.3)	1021(65.3)	1380 (87.6)	888 (56.8)	1317(83.6)
Kerala	Cattle+Buff.	48 (16.0)	208 (69.3)	43 (14.3)	213 (71.0)	52 (17.3)	210 (70.0)
Maharashtra	Cattle+Buff.	446 (44.6)	692 (69.2)	701 (70.1)	893 (89.3)	431 (43.1)	667 (66.7)
Punjab	Cattle+Buff.	413 (36.3)	650 (57.8)	260 (22.8)	472 (42.0)	376 (33.0)	521 (46.4)
Tamil Nadu	Cattle+Buff.	35(11.7)	210(70)	34(11.3)	231(77)	36(12)	226(75.3)
Uttar Pradesh	Cattle+Buff.	706 (24.9)	911 (43.9)	597 (21.1)	808 (38.9)	740 (26.1)	930 (44.8)



Fig . 44 Average post vaccinal seroconversion in Phase VII

Table 32. Phase VIII

Phase	Species	Number & % animals showing titres $\geq 1.8 \log_{10}$ against FMDV					
		Type O		Type A		Type Asia 1	
		Pre-vac	Post-vac	Pre-vac	Post-vac	Pre-vac	Post-vac
Andaman& Nicobar	Cattle+Buff.	53(21.1)	102(40.6)	18(7.2)	49(19.5)	47(18.7)	85(33.86)
Andhra Pradesh	Cattle+Buff.	356 (44.5)	594 (74.0)	415 (51.8)	624 (78.0)	333 (41.6)	527 (65.8)
Delhi	Cattle+Buff.	92 (92)	100 (100)	66 (66)	86 (86)	83 (83)	98 (98)
Gujarat	Cattle+Buff.	191 (23.9)	394 (49.3)	197 (24.6)	357 (44.6)	264 (33.0)	403 (50.4)
Haryana	Cattle+Buff.	949(61.3)	1289 (83.7)	877 (56.6)	992 (64.4)	765 (49.4)	1101(71.4)
Kerala*	Cattle+Buff.	226(37.6)	395(65.8)	265(44.16)	341(56.8)	260(43.3)	397(66.2)
Maharashtra	Cattle+Buff.	646 (64.6)	904 (90.4)	574 (57.4)	848 (84.8)	198 (19.8)	452 (45.2)
Punjab	Cattle+Buff.	580(58.94)	825(73.3)	410(41.66)	643(57.15)	452(45.93)	741(65.86)
Tamil Nadu	Cattle+Buff.	34(34)	74(74)	40(40)	60(60)	25(25)	78(78)
Uttar Pradesh	Cattle+Buff.	707(37.1)	1550(56.5)	502(26.4)	1310(47.7)	617(32.4)	1288(46.9)

* Kerala Phase VIII & IX data is combined

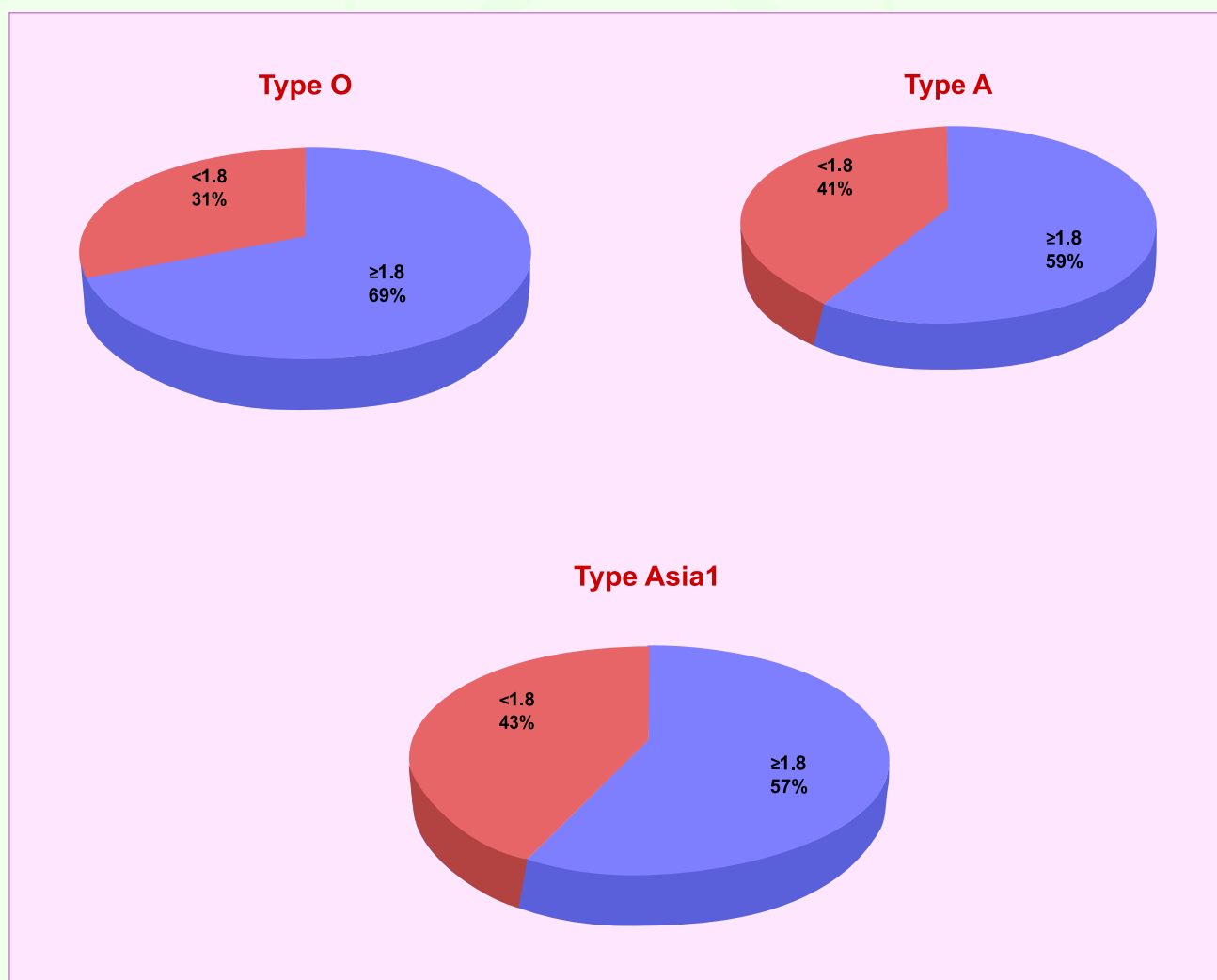


Fig . 45 Average post vaccinal seroconversion in Phase VIII

Table 33. Phase IX

Phase	Species	Number & % animals showing titres $\geq 1.8 \log_{10}$ against FMDV					
		Type O		Type A		Type Asia 1	
		Pre-vac	Post-vac	Pre-vac	Post-vac	Pre-vac	Post-vac
Andaman& Nicobar	Cattle+Buff.	73(32)	69(30.26)	31(13.59)	35(15.35)	56(24.56)	42(18.42)
Andhra Pradesh	Cattle+Buff.	422(52.8)	673(84.1)	329(41.1)	534(66.8)	287(35.9)	534(66.8)
Delhi	Cattle+Buff.	57(57)	NA	65(65)	NA	33(33)	NA
Gujarat	Cattle+Buff.	230(28.7)	618(77.2)	284(35.5)	572(71.5)	326(40.7)	595(66.9)
Haryana	Cattle+Buff.	647(43.2)	1140(77.2)	590(39.4)	1022(69.2)	410(27.4)	879(59.6)
Kerala*	Cattle+Buff.	226(37.6)	395(65.8)	265(44.16)	341(56.8)	260(43.3)	397(66.2)
Maharashtra	Cattle+Buff.	730(73)	951(95.1)	524(52.4)	817(81.7)	324(32.4)	695(69.5)
Punjab	Cattle+Buff.	1035(66.4)	1193(77.2)	831(53.3)	978(63.3)	926(59.4)	1132(73.2)
Tamil Nadu	Cattle+Buff.	40(40)	58(58)	45(45)	64(64)	33(33)	74(74)
Uttar Pradesh	Cattle+Buff.	334(45.9)	NA	134(18.4)	NA	177(24.3)	NA

