SOUVENIR

OIE/FAO FMD
Reference Laboratories Network Meeting 2009

NASC Complex, ICAR, New Delhi

23rd to 27th November 2009

Project Directorate on Foot and Mouth Disease
(Indian Council of Agricultural Research)
Mukteswar, Nainital 263 138
(Uttarakhand)

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Panoramic view of Himalayan snow peaks captured from PDFMD, Mukteswar

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डा. मंगला राय
सचिव एवं महानिदेशक
DR. MANGALA RAI
SECRETARY & DIRECTOR-GENERAL

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Message

I am pleased to know that the global meeting of the OIE/FAO FMD Reference Laboratories Network is being organized during 23-27 November 2009 in association with the Indian Council of Agricultural Research at New Delhi.

Foot-and-mouth disease, a contagious animal disease, is widespread throughout the year in our country and economic losses caused by the disease are hugel direct losses alone are estimated to be more than Rs 20,000 crore (4.45 billion USD) per year.

To safeguard international trade of animals or their derived products, an efficient global surveillance for FMD including constantly updated information on antigenic and genetic characteristics of the foot-and-mouth disease virus involved in outbreaks is required. This network monitors foot-and-mouth disease situation in the world throught joint surveillance, and harmonizes laboratory procedures that provide epidemiological information, and give control options through laboratory-based data on vaccine-matching.

I hope that the deliberations and strategic recommendations would provide suitable guidelines for planners to formulate and chalk-out agenda for controlling FMD globally.

I wish this Meeting a grand success.

(MANGALA RAI)

Dated : 19 November 2009
New Delhi

सचिव
भारत सरकार
Secretary
Government of India

सत्यमेव जयते

कृषि मंत्रालय
पशुपालन, डेयरी और मत्स्यपालन विभाग
कृषि भवन, नई दिल्ली - 110 014

Ministry of Agriculture
Department of Animal Husbandry, Dairying & Fisheries
Krishi Bhawan, New Delhi- 110 014

Message

I am happy to know that the Indian Council of Agricultural Research (ICAR) is hosting the Global OIE/FAO FMD Reference Laboratories Network Meeting, 2009 at New Delhi this year. The meeting is very important, since the livestock and fisheries sectors together account for nearly 30 % of the value output of agriculture and allied sector in India and contribute about 6.3 % of the national GDP.

Foot and Mouth Disease (FMD) poses the major constraint to our participation in international trade. The immediate objective in the coming years should be to control and ultimately eradicate FMD in India. The initial results of the FMD Control Programme are encouraging; however intensified efforts on a global scale alone will help to control the FMD meance. It is against this backdrop that the theme of the meeting assumes greater significance.

(Rudhra Gangadharan)

Dr. Gavin Wall
FAO Representative in India and Bhutan
New Delhi, India

Message

Foot-and-Mouth Disease (FMD) is the most infectious of animal diseases, affecting a wide range of the most important livestock species - cattle, buffalo, yaks, sheep, goats and pigs as well as vulnerable populations of endangered wildlife.

Despite intensive national control efforts in developed countries since the late 1800's, only Western Europe, Australasia, North and Central America are free of the disease. Over 100 countries remain directly affected, or have not yet managed to acquire 'free' status. The impact of the disease is greatest on poor farmers in all countries; in 'free' countries, a single outbreak can cripple exports for a year or more costing hundreds of millions in lost export. In the endemically affected countries, waves of infection, sometimes several per year, ensure that most ruminants meet infection in their productive lives. The direct impacts of infection include long term losses in milk production by cattle and buffalo and lameness affecting the use of livestock in traction. Because of trade bans, FMD also impacts on the sales of livestock products to other countries.

In the food insecure countries, which are of great importance in the work of FAO, FMD is of significance because of its contribution to the productivity loss that relates to the disease burden which is estimated to be at least 20%. This 20% loss in production translates into food shortages and loss of income for the poor. The scale of the problem is recognized when one realizes that over 700 million of the worlds poor are livestock owners.

The OIE/FAO FMD Reference laboratories network is one part of the approach used by FAO to provide its member states with improved services and information to assist their prevention and control of FMD. The constituent laboratories provide vital information to member states on the selection of appropriate inputs to combat the ever changing strains of FMDV that circulate in the seven major virus pools. The Annual Meeting is an important time to reflect on the threat of new virus variants to the control programmes operating in each region and on improvement of services to member states and to regional programs, including those of FAO.

In the past year FAO, together with OIE, has organized a Global Conference on FMD in Paraguay; this conference endorsed the importance of considering FMD control as a global public good and gave the mandate to FAO to promote regional programmes (Roadmaps) and regional technical networks to better deliver the guidance and services needed by member states in each region. This meeting is part of the follow-up and should help to link the regional technical leaders to international organizations which lead the global program. It is also anticipated that India will, year on year, step up its fight against FMD, and will be prepared to share the lessons learnt, and its scientific and technical achievements.

FAO is proud of the success of the Network and will continue to support the further development of the Global FMD laboratory network.

Gavin Wall

Dr. S. Ayyappan
Deputy Director General (Animal Science)
ICAR, Krishi Bhawan, New Delhi 110 114

भाकृअनुप
ICAR

Message

It is a matter of great pleasure to know that the Global OIE/FAO FMD Reference Laboratories Network Meeting, 2009 is being hosted by the Indian Council of Agricultural Research (ICAR) at New Delhi from November 23-27, 2009. The organization of this meeting is aptly timed as our needs are increasing and therefore, we need to think on improvement of livestock health and production with special emphasis on controlling FMD.