* Kerala Phase VIII & IX data is combined

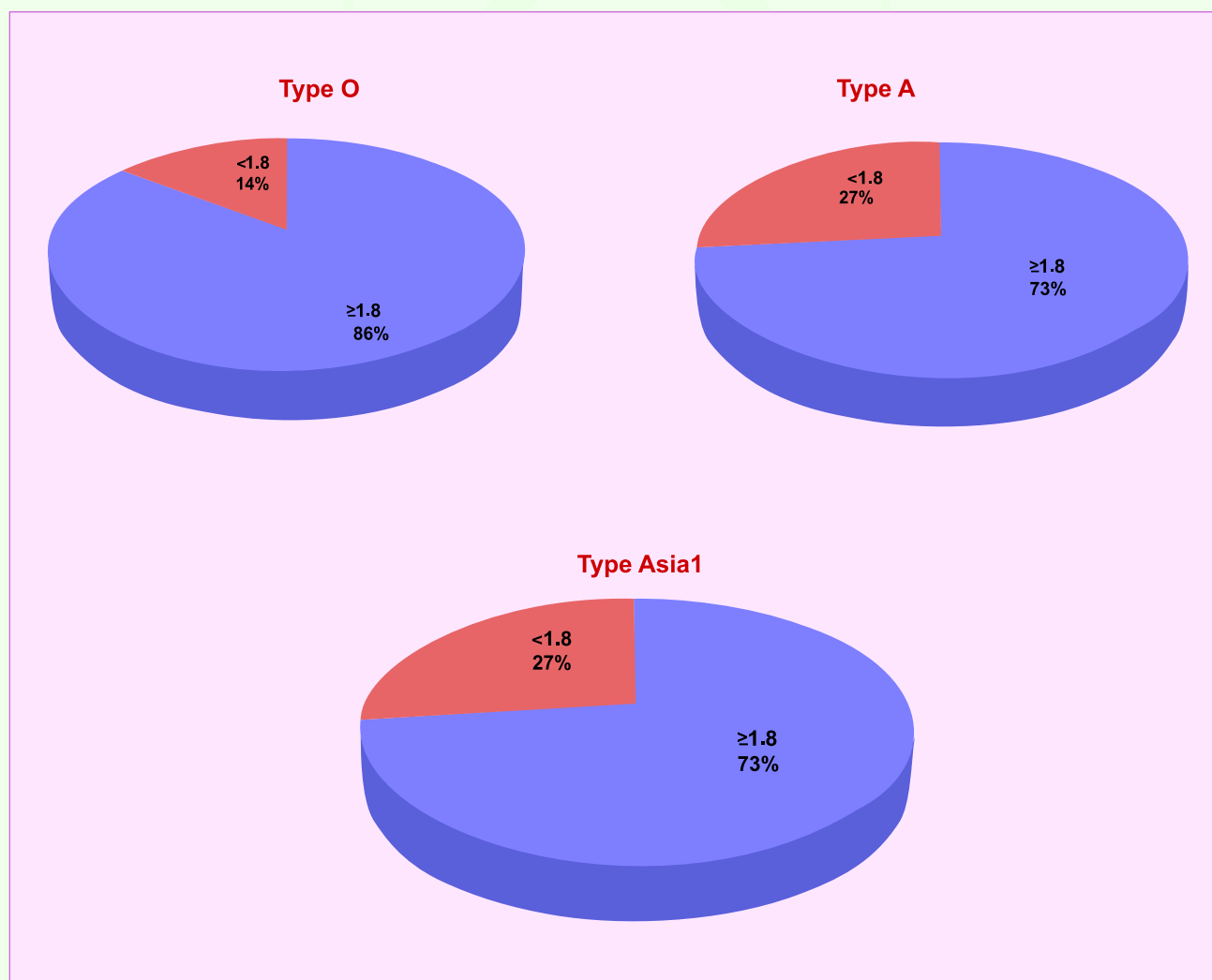


Fig. 46 Average post vaccinal seroconversion in Phase IX

Table 34. Phase X

Phase	Species	Number & % animals showing titres $\geq 1.8 \log_{10}$ against FMDV					
		Type O		Type A		Type Asia 1	
		Pre-vac	Post-vac	Pre-vac	Post-vac	Pre-vac	Post-vac
Andaman& Nicobar	Cattle+Buff.	Serum testing in Progress					
Andhra Pradesh	Cattle+Buff.	502(62.7)	635(79.3)	368(46)	575(71.8)	411(51.3)	602(75.2)
Delhi	Cattle+Buff.	Samples not available					
Gujarat	Cattle+Buff.	356(44.5)	620(77.5)	286(35.7)	525(65.6)	276(34.5)	535(66.9)
Haryana	Cattle+Buff.	851(59.9)	1350(93.8)	615(43.3)	1003(69.7)	587(41.3)	145(79.5)
Kerala	Cattle+Buff.	160(40)	59(59)	145(36.25)	66(66)	150(37.5)	53(53)
Maharashtra	Cattle+Buff.	785(78.5)	978(97.8)	686(68.6)	935(93.5)	607(60.7)	846(84.6)
Punjab	Cattle+Buff.	Serum testing in Progress					
Tamil Nadu	Cattle+Buff.	32(32)	62(62)	45(45)	63(63)	41(41)	70(70)
Uttar Pradesh	Cattle+Buff.	Serum testing in Progress					

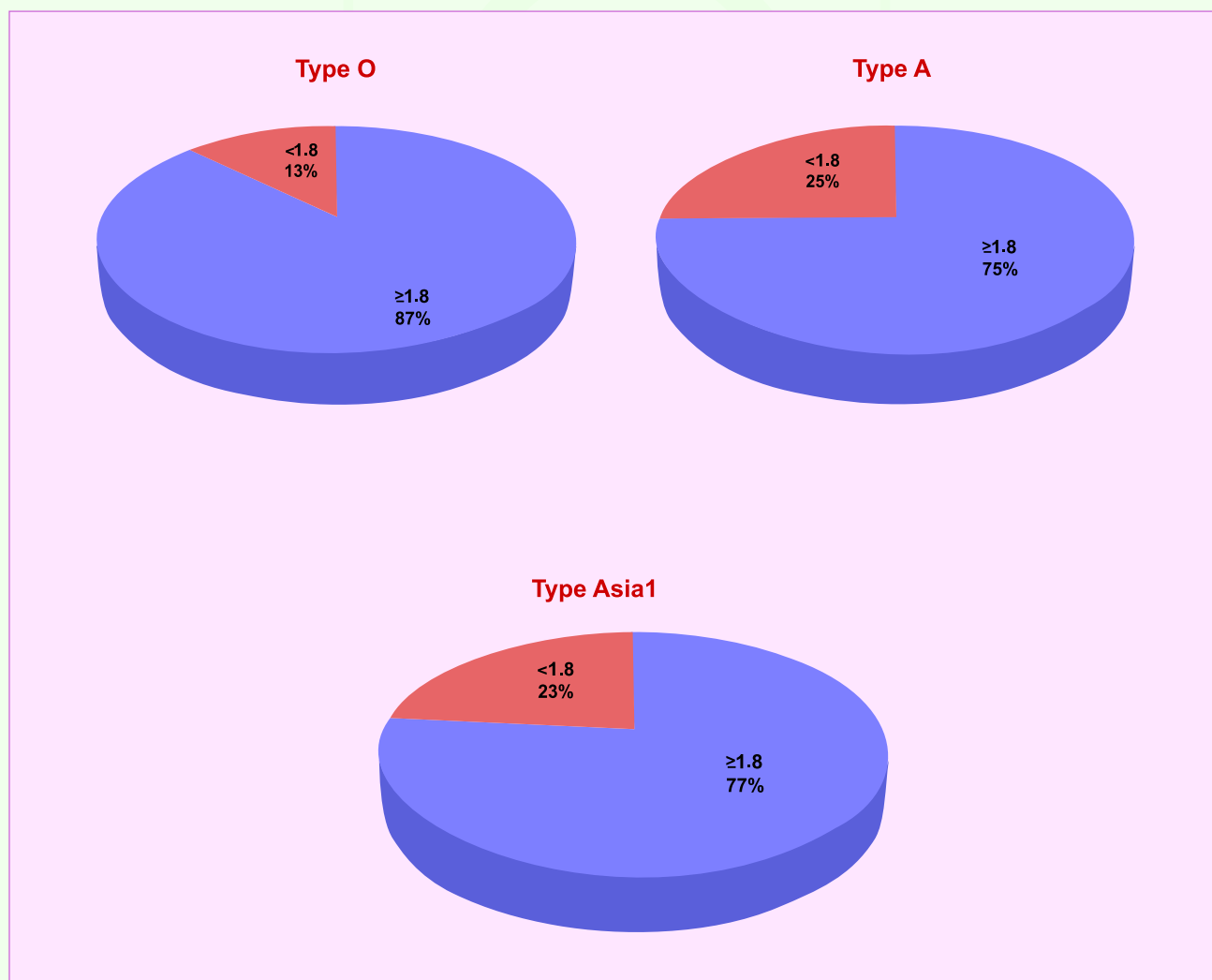


Fig. 47 Average post vaccinal seroconversion in Phase X

10.2.1 Summary of overall sero conversion in phases I to X against each serotype and impact of vaccine

Table 35. Percent animals showing post vaccinal antibody titers of $\geq 1.8 \log_{10}$ against FMD virus

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

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 these areas down to near zero. In recent times, there has been case of FMD in some 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. There has been certain problem in maintaining 6 month interval between successive vaccinations. This problem can be circumvented/compensated by using a vaccine having 6-8 PD50/dose. The results have been encouraging and should be further strengthened by constituting National FMD Commission.

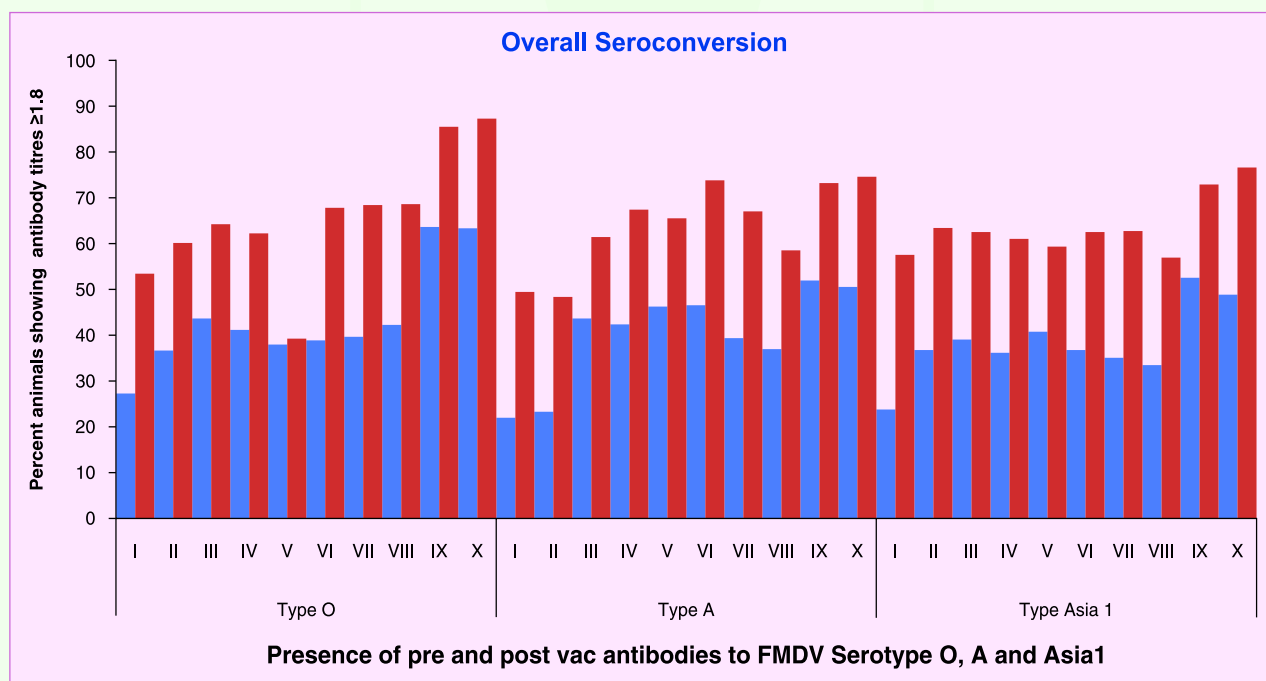


Fig. 48 Overall seroconversion of pre and post vac serum samples under FMD-CP

Table. 36 summary of total number of serum samples tested under FMD CP till 2010-11

State/UT	Phase I		Phase II		Phase III		Phase IV		Phase V		Phase VI		Phase VII		Phase VIII		Phase IX		Phase X	
	Pre	Post	Pre	Post	Pre	Post	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	251	251	228	228	-	-
Andhra Pradesh	800	800	-	800	800	800	800	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	100	100	100	-	-	-
Gujarat	382	259	-	-	442	357	497	456	195	202	395	395	800	800	800	800	800	800	800	800
Haryana	-	-	-	1558	-	1585	1589	1552	1600	1599	1496	1499	1562	1574	1547	1540	1497	1476	1420	1439
Kerala	483 (pre) and 496(post) of Phase I, II and IV								290	290	70	70	300	300	600 (pre)		600(post)		400	100
Maharashtra	844	761	-	834	753	799	789	797	802	772	901	928	1000	1000	1000	1000	1000	1000	1000	1000
Punjab	-	742	-	500	1084	1365	1291	978	1370	1139	1509	1568	1265	1432	984	1125	1558	1546	-	-
Tamilnadu	100	100	100	100	180(pre)		330(post)		-	-	160	130	300	300	100	100	100	100	100	100
Uttar Pradesh	-	-	139	407	1155	1584	1910	1770	1440	1591	1488	1579	2833	2075	1904	2744	728	-	-	-
subTotal	2176	2712	263	4223	4438	6707	7075	6545	6667	6568	7187	7337	9175	8596	8086	8460	6811	5950	4520	4239
Total	4888*		4486*		11145*		13620*		13235		14524		17771		16546*		12761*		8759	
Grant total	Pre-vac 57481**										post-vac-62433**									

* 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 labelling

** this includes all the samples tested

Table 37. Sero-monitoring of post vaccinal immunity against serotypes O, A and Asia1 in animals vaccinated under ASCAD/RKVY programme: sampling was done at random, and not as per FMD-CP format

State	Number of sample tested	Species	Number & % animals showing titres $\geq 1.8 \log_{10}$ against FMDV					
			Type O		Type A		Type Asia 1	
			Pre-vac	Post-vac	Pre-vac	Post-vac	Pre-vac	Post-vac
Tripura	100+100	C+B	15(15%)	77(77%)	9(9%)	79(79%)	4(4%)	62(62%)
Orissa	480+380	C+B	234(48.8%)	261(68.9%)	227(47.3%)	272(71.6%)	266(55.4%)	294(77.4%)
Bihar	570+570	C+B	97(17%)	190(33.3%)	20(3.5%)	103(18%)	3(0.5%)	81(14.2%)
AP(RKVY)	1074+1074	C+B	575(53.5%)	952(88.6%)	281(26.1%)	686(63.8%)	242(22.5%)	650(60.5%)
AP(ASCAD)	828+828	C+B	412(49.7%)	648(78.2%)	175(21.1%)	425(51.3%)	202(24.3%)	451(54.4%)
Manipur	180+180	C+B	96(53.3)	168(93.3)	91(50.5)	154(85.5)	4(2.2)	6(3.3)
Mizoram	160+160	C+B	16(10)	78(48.75)	12(7.5)	92(57.5)	9(5.6)	37(33.6)
Nagaland	60+60	C+B	10(16.6)	48(80)	5(8.3)	30(50)	1(1.6)	13(21.6)

The result obtained for Orissa and Andhra Pradesh is satisfactory looking at the random nature of sampling in time and space

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. Inactivated virus antigen was also outsourced from a FMD vaccine production house in the country to meet demand. Antibodies against purified virus was raised and titrated against homologous as well as heterologous virus. Freeze dried and standardized serum antibodies and known positive antigen (killed) of all three serotypes were supplied to all the

centers and network units for use in virus typing ELISA and LPB-ELISA. Viruse serotyping Kits for 9000 tests were supplied to 14 centers/network units. LPB-ELISA Kits for 82,800 tests supplied to the 15 centers/ network units and other government agencies and industry to ensure uniformity in test results. r3AB3 DIVA Kit for FMD to test a total number of 45,000 serum samples was produced and reagents to test 58,440 samples have been supplied to the network units and vaccine manufacturing companies.