Foot and mouth disease (FMD) is wide spread in our country and occurs throughout the year. Earliest description of FMD in India dates back to 1868. Research on FMD in India has a long history since 1929. The disease situation is complicated by the fact that there is a large susceptible population of 480 million animals in the country, the virus occurs in three immunologically and genetically distinct forms (serotypes O, A and Asia1), which do not confer cross protection between them, unrestricted animal movement and limited vaccination.

In my opinion the theme and the venue of the seminar has been wisely chosen as per the need of time. I hope the deliberations and discussions held during the meeting will be of immense value and culminate into fruitful suggestions for the cause it is being organized.

I wish this International meeting a great success.

(S. Ayyappan)

Dr. Lal Krishna
Animal Husbandry Commissioner &
Assistant Director General
(Animal Health)

सत्यमेव जयते

Government of India
Ministry of Agriculture
Department of Animal Husbandry, Dairying & Fisheries &
Department of Agricultural Research & Education
(Indian Council of Agricultural Research
Krishi Bhavan, New Delhi–110 001

Message

In a scenario where majority of our farmers have small land holdings, animal husbandry plays a major role in their economic upliftment and welfare. Foot and Mouth Disease (FMD) has for centuries been known as a serious threat to the health and welfare of the domestic and wild ruminant animals with negative impacts on the livelihoods of animal keepers.

India is endemic for FMD and to control this disease in the country, Government of India initiated FMD Control Programme in 2003 in 54 districts, in addition to other programmes where FMD vaccination is also a component. Sincere efforts made under the programme culminated into the reduction in the incidence of the disease and even has gone down near to zero in some of the Northern states of the country. Indian Council of Agricultural Research (ICAR) through its Project Directorate on FMD is playing a vital role in providing companion diagnostics, sero-monitoring services and active disease surveillance.

It is my great pleasure to learn that ICAR is organizing the Global OIE/FAO FMD Reference Laboratories Network Meeting at New Delhi from 23-27 November 2009. The outcome of this meeting will certainly stimulate FMD researchers to work further in different areas of vaccines, diagnostics and early infection process.

On this occasion, I greatly appreciate the efforts made by the organizer, and extend my very best wishes for the success of this meeting.

(LAL KRISHNA)

Dr. Mohinder S Oberoi
Sub Regional Manager
Emergency Centre for Transboundary Animal Diseases Unit (SAARC)
Food and Agriculture Organization of the United Nations
PO Box 25, Pulchowk, Kathmandu, Nepal

Message

On behalf of FAO Chief Veterinary Officer I am glad that the Global OIE/FAO FMD Reference Laboratories Network Meeting is being organized by Indian Council of Agricultural Research at New Delhi from 23-27 November 2009.

Foot and mouth disease (FMD) is endemic and remains a major disease of livestock in the SAARC countries causing huge production and economic losses. In the year 2005 the SAARC member countries resolved to have a FMD leading laboratory hosted by India under the Global Framework for the Progressive Control of Transboundary Animal Diseases (GF-TADs). It is expected that the FMD Reference laboratory in India assumes a leading role along with the participation of the Global OIE/FAO FMD Reference Laboratories Network to monitor the FMD situation in the region and initiate the harmonization of vaccine quality and strain matching in the region. I appreciate the initiative taken for organizing this global meeting in India and hope that the scientific discussions and recommendations will provide appropriate guidelines for devising strategy to successfully control the disease in the South Asian region.

On this occasion, I on behalf of FAO-CVO extend good wishes for the success of this global meeting and appreciate the efforts made by the organizers to bring people from across the globe for discussions and recommendations.

Mohinder S Oberoi

Dr. David J. Paton
OIE/FAO FMD Ref Labs Network Secretary
Head of Epidemiology Section and Reference Laboratories
Pirbright Laboratory

Foreword

The Foot-and-Mouth Disease Reference Laboratories Network is a network of OIE and FAO reference laboratories for Foot-and-Mouth Disease (FMD) that has been established under the secretariat of the OIE Reference Laboratory and FAO World Reference Laboratory for FMD, IAH-Pirbright, UK (WRLFMD).

The Network provides an awareness of the global FMD situation through its joint surveillance activities, it gathers and shares antigenic and genetic characteristics of FMD virus in current circulation, harmonizes laboratory procedures that provide such information and provides control options through laboratory based data on vaccine matching.

This year the annual meeting of the Network in Delhi will bring together WRLFMD; the FAO/OIE Regional Reference Laboratory for FMD, FGI-ARRIAH, Vladimir, Russia; OIE FMD Reference Laboratory for the Sub-Saharan continent, Gabarone, Botswana; the FAO/OIE Reference Laboratory for FMD, Centro Panamericano de Fiebre Aftosa OPS/OMS, Rio de Janeiro, Brasil; OIE Reference Laboratory for Foot and Mouth Disease, Laboratorio de Fiebre Aftosa de la Dirección de Laboratorios y Control Técnico, Argentina; FAO FMD Reference Centre, Exotic Animal Health, ARC-Onderstepoort Veterinary Institute, South Africa; FAO FMD Reference Laboratory, Foreign Animal Disease Diagnostic Lab, Plum Island Animal Disease Center, Greenport, USA; OIE FMD Reference Laboratory, Pakchong, Thailand; FAO Reference Centre for FMD, Indian Council of Agricultural Research, Mukteswar, Nainital (Uttarakhand), India; OIE Collaborating Centre for Validation, Quality Assessment and Quality Control of Diagnostic Assays and Vaccine Testing for Vesicular Diseases in Europe, CODA-CERVA-VAR, Belgium; National FMD Laboratory, Lanzhou Veterinary Laboratory, Gansu, China. Other FMD reference laboratories will be invited to join in the future.