Table 38. Serotyping and LPBELISA Reagents supplied during April 2010 to March 2011

Date of Supply	Party to which supplied	Typing ELISA (No. of test)	LPB ELISA (No. of test)
07/04/2010	IVRI, Bangalore		500
19/04/2010	Guwahati Center	500	
20/04/2010	Orissa Network Unit	500	1500
01/05/2010	Gujarat Network Unit	500	2000
01/05/2010	Jaipur Network Unit		800
18/05/2010	Jammu Network Unit		1400
22/05/2010	Hyderabad Center		2000
25/05/2010	Pune Center		1000
29/05/2010	Kolkata Center	500	1000
03/06/2010	Imphal Network Unit		700
26/06/2010	Hyderabad		2000
26/06/2010	Agartala Network Unit		700
30/06/2010	Shimla Network Unit	500	
17/07/2010	Pune Center		2000
23/07/2010	Mathura Center		1000
27/07/2010	Guwahati Center	500	2000
17/08/2010	Jalandhar Network Unit		1000
20/08/2010	Ahmedabad Network Unit		1000
04/09/2010	Hisar	500	3500
10/09/2010	Intervet		1000
10/09/2010	Nirjuli Network Unit	500	
17/09/2010	Bangalore enter	500	3000
21/09/2010	Pune Center	500	2000
22/09/2010	Kerala Network Unit		1000
01/10/2010	Patna Network Unit	500	1000
12/11/2010	Hyderabad Center	500	3000

Date of Supply	Party to which supplied	Typing ELISA (No. of test)	LPB ELISA (No. of test)
19/11/2010	Mathura Center		1000
01/12/2010	Orissa Network Unit		1200
13/12/2010	Imphal Network Unit	500	700
20/01/2011	NIAH, Baghpat		500
24/12/2010	Bhopal Network Unit		2000
07/02/2011	Bangalore	500	3000
09/02/2011	Kolkata Center		2300
11/02/2011	Hyderabad Center		5000
15/02/2011	Pune Center		2000
19/02/2011	Mathura Center		5000
25/02/2011	Hisar Center		8000
26/02/2011	Kerala Network Unit	500	1900
03/03/2011	Ranipet enter	500	4000
05/03/2011	NIAH, Baghpat		500
10/03/2011	Gujarat Network Unit		4000
10/03/2011	Jalandhar	500	4000
29/03/2011	Guwahati	500	1000
29/03/2011	Nagaland Network Unit		1100
29/03/2011	Mizoram Network Unit		500
	Total	9000	82,800

Table 39. DIVA Reagents supplied during April 2010 to March 2011

Date of Supply	No of tests	Party to which supplied
22-03-2010	2160	Jalandhar
27-03-2010	3060	Bangalore
27-03-2010	2700	Hyderabad
13-04-2010	900	Mathura
20-04-2010	3060	Orissa
01-05-2010	3060	Ahemdabad
01-05-2010	3060	Jaipur
18-05-2010	1800	Jammu & Kashmir
29-05-2010	900	Kolkata
03-06-2010	900	Manipur
26-06-2010	900	Tripura
27-07-2010	2700	Guwahati
04-09-2010	2160	Hisar
04-09-2010	1800	Bhopal
10-09-2010	1800	Himachal Pradesh
10-09-2010	900	Itanagar
17-09-2010	2700	Bangalore
22-09-2010	1350	Kerala
01-10-2010	4500	Patna
12-10-2010	1800	Mathura
21-10-2010	450	Intervet, Pune
12-11-2010	1800	Hyderabad
13-12-2010	900	Manipur
24-12-2010	1800	Bhopal
07-02-2011	3000	Bangalore
15-02-2011	2700	Jalandhar
15-02-2011	3600	Pune

Date of Supply	No of tests	Party to which supplied
19-02-2011	900	Mathura
25-02-2011	3600	Hisar
25-02-2011	1800	Kerala
03-03-2011	3600	Ranipet
03-03-2011	900	Agartala
10-03-2011	1800	Ahemedabad
29-03-2011	900	Guwahati
29-03-2011	1080	Nagaland
29-03-2011	900	Mizoram
Total	71940	



Reports and Recommendations

12.1 Proceedings of the 21th Annual scientists meet of the PDFMD (ICAR)

The 21st Annual Scientists Meet of the Project Directorate on Foot and Mouth Disease (PD on FMD) for the year 2009-10 was held on 27-28 September 2010 at NAAS Complex, New Delhi. Scientists of all the Regional centers (8) and Network units (14) participated in this meet. The Network Unit, Lucknow did not participate. There were special invitees who also participated in the meeting. There were 45 participants.

The meeting was chaired by Prof. KML Pathak, DDG (AS), ICAR in the presence of Prof. Gaya Prasad, ADG (AH), ICAR. The special invitees were Dr. Lal Krishna (Former AHC and ADG (AH), ICAR), Dr. R K Singh (Director, NRC on Equine), Dr. G K Sharma (DGM, AH, NDDDB) and Dr. R. Venkataramanan (Joint Director, IVRI Campus, Bangalore).

Dr. B Pattnaik, Project Director welcomed the Chairman, the dignitaries and participating scientists of Regional centers and network Units. He emphasized that AICRP on FMD is in-built component of PDFMD and under AICRP there are 8 Regional Centers and 15 network Units catering to the need of epidemiology, surveillance, diagnosis, National seromonitoring and eromonitoring of FMD control programme running in the country. PDFMD is also supplying diagnostic reagents with SOP for uniformity in diagnosis and monitoring of FMD in the country. PDFMD is also providing specialized service and training on FMD diagnosis in the country.

Prof. Gaya Prasad, ADG (AH), ICAR informed that AICRP on FMD is the oldest coordinated research programme in the country which is

testimony to the fact that FMD is the biggest threat to animal health and poverty alleviation in the country. FMD virus continues to be a difficult virus and challenge to policy makers and scientists. Now India cannot trade on livestock and its product to FMD free countries, and there are certain other challenges before us to be addressed like short duration of immunity of FMD vaccine, thermo stability of the vaccine and developing test systems to detect persistence of virus in vaccinated animals.

Prof. KML Pathak in his remark congratulated the Project Director and his team for the release of the DIVA kit by the Honourable Union Minister of Agriculture during the ICAR foundation day ceremony on 16th July 2010. Then he asked the Project Director to present the report for the year 2009-10 alongwith action taken report on the recommendations of the 20th Annual Scientist Meet held at Imphal.

Dr. Pattnaik presented the action taken report of the 20th ASM that was accepted. During the discussion Prof. Pathak enquired about the transfer of trained scientist(s) from the Regional Centers and Network units. Dr. Pattnaik informed that it was mostly implemented and constant effort being made to make understand the state Government authorities/SAUs/SVUs about the necessity and importance of continuance of trained scientific manpower at the Regional Centers and Network Units. He further informed that the transfer problem at the Pune Regional Center has been settled by discussion with the competent authorities. He also informed to the house that Himachal Pradesh, Punjab, Haryana and Delhi have recorded nil or sporadic cases of FMD, and these states in the North hold the

promise for being identified as "Potential FMD free zones (DFZ)".

Dr. A. Sanyal, Principal Scientist, presented the report of the Central FMD Laboratory, Mukteswar and highlighted the achievements.

- A total of 1624 clinical specimens were collected from 799 outbreaks by the Regional Centers and Network Units of AICRP and some of these are duplicate samples from the same outbreak collected at different times. 610 outbreaks could be confirmed for FMD. Dominance of type O virus was noticed in causing FMD in the country.
- During 2009-10, a total of 549 outbreak clinical materials were received and processed for serotype identification by the Central FMD Laboratory from all the Regional Centers and Network Units of AICRP for reconfirmation and subsequent characterization of field outbreak viruses. Serotype O could be identified in 423 samples, serotype A in 15 samples and serotype Asia1 in 7 samples.
- A total of 549 clinical materials were passaged three times in BHK21 and IBRS2 cells for virus revival. A total of 133 field viruses (O-97, A-24 and Asia1-12) were revived as cell culture adapted virus.
- The central FMD laboratory maintains the National FMD virus Repository that is upgraded annually with latest virus isolates. All the 133 field viruses adapted in cell culture were added to National FMD virus Repository. At present the repository has 1687 field isolates.
- Analysis of antigenic relationship of the field outbreak strains with in-use vaccine strains is a regular exercise to monitor antigenic variation, if any, occurring in the field. This year, a total of 33 virus isolates including 20 serotype O, 7 serotype Asia1 and 6 serotype A field isolates were subjected to one-way antigenic relationship study using bovine vaccinate serum (BVS) in two-dimensional

micro-neutralization test. All of them showed close antigenic match with respective vaccine strains indicating their appropriate antigenic coverage.

- For Genetic characterization of field isolates and to understand molecular epidemiology of the disease in the country, 1D genomic region was used as the target sequence. The nucleotide sequences were analyzed using appropriate computer software. In case of serotype O, compared to recent past, the year saw a major shift in genetic lineage of serotype O viruses circulating in India. The 'Ind2001' lineage viruses gained upper hand after a gap of 8 years and outcompeted PanAsia II lineage. Nevertheless the disease due to PanAsia II, PanAsia I and Branch C-II viruses was also noticed. There were outbreaks of FMD in Mithun in Arunachal Pradesh due to PanAsia I; this lineage also caused outbreaks in Bihar. In case of serotype A, co-circulation of both the VP3⁵⁹-deletion and non deletion mutants belonging to genotype VII was observed. Viruses from Punjab and Uttarakhand clustered in the VP3⁵⁹-deletion lineage-VIIIf, where as viruses from Orissa and Andhra Pradesh clustered in non-deletion group. Serotype A continued to be genetically heterogeneous. In case of serotype Asia1, Outbreak was recorded in Gujarat, Madhya Pradesh, Maharashtra, Uttar Pradesh and West Bengal. Field isolates were grouped with lineage C reiterating the supremacy of this lineage in the field since 2005.
- Under FMD Control Programme ~95,000 pre and post vaccinated serum samples from Phase I were tested. After 8th Phase the post vaccinal serum antibody titre $e'' \log_{10} 1.8$ was found in 79.5% samples against serotype O, 71.6% against serotype A and 66.2% against serotype Asia1 as compared to 53.5% samples against serotype O, 49.5% against serotype A and 57.6% against serotype Asia1 after first phase. Over the year as more

rounds of vaccination are completed, there has been marked improvement in the antibody status (herd immunity) of the animal population in FMDCP areas.

- For National FMD Serosurveillance, level of serum antibody in 11,560 random serum samples from different states of the country was estimated by Liquid Phase Blocking ELISA (LPB-ELISA). 42.3% percent of animals against serotype O, 40% against serotype A and 30.5% against serotype Asia1 showed antibody titre of $e'' \log_{10} 1.8$. A total number of 29,763 random bovine serum samples collected from 335 districts covering 21 different states of the country were tested by 3AB3 DIVA ELISA in an exercise to estimate prevalence of FMD virus infection in the country. This revealed 27.9% of the bovine population in the country to be FMD virus positive during 2008-2009 at a confidence level of 95%. In many states like Kerala and Punjab, a clear and direct correlation could be made in the prevalence figure and the natural outbreak history for the districts.
- Selection of alternate candidate panel for serotype O was undertaken because of changing scenario of type O outbreaks caused by "IND2001" strains and co-circulation of PanAsia lineage/genotype in India. Seven FMDV type O strains from a pool of 150 previously characterized FMDV type O field isolates were short listed for evaluation as alternate candidate vaccine strains. A total of 18 FMDV serotype O field isolates from different parts of country were used to study antigenic relationship with the candidate panel (BVS) by micro-neutralization test. The study revealed that the current in-use vaccine strain IND R2/75 is still the best and covers all the circulating outbreak strains in the country. Indian FMD vaccine industry collaborated in this study.
- New research work was initiated for detection

of FMD Virus in semen samples by multiplex PCR for which Standard Operating Procedure (SOP) was developed.

- Work was also initiated for development of one-step, reverse transcription loop-mediated amplification (RT-LAMP) assay for diagnosis of FMD virus. The assay once developed (targeting an across serotype conserved fragment of the 3D RNA polymerase gene) can detect FMD virus in under an hour in a single tube without thermal cycling.
- Produced, Standardized and Supplied Kits for testing 7000 clinical materials using sandwich ELISA, and for testing 80,000 serum samples by LPB-ELISA. DIVA Kit to test a total number of 75,000 serum samples was produced and kit for testing of 3,27,000 serum samples have been supplied so far to the Regional Centers and Network units and vaccine manufacturing companies for testing random serum samples.
- PD FMD participated in 2008 Inter-Laboratory Comparative Testing Exercise for FMD vaccine matching between members and observers within the OIE/FAO Global Network of FMD Reference Laboratories. The LPB ELISA developed at PDFMD was employed in the study and the results accrued matched with the best in the network. This also revealed appropriateness of the indigenously developed LPB ELISA at the Global level.
- Collaborative programme with USDA-ARS on "Effective Molecular Vaccines against Foot-and-Mouth Disease" has been finalized and initiated under Global FMD Research Alliance (GFRA). IVRI, Bangalore Campus also participates in this programme. The outcome of the study is expected to be a viral vectored molecular vaccine with certain advantages.
- Under Human Resource Development, nine training *cum* workshop sessions consisting of total of 49 days on "recombinant 3AB3 nonstructural protein based indirect ELISA

for differentiation of FMD infected from vaccinated animals (DIVA)" for 60 researchers from network units and private FMD vaccine manufacturing companies were organized at Central FMD laboratory. A total of twenty-five scientists from regional centers and network units have been trained to carryout LPBELISA at Central FMD laboratory of PDFMD, Mukteswar.

Presentations were made by the In-charges of 08 Regional Laboratory and 14 Network Units.

After detailed discussion under the chairmanship of Prof. Gaya Prasad in the presence of Dr. Lal Krishna, Former ADG (AH), and other special invitees, the following recommendations were made:

Recommendations

1. Retrospective diagnosis of FMD should be done in case of delayed reporting of FMD outbreak(s). **[Action: Regional FMD Centers and Network Units / PDFMD].**
2. The centers and units should determine the circulation of FMD virus serotypes in the states by NSP and SP antibody assay. **[Action: Regional FMD Centers and Network Units / PDFMD].**
3. Lucknow network unit may be activated/ closed/shifted. **[Action: ADG(AH)/ PDFMD].**
4. PDFMD to continue to supply the diagnostic kits till the technologies transferred to Industry on non-exclusive basis. **[Action: PDFMD].**
5. Alternate test system needs to be developed to assess efficacy/potency of FMD vaccine batches. **[Action: ICAR/PDFMD].**
6. Faster mode of communication to be used for reporting of disease. **[Action: DADF/ State AH Dept./ICAR].**
7. Research need to be initiated for development of FMD vaccine with better thermostability in the context of Global Warming and also for enhanced duration of immunity. **[Action: ADG(AH)/PDFMD/IVRI].**
8. Serosurveillance to be intensified in Punjab, Haryana, Himachal Pradesh and Delhi to identify these states as potential FMD free zones. **[Action: Regional FMD Centers and Network Units / PDFMD].**
9. Bi- Annual 100% vaccination coverage under ASCAD/ RKVY to be undertaken as in FMDCP, followed by seromonitoring. **[Action: DADF/ State AH Dept.].**
10. Centralized Quality control of FMD vaccine may be carried out. Pay load of serotype specific virus antigen per dose in each batch of vaccine to be determined. **[Action: DADF /ICAR].**
11. Rajasthan state should be included under the FMD CP programme as it borders with Haryana and Punjab where incidence of FMD is under control. In this connection a meeting of Directors of Animal Husbandry, Departments of Punjab, Haryana, U.P., Rajasthan, Delhi and Himachal Pradesh to be convened at Delhi under the Chairmanship of Prof. KML Pathak, DDG (AS) to draw strategy for control of FMD and control migration of FMD unvaccinated animals from Rajasthan to neighboring states. **[Action: DADF /ICAR].**
12. Discussion need to be initiated for use of type O monovalent vaccine alternatively with trivalent vaccine in serotype O preponderant areas. **[Action: DADF /ICAR].**
13. Each center and network unit should adopt one village with 100% Bi-annual FMD vaccination for post vaccinal sero-monitoring. The villages to be selected in consultation with the state Animal Husbandry Department. **[Action: Regional FMD Centers and Network Units / State AH Dept].**