Reference Laboratories Information System for the OIE/FAO Foot and Mouth Disease Reference Laboratories Network

Facilitation of the exchange of epidemiological data and informatics are through the provision of a web-based Reference Laboratories Information System (ReLaIS).

http://www.foot-and-mouth.org/

ReLaIS provides public and private web-interfaces that contain up-to-date information on FMD. Global surveillance information is visualised on a geographical interface and list. From here a cascade of laboratory information including virus isolation, serotyping, antigenic/vaccine matching and molecular phylogenies can be accessed for each sample. Tools on ReLaIS also enable registered users to input molecular sequences from their own laboratories (in a private secure area) and manipulate the data to create specific phylogenies.

Purpose of the Network

Safeguarding the international trade of animals or their derived products requires an efficient global surveillance for foot-and-mouth disease (FMD) including constantly updated information on antigenic and genetic characteristics of FMD virus (FMDV) involved in current outbreaks. The exchange of FMDV isolates and data relating to them is also desirable for the development and selection of vaccines and other tools for surveillance and control of FMD, as well as for harmonisation of such approaches. Strengthening of reference laboratory capabilities supports Regional FMD Control Schemes under the auspices of the OIE/FAO Global Framework for the Progressive Control of Transboundary Animal Diseases (GF-TADS).

Objectives of the Network

1. To gather, generate, analyse and make available laboratory information on the global occurrence and spread of FMD and on the characterisation of FMD viruses.
2. To provide recommendations on vaccine strain selection for implementation of control schemes and for vaccine antigen reserves.
3. To offer expertise to OIE, FAO and Member Countries to assist in the control of FMD.
4. To harmonise approaches to the characterisation of FMD viruses.
5. To increase the competence of reference laboratories, to identify constraints to the functioning of the network and to propose solutions.

The network will

1. Meet at least annually to review progress and to agree plans of the network.
2. Develop processes based on best practices to achieve equivalence in FMD laboratory outputs.
3. Collect, characterise (antigenically and genetically), archive and safeguard FMD viruses representing the global diversity of strains.
4. Agree a memorandum of understanding for exchange of materials and information and if necessary a materials/information transfer agreement.
5. Develop a web-based tool for the network to share and make available laboratory information including vaccine matching results, as close to real time as possible.
6. Provide an annual network report to OIE/FAO.
7. Facilitate training and scientific exchange on FMD laboratory activities.

8. Identify research requirements and where appropriate develop joint research projects, for example on validation of diagnostic methods.
9. Maintain a database of FMD laboratory experts and their field of expertise.

Coordination

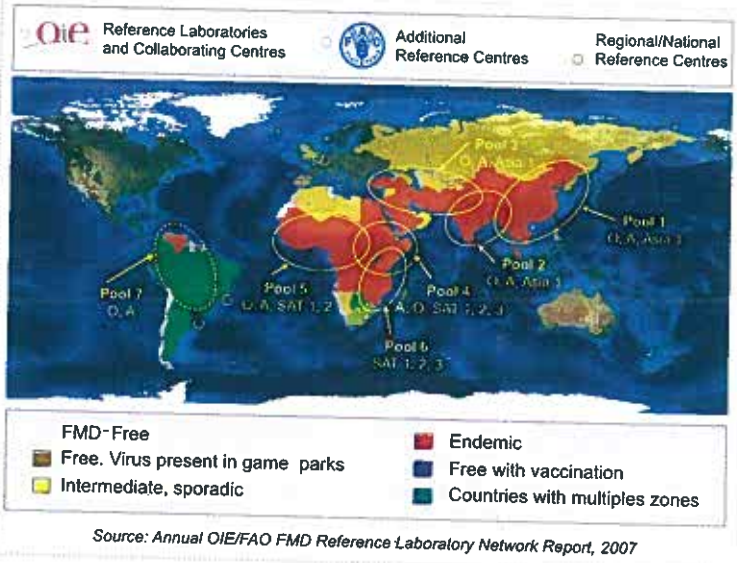
A secretariat is needed to organise the annual meeting and reporting, to establish and maintain ReLaIS and to facilitate the implementation of the agreed plan of work. The Pirbright Laboratory provides the current secretariat.

Steering Committee

A Steering Committee is established comprising OIE, FAO and a representative of each participating laboratory, with the Network Secretariat in attendance, chaired by OIE.