14. Creation of FMD Network units in Chattisgarh, Jharkhand and Goa, and Shifting of FMD Network Unit at Lucknow to any other place in the state. **[Action: ICAR/PDFMD].**
15. The prevalence of type A and Asia 1 serotypes in certain states of the country has to be studied. **[Action: Regional FMD Centers and Network Units /PDFMD].**
16. The studies on epidemiology, surveillance and monitoring have to be intensified in Andaman Nicobar Islands to make it free from FMD. The Kolkata Regional Center should work jointly with CARI, Portblair for this purpose. **[Action: Kolkata Regional FMD Center/ AH Dept. A&N Islands/PDFMD/CARI, Portblair].**
17. FMD vaccine industry and other stake holders may be invited to attend the ASM for broader interaction. **[Action: Regional FMD Centers and Network Units /PDFMD].**
18. BSL2 + facility have to be provided to each Regional FMD Centers. **[Action: ADG (AH)/ PDFMD].**
19. One needle per animal to be used during FMD vaccination to check the spread of disease during vaccination. **[Action: DADF /State AH Depts.].**
20. The National FMD sero-surveillance programme should be continued as annual activity. **[Action: Regional FMD Centers and Network Units /PDFMD].**
21. The PI of each center/ network units should specify the percentage of time devoted or contributed for FMD work. **[Action: Regional FMD Centers and Network Units /PDFMD].**
22. Persuasion with the Directors of State AH department for timely reporting of the disease. **[Action: DADF/Regional FMD Centers and Network Units /PDFMD/ ADG(AH)].**
23. Uniform format to be used by all Regional Centers and Network Units for presentation of data and achievements with inferences drawn. **[Action: Regional FMD Centers and Network Units /PDFMD].**
24. Status of FMD in coastal areas has to be assessed. **[Action: Kolkata, Hyderabad, Ranipet, Bangalore, Pune Regional FMD Centers and Odisha, Ahmedabad and Thiruvananthapuram Network Units / PDFMD].**
25. Each center/unit should revalidate their balance amount/fund of the previous years immediately from the ADG (AH) or surrender the unspent amount of previous years. **[Action: Regional FMD Centers and Network Units /PDFMD].**
26. Immediate laboratory requirements of the Regional Centers and network Units were discussed and the following requirements were approved from within the total XIth Plan allocation **(Action: ADG, AH and PDFMD).**
 - Each Regional Center and Network Unit will be supplied with two Immunowash (8-12 channel) (Nunc) (total 44) and 3 for the Central FMD Laboratory (One each for LPBE, Sandwich and Indirect DIVA ELISA (Grand total 47).
 - Each center and regional units has to be supplied with two liquid handling system (multichannel/multistepper) (total 44) for use in diagnostic assays.
 - Recruitment of RA/SRF on contract basis by walk-in-interview by PDFMD, Mukteswar for the Regional centers / Network units as per the approved manpower in EFC, and on need basis.
 - Details of Requirements of AICRP on FMD Regional centers and Network Units to be provided on priority:

Name of Center/Units	Requirements	
Hisar	1. -20°C Deep Freeze. : Four	
Pune	1. -20°C Deep Freeze. : Four	
Mathura	1. -20°C Deep Freeze : two	
	2. Autoclave : one	
Guwahati	1. -20°C Deep Freeze : two	2. Refrigerator : two
	3. Autoclave : one	4. Computer with Accessories : one
Ranipet	1. -20°C Deep Freeze : two	2. Computer with Accessories : one
Kolkotta	1. -20°C Deep Freeze : one	2. ELISA Reader; one
Bangalore	1. -20°C Deep Freeze : two	
Hyderabad Unit	1. -20°C Deep Freeze : two	
Jalandhar Unit	1. -20°C Deep Freeze : two	
Patna Unit	1. -20°C Deep Freeze : two	2. Refrigerator : one
	3. Computer with Accessories : one	
Cuttack Unit	1. -20°C Deep Freeze : one	
Tripura Unit	1. -20°C Deep Freeze : two	2. ELISA reader : one
Imphal Unit	1. ELISA reader : one	2. Generator : one
Jammu Unit	1. -20°C Deep Freeze : two	2. ELISA Reader : one
Bhopal	1. -20°C Deep Freeze : two	
Thiruvananthapuram	1. -20°C Deep Freeze : two	2. ELISA Reader : one
	3. Refrigerator : one	
Jaipur	1. -20°C Deep Freeze : two	
Aizwal	1. -20°C Deep Freeze : one	2. Refrigerator : one
Itanagar	1. -20°C Deep Freeze : two	
Ahemedabad	1. -20°C Deep Freeze : two	
Kohima	1. -20°C Deep Freeze : one	2. Refrigerator : one
	3. Generator : one	
Shimla	1. Computer with Accessories : one	

12.2 Proceedings of the 1st Meeting of the Second RAC

The first meeting of Second RAC of the PD on FMD was held on 14.09.2010 in the

Committee Room No. 2 at ICAR, Krishi Bhawan, New Delhi under the chairmanship of Dr. S.K. Garg, Former Vice-chancellor, DUVASU, Mathura, UP.

Sl. No.	Recommendation of RAC	Director's Comment	Comments of the Council
1.	PDFMD should continue to produce and supply diagnostic reagents along with the SOP to achieve uniform diagnosis and surveillance of FMD in the country	This is a continuous activity and all required diagnostics are regularly produced, standardized and supplied to all laboratories involved in FMD diagnosis as well as to the FMD vaccine industry.	Agreed
2.	Creation of an interactive web site on FMD at the earliest possible time. The interactive website shall provide information pertaining to FMD outbreaks in different parts of the country, molecular characterization data and vaccine matching details.	Steps have been initiated with the help of DIPA and ERNET. Technicalities and estimate to hook up all the centers, network units and the Central FMD laboratory have already been worked out by ERNET, and administrative approval is being obtained from the Council.	Agreed

Sl. No.	Recommendation of RAC	Director's Comment	Comments of the Council
3.	Representatives from DADF, GOI may be invited to the meetings of RAC as special invitees for better coordination in the areas of FMD surveillance and control.	It has been noted and will be followed in subsequent meetings of RAC.	Not agreed. RAC is a constitutional committee. The outsiders should not be invited
4.	Process should be initiated to declare/certify FMD free zones in the country as per OIE recommendations.	It has been noted and necessary action will be initiated with the help of DADF.	Agreed
5.	In the Annual Scientists Meet on FMD, the members of RAC and representatives of FMD vaccine industry may be invited for greater interaction and understanding requirements of the industry and the country as a whole.	It has been noted and will be followed in subsequent meetings of RAC.	Not Agreed
6.	Use of mathematical model/in-silico analysis to correlate genetic data with antigenic relationship among field isolates to quickly understand the antigenic coverage of the in-use vaccine strains.	It has been noted and necessary action will be initiated for procurement of appropriate software and training of the scientists in the field from within available fund.	Agreed
7.	Intensification of FMD surveillance programme in the areas where the incidence of FMD outbreak is low or reduced.	It has been noted and necessary action will be initiated with the help of AICRP regional center at Hisar and network units of Shimla and Jalandhar.	Agreed
8.	Studies need to be initiated to understand early infection process (early pathogenesis) of FMD in order to develop virus specific markers for early diagnosis of the disease	A research project on the subject is already underway.	Agreed
9.	Use of FTA cards for collection of difficult clinical samples for diagnosis and molecular analysis of outbreak viruses. This will be helpful in investigation of the outbreaks reported late in the clinical stage.	It has been noted and necessary action will be initiated for procurement of FTA cards and training of the scientists of the AICRP on its use.	Agreed
10.	Study need to be undertaken to find out the primary foci of FMD virus serotypes A and Asia1 as these 2 serotypes are localized in certain parts of the country.	It has been noted and necessary action will be initiated with the help of AICRP regional centers of Hisar and Mathura and network units of Ahmedabad, Bhopal, Shimla and Jalandhar.	Agreed
11.	A pilot study need to be undertaken regarding use of monovalent type O vaccine and evaluation of different antigenic pay loads in the trivalent vaccine to understand dose-dependent protection level.	It has been noted and necessary action will be initiated with the help of AICRP regional centers of Hisar and Mathura and network units of Ahmedabad, Bhopal, Shimla, Patna and Jalandhar. Dr.V.A. Srinivasan, member, RAC has agreed to supply the required vaccine doses for the experiment.	Agreed