The terms of reference for the steering committee

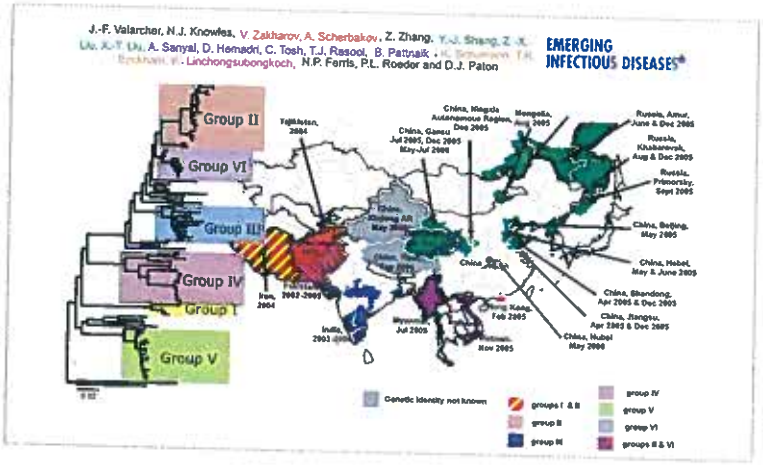
1. Review priorities, organisational relationships and progress of the Network
2. Seek funding for Network activities.
3. Provide logistical support.
4. Resolve disagreements between network members



Source: Annual OIE/FAO FMD Reference Laboratory Network Report, 2007

Figure 1. Global distribution of FMD and FMDV pools in relation to reference laboratory network participants

Multiple origins of a FMD V Serotype Asia 1 epidemic



INTRODUCTION TO OIE/FAO FOOT AND MOUTH DISEASE REFERENCE LABORATORIES NETWORK

The Network is a formal collaboration of designated OIE and FAO reference laboratories and collaborating centres that work on foot-and-mouth disease (FMD). The Network has been established with two principal goals, namely:

- To improve surveillance of the global distribution and emergence of FMD virus strains and on the appropriateness of different vaccine strains for use or for retention in banks in different regions; and,
- To provide expertise and advice to OIE, FAO and other international disease control agencies as well as assistance to national FMD Reference laboratories.

ABOUT THE NETWORK

The Foot-and-Mouth Disease Reference Laboratories Network is a network of OIE and FAO reference laboratories for Foot-and-Mouth Disease (FMD) that has been established under the secretariat of the OIE Reference Laboratory and FAO World Reference Laboratory for FMD, IAH-Pirbright, UK (WRLFMD).

The Network has members from 10 different participating laboratories/countries (OIE/FAO Reference Laboratories/Centres) and are as follows:

1. OIE Reference Laboratory and FAO World Reference Laboratory for FMD, IAH-Pirbright, UK
2. OIE FMD Reference Laboratory for the Sub-Saharan continent, Gabarone, Botswana
3. FAO/OIE Regional Reference Laboratory for FMD, FGI-ARRIAH, Vladimir, Russia
4. FAO/OIE Reference Laboratory for FMD, Centro Panamericano de Fiebre Aftosa OPS/OMS, Rio de Janeiro, Brasil
5. OIE Reference Laboratory for Foot and Mouth Disease, Laboratorio de Fiebre Aftosa de la Dirección de Laboratorios y Control Técnico, Argentina
6. FAO FMD Reference Centre, Exotic Animal Health, ARC-Onderstepoort Veterinary Institute, South Africa
7. FAO FMD Reference Laboratory, Foreign Animal Disease Diagnostic Lab, Plum Island Animal Disease Center, Greenport, USA
8. OIE FMD Reference Laboratory, Pakchong, Thailand
9. FAO Reference Centre for FMD, Indian Council of Agricultural Research, Mukteswar, Nainital (Uttarakhand), India
10. OIE Collaborating Centre for Validation, Quality Assessment and Quality Control of Diagnostic Assays and Vaccine Testing for Vesicular Diseases in Europe, CODA-CERVA-VAR, Belgium

International Observers

1. National FMD Laboratory, Lanzhou Veterinary Laboratory, Gansu, China
2. Friedrich Loeffler Institute, Riems, Germany
3. Embakasi, Kenya

The Network provides an awareness of the global FMD situation through its joint surveillance activities, it gathers and shares antigenic and genetic characteristics of FMD virus in current circulation, harmonizes laboratory procedures that provide such information and provides control options through laboratory based data on vaccine matching.

Facilitation of the exchange of epidemiological data and informatics are through the provision of a web-based Reference Laboratories Information System (ReLaIS). ReLaIS provides public and private web-interfaces that contain up-to-date information on FMD. Network documents can be found in the Network Members area. Global surveillance information is visualised on a geographical interface and list (see FMD Database access). From here a cascade

of laboratory information including virus isolation, serotyping, antigenic/vaccine matching and molecular phylogenies can be accessed for each sample. Tools on ReLaIS also enable registered users to input molecular sequences from their own laboratories (in a private secure area) and manipulate the data to create specific phylogenies.

The members of the Global FAO/OIE Network of FMD Reference Laboratories meet every year to update Global and Regional FMD situation. The Global FMD Network Meeting, 2009 is being hosted by ICAR in New Delhi. The expected outcomes of the meeting are:

- Update on global and regional FMD situation during 2009
- Regional FMD vaccine strain recommendations
- Conclusions on inter-laboratory vaccine matching studies conducted in 2009
- Plans for future inter-laboratory vaccine matching studies
- Update and draft report on regional quality assurance undertaken in support of FMD laboratory testing
- Agreement on timeframes and actions to develop Annual Network report for 2009
- Workplan for Network during 2010

FMD RESEARCH IN INDIA

Research on FMD by ICAR dates back to 1929. The Project Directorate on Foot and Mouth Disease (PDFMD), which is the premier Institute for FMD in the country under the ICAR, was established as an All India Coordinated Research Project (AICRP) for FMD in 1968. The Project Directorate has 23 regional centers and network units covering all the major regions of the country with the Central FMD laboratory at Mukteswar. An International Center for FMD with BSL3+ facility to cater to the SAARC Region is being established shortly. The PDFMD has developed scientific expertise in conventional as well as in cutting edge areas, second to none in the world and the leader in South Asia, in the field of FMD diagnosis, epidemiology and research. The primary mandate of the institute is to carry out research on the epidemiology of FMD out breaks in the country. It is also entrusted with the duty of providing technical support to the FMD control programme currently undergoing in the country.

The PD FMD has been carrying out major investigations on the nature of the causative virus that exists in diverse serological/antigenic and genetic forms and cause FMD in each and every nook and corner of the country.

The major contributions of the institute are:

- Making available the epidemiologic data about the disease for use in its control.
- Development of indigenous kits and reagents for FMD diagnosis and research.
- Development of FMD vaccine production technology and diagnostics.
- Adoption of globally tested techniques and methods for FMD research in India.
- Extending disease monitoring and sero-surveillance services.
- Developing a skilled work force and a network for effective maintenance of alert- report-monitor chain.
- Making available the best strains for vaccine production and its constant monitoring for effective coverage of emerging field viruses.

MANDATE OF THE PROJECT DIRECTORATE ON FOOT AND MOUTH DISEASE

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.

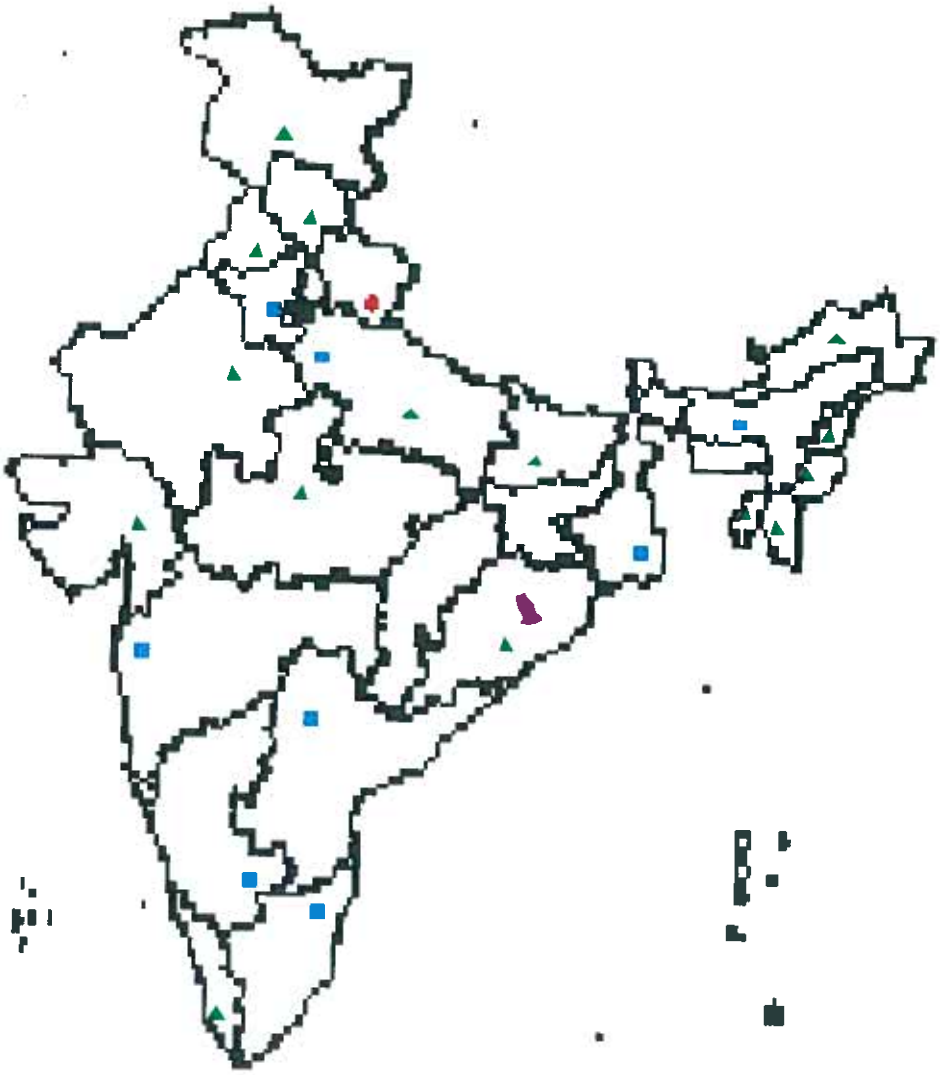
ORGANIZATIONAL SET-UP

Bangalore
Guwahati
Hisar
Hyderabad
Kolkata
Mathura
Pune
Ranipet

Agartala
Ahmedabad
Aizwal
Bhopal
Cuttack
Imphal
Itanagar
Jaipur
Jalandhar
Jammu
Kohima
Lucknow
Patna
Shimla
Thiruvananthapuram

Project Directorate on FMD

- Regional centers (8)
- Central Laboratory Mukteswar
- International Center Bhubneswar
- Network units (15)



OBJECTIVES OF THE INSTITUTE

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 FMDV.
3. Production, standardization and supply of diagnostic reagents for FMD virus serotyping and post-vaccinal sero-conversion.
4. Development of newer diagnostic techniques using cutting-edge technologies in molecular biology and Hybridoma.
5. 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.
6. 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 FMDV.
7. Production, standardization and supply of diagnostic reagents for FMD virus serotyping and post-vaccinal sero-conversion.
8. Development of newer diagnostic techniques using cutting-edge technologies in molecular biology and Hybridoma.
9. Analysis of economic impact of FMD on livestock industries
10. To act as referral laboratory for FMD in South Asia.