Sl. No.	Recommendation of RAC	Director's Comment	Comments of the Council
12.	Differential diagnosis of FMD with other vesicular diseases needs to be initiated.	It has been noted and work on this aspect will be initiated as soon as the BSL3+ laboratory facility is established at the International Center for FMD.	Agreed
13.	Each Regional center and Network Unit should adapt 1-2 villages to determine the impact of vaccination and resultant antibody response in the animals that will be used as the benchmark/standard to evaluate sero-conversion following vaccination in the particular state.	It has been noted and necessary action shall be immediately initiated by all the regional centers and network units of the AICRP under the guidance of the Central FMD Laboratory, Mukteswar.	Agreed
14.	The turnkey agreement with NDDDB should be expedited at the earliest to initiate the work of International Center for FMD at Bhubaneswar.	The turn key agreement is under process at the Council.	Under process
15.	AICRP Regional Centers and Network Units should have minimum BSL2 facilities on immediate basis for safe handling of FMD virus.	It has been noted and necessary equipment supplies shall be made under centralized purchase (to ensure uniformity and quality of the make) once plan fund is available.	Agreed
16.	Liquid handling system need to be provided to each Regional Centers and Network units of the project to enhance their working capability.	It has been noted and necessary equipment supplies shall be made under centralized purchase (to ensure uniformity and quality of the make) once plan fund is available.	Agreed
17.	<p>Following nine research programmes should be continued.</p> <ul style="list-style-type: none"> • Antigenic and molecular epidemiology of Foot and Mouth Disease virus serotype O in India • Antigenic profiling and molecular epidemiology of foot and mouth disease attributed to serotype A virus in India. • Antigenic analysis and Genetic characterization of Foot and Mouth Disease virus serotype Asia1 in India. • Maintenance of National Foot and Mouth Disease Virus Repository. • Development of a lateral flow chromatographic strip for rapid pen-side diagnosis of foot and mouth disease. • Molecular Diagnosis of Foot and Mouth Disease Virus. • National Foot and Mouth Disease sero-surveillance and epidemiological analysis of Foot and Mouth Disease outbreaks. • Production, Standardization and Supply of diagnostic reagents for Foot and Mouth Disease diagnosis and surveillance. • Seromonitoring under Foot and Mouth Disease control programme. 	Noted and complied.	Already in practice

Sl. No.	Recommendation of RAC	Director's Comment	Comments of the Council
18.	<p>Following six new research proposals were approved</p> <ul style="list-style-type: none"> • Development of alternate assay system for FMD vaccine matching and efficacy • Pathogenesis of Foot and Mouth Disease Virus and its early diagnosis in Mice model. • Construction of an Infectious cDNA Clone for a Serotype Asia 1 Foot and Mouth Disease Virus • Development and application of "Loop-mediated isothermal amplification" (LAMP) assay for FMD diagnosis • Antigenic and Genetic variation of Foot and Mouth Disease virus serotype O and A in the presence and absence of immune selection • Surveillance and Monitoring of Foot and Mouth Disease in ovine, caprine and porcine species in India 	Noted and complied.	Agreed

12.3 Recommendations of QRT in respect of Project Directorate on Foot and Mouth Disease, Mukteswar for the period 2004-2008

Sl No	Recommendations of QRT	Comments of the Council
1	Construction of BSL3 + FMD Lab in Bhubaneswar, Orissa should be made in fast track mode.	Agreed, action will be initiated
2	On the issue of the implementation of FMDCP there is a need to have direct and focused interaction between DADF and ICAR.	Agreed. Already there is direct and focussed interaction between DADF and ICAR.
3	There is an urgent need to integrate the activities of PDFMD and the Bangalore campus of IVRI to provide a more useful support to the DADF in view of the massive FMD control programme.	Agreed, action will be initiated.
4	Although PDFMD and Indian Veterinary Research Institute have the necessary technologies in the area of (a) Diagnostics and (b) vaccines, and the industry has the necessary capacity to manufacture the required doses of vaccines needed, the coordination and regulatory systems are not in place. Nor is there any watch dog to study if the desired results (of FMDCP) and targets are achieved.	Agreed. Action will be taken to commercialize FMD diagnostics and vaccines. However, Watch dog committee to monitor success of FMDCP has to be formed by DADF.
5	The system of reporting the outbreaks needs to be improved to highly specific and specialized outbreak reporting system, using most advanced systems of the telecommunication.	Not agreed. Disease reporting is not part of the mandate of PDFMD.
6	In six phases of vaccination under FMDCP, needed numbers of sample has not been tested, and reliability of this date based on small sample size can hardly be relied upon globally. In most phases of vaccination the sero conversion remained at a low level between 40-70%.	Not agreed. The herd immunity of 40-70% only after 6 rounds of vaccination in an endemic country is not poor. It will gradually progress after subsequent regular vaccinations. It will require further rounds of vaccination to achieve 80% herd immunity.

SI No	Recommendations of QRT	Comments of the Council
7	DADF is requested to setup a separate, independent unit on the pattern of NREP (National Render pest Eradication Programme) to monitor different components of the FMDCP with defined authority and accountability.	Not agreed. The recommendation pertains to DADF.
8	The sample size (under FMDCP) to be raised to a minimum of 10%.	Not agreed. Because 10 percent sampling of animals (11 million animal pre- vaccination and 11 million animals post vaccination in each round of vaccination) is too huge task. However, the sampling frame has to be changed by DADF as the existing sampling frame was done by them.
9	Impact analysis of the programme (FMDCP) is not available and needs to be commissioned urgently	Agreed. Calculation of economic impact is being done by a collaborative research programme with NCAP
10	The creation of a National Quality Control Laboratory for the FMD vaccines used in the country on the lines of the PANAFTOSA laboratory located at Rio in Brazil, South America. The vaccine testing under the control of regulator (GoI/DADF-PDFMD) should be immediately started, before the vaccine is released for field use. This problem needs to be resolved on priority.	Agreed. Necessary action will be taken
11	The PDFMD should initiate research to increase the duration of immunity of the conventional vaccines by using newer adjuvant and delivery systems, optimize the final vaccine dose, to improve the thermo stability of the conventional vaccines, refine conventional vaccine technology to produce the vaccine free from Non Structural Proteins and develop better new generation vaccine.	Agreed. Necessary action will be taken.
12	An epidemiology section is created for monitoring, surveillance and design of epidemiological studies on the disease.	Agreed. Necessary action will be taken
13	Convert research findings into technology.	Agreed. Necessary action will be taken
14	Explore the possibility of testing by private parties.	Agreed. Necessary action will be taken
15	Strengthen centers under PD-FMD.	Agreed. Necessary action will be taken
16	Explore the possibilities of FMD vaccine quality control.	Agreed. Necessary action will be taken
17	Explore the possibilities of putting all FMD activities under one umbrella.	Agreed. Necessary action will be taken

12.4 Proceedings/Recommendations of the 8th Meeting of the IMC held in the ICAR Committee Room II, New Delhi on 23.03.2011

Item No	Agenda/recommendation of the IMC	Comments of the Members	Comments of the Director	Comments of the Council
1	Approval of the proceedings of the 7th Meeting of the Institute Management Committee of the Project Directorate on FMD Proceedings of 7 th IMC were discussed at length and ATR was presented by the Project Director. It was observed that appropriate action is being taken / initiated on all the recommendations of 7 th IMC.	It was noted by all the members and were satisfied with the action taken, and it was observed by the members that the matter of making available a medical doctor at Mukteswar should be taken seriously for welfare of the employees.	Delay in procurement of a photocopier under non-plan is due to some problem in the local market arising due to confusion in excise duty. This will be solved soon. It has been difficult to engage a medical doctor on contract with the salary of a RA (Rs.26,000 pm). Attempt is still on to find a doctor. Having an AMA at Haldwani (foot hills) is of no use as cases of emergency at Mukteswar cannot be handled. In recent past there have been some deaths due to non-availability of immediate life saving medical aid in time. Under the situation/ circumstance, a position of T-6 may be provided to PDFMD against which a MBBS graduate can be selected by the institute/ ASRB.	A separate proposal for T-6 post (MA) with detailed justification may be sent to the council for consideration.
2	Budget Utilization The IMC reviewed the budget utilization in the 11 th Plan period till 2010-11 and found it optimum. The IMC emphasized timely utilization of budget allocation.	Budget utilization is appropriate.	Budget utilization is as per annual allocation(s).	Noted
3	Vehicle for International Center for FMD at Bhubaneswar A new vehicle for this purpose is already approved in 11 th Plan EFC; as there is ban on purchase of new vehicle the same could not be procured. A vehicle was condemned and order was placed (date?) with Kirloskar Toyota for Kolkata Center; but due to administrative problems at Kolkata its delivery could not be taken. Since money has already been paid to the manufacturer and construction work of IC-FMD is to start soon, the IMC was requested to approve use of this vehicle for IC-FMD.	The IMC agreed to the proposal of taking delivery of the vehicle that was ordered with Kirloskar Toyota, Bangalore (date??) during 'no-ban' period for Kolkata center of the AICRP as replacements only, and use it for IC-FMD.	There is EFC approval to provide replacement for one vehicle each to the regional centers Pune, Guwahati and Kolkata of the AICRP. Accordingly, 3 vehicles were booked and payment was made to the manufacturer as per DGS&D rate during the FY———. The regional centers Pune and Guwahati have already taken delivery of their vehicle(s), and Kolkata center could not take delivery due to some problem at their end. As money has already been paid, and construction work of the IC-FMD is going to start shortly, delivery of the vehicle may be allowed at the institute head quarter for use.	A separate proposal with justification may be sent to the council.

Item No	Agenda/recommendation of the IMC	Comments of the Members	Comments of the Director	Comments of the Council
4	Hiring of accommodation for camp office & contractual manpower for International Center for FMD (IC-FMD) at Bhubaneswar. This was approved in the 5 th meeting of the IMC, but was not implemented as other related works were not completed. Now that revision of EFC for IC-FMD has been agreed and turnkey agreement to be signed soon with NDDB for execution of the project, it is now required to have a camp office at Bhubaneswar along with minimum contractual manpower.	Hon'ble members agreed.	Hiring of accommodation at Bhubaneswar for camp office of IC-FMD is now required as the construction is to start soon. Contractual manpower will be engaged as per the need of the camp office. One scientist will be posted there soon from mukteswar.	Sister institute in Bhubaneshwar may be approached in regard to camp office. A regards contractual manpower Director is competent authority to engage need based contractual workers as per guidelines issued by the council from time to tie.
5	Discipline-wise revision of cadre strength of scientists. One position of scientist each in the disciplines of Economics, Statistics, Computer Application and Veterinary Biochemistry is proposed to be converted to 04 positions of scientists in the discipline of Veterinary Microbiology.	The members agreed. The ADG (AH) stressed the need of 2 positions of Senior Scientists, one each for Epidemiology and Bioinformatics.	There are only three positions of scientists in the discipline of Veterinary Microbiology in cadre strength of 15. This number is not enough to meet the mandate of the institute. As this institute works on a viral disease (FMD), there is requirement for more number of scientists in the discipline of Veterinary Microbiology. Further, scientists in the disciplines of Economics, Statistics, Computer Application and Veterinary Biochemistry are not required, and these 04 positions of scientists may be converted/ re-appropriated to Veterinary Microbiology.	As per approved cadre strength circulated by the council from time to time. In case of any change separate proposal be sent for consideration of the council.
6	Requirement of additional post of Principal Scientist in the discipline of Veterinary Microbiology. The post already approved in 11 th Plan EFC to be filled by re-deployment. As till now a suitable redeployment has not been possible due to shortage in the number of such scientists in the system, it is requested that a post of Principal Scientist (Vet. Microbiology) is created/ transferred and filled up through ASRB.	Hon'ble members agreed.	There is currently only one post of Principal Scientist (Vet Microbiology) in the cadre strength, and the incumbent is looking after the Central FMD Laboratory of the institute. An additional post in this cadre and discipline is required to look after Biosafety and Biosecurity aspects of International Center for FMD, construction of which is to start soon. As it will be a specialized containment laboratory of level BSL3+, one more Principal Scientist in the discipline of Vet Microbiology is required to look after biosafety and biosecurity aspect of the laboratory since beginning of construction. This post may be provided and filled through ASRB.	Noted. A separate proposal be sent with full justification for re-employment/creation of post.

Publications/ Abstracts/Presentations in Conferences

1. J. K. Mohapatra, S. Subramaniam, L. K. Pandey, S. S. Pawar, A. De, B. Das, A. Sanyal, B. Pattnaik (2011). Phylogenetic structure of serotype A foot-and-mouth disease virus: Global diversity and the Indian perspective. **Journal of General Virology** Vol. 92, 873-879.
2. J. K. Mohapatra, L. K. Pandey, G. K. Sharma, S. K. Barik, S. S. Pawar, R. Palsamy, B. Pattnaik (2011). Multiplex PCR for rapid detection of serotype A foot-and-mouth disease virus variants with amino acid deletion at position 59 of the capsid protein VP3. **Journal of Virological Methods** Vol. 171, 287-291.
3. S. Ranabijuli, J. K. Mohapatra, L. K. Pandey, M. Rout, A. Sanyal, B. B. Dash, L. N. Sarangi, H. K. Panda, B. Pattnaik (2010). Serological evidence of foot-and-mouth disease virus infection in randomly surveyed goat

population of Orissa, India. **Transboundary and Emerging Diseases** Vol. 57, 448-454.

Abstracts/Presentations in Conferences

B. Pattnaik, M. Rout, J. K. Mohapatra (2010). Foot and Mouth Disease in India: A Status Update. **National Seminar on 'Animal Resource Development and Poverty Alleviation'**, June 8-9, 2010, OUAT, Bhubaneswar, Orissa.

External guest faculty in training programmes

J. K. Mohapatra (2010). DIVA Diagnostic Strategy for Viral Disease of Animals. Short term training course on "Application of Molecular Techniques in Modern Biotechnology Research". Nov 22-Dec 2, 2010, Sponsored by **State Biotech Programme, Govt. of Uttarakhand**, Organized by IVRI, Mukteswar, India.

Participation in Meetings/Conference/Symposium/Training

1. Scientists participated in two days training programme on strengthening of statistical computing for NARS in IVRI, Izatnagar during 18-06-10 and 19-06-10 and training programme, "Researchers' Training I: Data Analysis using SAS", "Researchers' Training II: Data Analysis using SAS", "Researchers' Training III: Data Analysis using SAS", on strengthening of statistical computing for NARS at IVRI, Bareilly.
2. Scientists of PDFMD participated in three days conference, "X Agricultural Sciences congress", at NBFGR, Lucknow from 10- 12th February, 2011.
3. Scientists of PDFMD participated in GF-TADS PCP-FMD & Diagnostic Lab Network co-ordination meeting on 7/1/2011 in New Delhi.



Education and Training

The Scientists of PD FMD are involved in teaching various courses for the M.V. Sc., and Ph.D. students of Division of Veterinary Virology for their degree at Indian Veterinary Research Institute. During the year the following courses were offered by the scientists of the project: Viral Pathogenesis, Immunity to Viral Infection, Viral vaccines, Diagnostic Virology, Virological Technique, Advanced Virological Technique, Slow Viruses and Virus like agents, Advanced General Virology, Avian viruses.

Training organized

Seven training sessions consisting of a total of 41 days on “recombinant 3AB₃ nonstructural protein based indirect ELISA for differentiation of FMD virus infected from vaccinated animals (DIVA)” were organized, in which 9 scientists from network units/regional centres and three scientists from FMD vaccine manufacturing companies participated.



Visiting Dignitaries

1. Prof.K.M.L Pathak, DDG(AS), ICAR visited PD-FMD on 07-07-2010
2. Dr. Keith Sumption, Secretary of the European Commission for the Control of Foot-and-Mouth Disease (EUFMD), Animal Health Service, Food-and-Agriculture Organization of the United Nations, Rome visited PD-FMD from 30-10-2010 to 1-11-2010.
3. Dr.A.K.Srivastava, Director, NDRI, Karnal visited PD-FMD on 12-11-2010



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