OBJECTIVE OF THE INTERNATIONAL CENTER FOR FMD FOR SAARC/SOUTH ASIA UNDER THE INSTITUTE

1. Training of the personnel from the countries in the SAARC / South Asia region for FMD diagnosis and epidemiology.
2. Supply of quality reagents and diagnostic kits to the member countries for use in diagnosis and maintain uniformity.
3. Providing consultancy for establishment of FMD laboratories in the member countries and plan FMD control programme for South Asia.
4. Maintenance of Regional FMD virus repository for retrospect use.
5. Regional vaccine matching exercise for development of unified FMD vaccine for the South Asian region.
6. To function as Central FMD Vaccine Quality Assurance Agency.

RESEARCH PROGRAMMES AND ACTIVITIES OF THE INSTITUTE DURING 2007-2012

The research programmes are intended to be demand driven. The major research thrust areas identified emphasizes the recent issues and need. Continuous active and passive surveillance and monitoring has yielded valuable epidemiological data for planning and executing disease control strategies. Thrust is also on making newer diagnostics for use in the country for surveillance and monitoring of FMD. Various research programmes and activities of the institute scheduled during 2007-2012 are as follows:

Program I: Surveillance, Epidemiology and molecular epidemiology of FMD

Activities

- Monitoring & Surveillance of FMD through active diagnosis:
- Application of newer tools for molecular epidemiology along with full length genome sequence to assess genetic make up of outbreak strains.
- Application of GIS mapping and occurrence of FMD outbreaks.

Program II: Molecular/ genetic typing of Virus strains and making gene constructs

Activities

- Development and application of nucleic acid hybridization based techniques for rapid and precise detection of mutants/ variants of virus.
- Development and application of newer genome based techniques for simultaneous FMDV detection and strain differentiation.
- Production and evaluation of recombinant structural and non-structural proteins of virus serotypes for possible use as antigen in serological tests.
- Production of DNA clones for raising serotype specific antibodies for use in diagnostics.

Program III: Refinement and Development of quality diagnostics

Activities

- Development of "Lab-on-site" test using principles of Chromatographic Immunoassay for pen-side detection of FMDV serotype.
- Development of DIVA assay targeting non-structural FMDV proteins for differentiation of infected from vaccinated animals.

Program IV: Establishment of Containment Laboratory

Activities

- Establishment of International (South Asian) Center for FMD.

Program V: Economic impact analyses of Diseases

Activities

- Development of modules for economic impact analysis and estimation of economic loss due to FMD in collaboration with NCAP. A research programme on the subject has already been taken up in collaboration with NCAP, New Delhi.

ACHIEVEMENTS OF PROJECT DIRECTORATE ON FMD DURING 2007-2009

- FMD outbreaks data in regular basis have been compiled. 2022 outbreaks during the period 2007-2009 were investigated, diagnosed and the causative virus strains were characterized at the level of genome and antigenicity and the stakeholders were advised accordingly.
- Regular vaccine matching exercise has resulted in the identification of a new vaccine candidate for FMD virus serotype A for incorporation in the trivalent vaccine to take care of the antigenic divergence that has recently arisen.
- Country wide survey/sero-monitoring has been initiated for the first time in the country to estimate the prevalence of FMD. This has given meaningful information on impact of regular vaccination in restricting circulation of virus in susceptible livestock population. Prevalence of the disease varied from 5 to 46% in different states.
- The complete genome sequence (~8.2 Kb) of more than 40 isolates of serotypes O, A and Asia1 was generated to understand the molecular evolution of FMD virus in the country. A substantial amount of data raised on full length genomes added value to the FMD virus genomics/characterization.
- A non-isotopic RNA dot hybridization assay with colorimetric detection, targeting both the internal ribosome entry site (IRES) and the 3D genomic region has been developed and validated as a diagnostic.
- A multiplex PCR (mPCR) protocol for serotyping of FMD clinical samples was developed. The test was found to be sensitive and reliable for differentiating Indian FMDV serotypes. This is an important molecular diagnostic.
- A simple, fast, relatively cost-effective multi-

primer RT-PCR assay to differentiate genetic lineages of different serotypes of FMD virus prevalent in India was developed. This specialized PCR is a tool that gives a fast preliminary indication on the genetic lineage before proceeding with thorough nucleotide sequencing.

- ART-PCR (oligo-probing) ELISA in both solid and aqueous phase hybridization formats targeting an across serotype conserved site was developed. The assay proved to be successful in detecting viral genomes in samples undetectable in conventional ELISA, thereby demonstrating 'proof of sensitivity' for such assays. The test has the potential to be adapted for comprehensive surveillance of foot-and-mouth disease in India.
- Prokaryotic expressed recombinant 3AB3 NSP based indirect ELISA for DIVA is designed, developed and evaluated using in-house produced and standardized reagents at PDFMD. Differentiation of infection from vaccination based on detection of the antibodies to NSPs, in particular 3ABC or 3AB has been established to be a single reliable indicator of FMD virus infection or exposure. The usefulness of this diagnostic test is more in context of herd based surveillance rather than for judging individual status. This kit has been made available to all users.
- Development of diagnostic kits has made the country self sufficient in FMD surveillance and monitoring. The diagnostic kits has made available to the reach of AICRP on FMD. FMD virus typing ELISA kit for testing 7000 clinical materials was supplied to FMD centers/ network units. Seromonitoring kit for testing 80,000 serum samples by LPB-ELISA were produced and supplied to centers/ network units and other government agencies and industry. DIVA Kit to test a total number of 50,000 serum samples was produced and reagents for a total of 30,000 samples have been supplied so far to the AICRP network and vaccine manufacturing companies for testing random serum samples for estimation of FMD prevalence. Production and supply of these diagnostic kits ensured uniformity in application and test result across the country.
- Regular training and refresher courses for the scientific staff of Regional Centers and Network units and industry were conducted on use/application of virus typing ELISA, LPB-ELISA and DIVA ELISA.
- Collaborative programme on economics of FMD has already been taken up with National Centre for Agricultural Economics and Policy Research (NCAP).
- Post-vaccinal sero-monitoring after each round/phase of vaccination under the FMD control programme being run by the Department of Animal husbandry, Dairying and Fisheries, GOI in selected 54 districts of the country has been completed up to sixth phase by this institute. Gradual increase in protective antibody response was observed. The results accrued have revealed successful beginning of the control programme and need for continuance of vaccination till zonal freedom is achieved.
- Linkage is established with DAHD&F for control of FMD in India and creation of disease free zones. Technical and logistic support is provided in the form of diagnostic kits for testing and analyzing pre and post vaccinate serum samples after each round of vaccination using indigenously developed LPB ELISA, thereby, assessing the impact of vaccination.
- Linkage with Indian FMD vaccine Industry has already been initiated for FMD research and development with satisfactory outcome.
- Linkage is also established with the Animal Husbandry Departments of different state governments for surveillance and monitoring.

GENETIC TRANSITIONS OF INDIAN SEROTYPE O FOOT AND MOUTH DISEASE VIRUS ISOLATES RESPONSIBLE FOR FIELD OUTBREAKS DURING 2005-2009: A BRIEF NOTE

Tamilselvan RP, Ankan De, Sanyal A and Pattnaik B

PD on FMD, Mukteswar

Summary

Scenario of serotype O epidemiology in the country indicates complex situation with predominance of PanAsia II lineage during 2003-2008 and dominance of 'Ind2001' during 2009 especially in Northern India. With a wide host range (both domestic and wild) and large number of susceptible animals, Serotype O virus is continuing to evolve as heterogenous genetic group and co-circulate in the population. In spite of genetic diversity observed among the circulating outbreak strains, the in-use vaccine strain continues to offer adequate antigenic coverage over them.

Foot and Mouth Disease (FMD) is endemic in India and serotype O of Foot and Mouth Disease virus (FMDV) is responsible for nearly 75-80% of field outbreaks reported. The advent of nucleotide sequencing has changed the way in which infectious disease was dealt in road towards their control and eradication and FMD is not an exception to this rule.

In India, systematic study on molecular epidemiological scenario of FMD by nucleotide sequencing of major immunogenic protein (VP1) coding genomic region (1D) of FMD virus (FMDV) was initiated way back in 1996. With the observation that the Indian isolates are different from serotype O viruses circulating in other parts of the world and were genetically heterogeneous. Subsequently, global analysis of FMD virus serotype O isolates shown viruses circulating in India are grouped with Middle East and other South Asian Nations which constituted single genotype.

In the light of increasing number of outbreaks due to serotype O in India during 2000-2001 and FMD epidemics in Europe in early 2001 prompted a comprehensive retrospective analysis of type O viruses circulated in India during the 1987-2001. The isolates of this period grouped themselves in three branches (Branch A, B and C), the branch C further subdivided into 4 groups (C-I, C-II, C-III and C-IV). The Indian origin PanAsia viruses (Branch C-IV) were clustered separately from the rest of the isolates in Branch C and another new comer (Branch C-III) was identified (New Strain or Ind2001). During the period 2000-2005 four genetically different clusters of antigenically homogenous population of serotype O viruses co-circulated in India. From 2003, dominance of PanAsia II viruses was observed. We further investigated the genetic pathway of FMDV serotype O isolates in each year from 2005-2009 as a part of regular molecular epidemiological surveillance.

Trend in involvement of serotype O as a causative agent of FMD outbreaks from 2005-2009

Period (April-March)	No of samples tested	Type O virus Identified by Sandwich ELICA	Percentage of Type O total outbreaks to total outbreaks
2005-2006	2319	1238	83.0
2006-2007	1685	748	62.0
2007-2008	2258	1042	82.1
2008-2009	640	334	83.3

Source: Annual Reports 2005-2009, PDFMD

Year 2005-2006

The serotype O viruses responsible for field outbreaks collected from 15 states (Arunachal Pradesh, Andhra Pradesh, Assam, Bihar, Gujarat, Himachal Pradesh, Jammu and Kashmir, Karnataka, Kerala, Manipur, Tamil Nadu, Uttar Pradesh and West Bengal) were analyzed with field isolates recovered during 2004 also. The viruses were grouped in 2 predominant lineages/clusters, namely PanAsia II and PanAsia I. Of 49 serotype O viruses sequenced at 1D region, 23 of them belonged to PanAsia II lineage and PanAsia I was next predominant lineage responsible for field outbreaks during this period. During early 2005, two isolates (IND 60/05 and IND 61/05) recovered from wild Gaur (Tekkadi wild life sanctuary, Kerala) were also nucleotide sequenced and were found to belong to PanAsia II strain Indicating wide host range of these viruses similar to that of their parental lineage (PanAsia I). There was limited circulation of Ind2001-like viruses.

Year 2006-2007

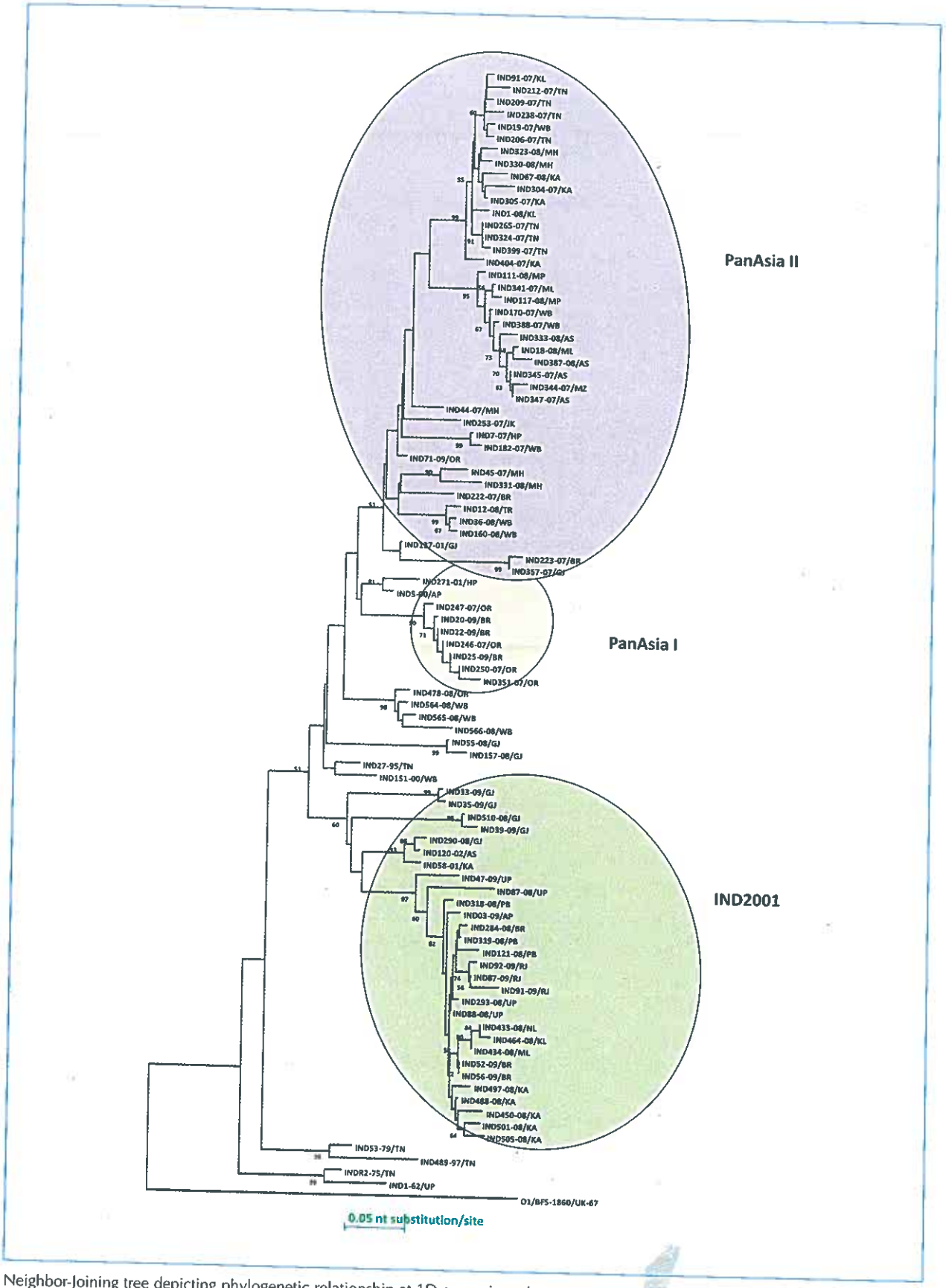
In 2006-07, phylogenetic analysis of twenty five serotype O isolates revealed similar molecular epidemiological situation that of the 2005-2006. PanAsia II strains dominated the scenario followed by its parent strain (PanAsia I). The Ind2001 strain could not be detected during this period, indicating a further decline in its circulation. During July, 2007, PanAsia II viruses caused outbreak in a Zoological Park with mortality in Mithun and Wild boar. Comparison of type O isolates from India with the isolates of other countries available in the public domain sequence database indicated the presence of PanAsia II strain in our neighbouring countries viz; Bhutan, Nepal and also in Malaysia.

Year 2007-2008

In 2007-08, phylogenetic analysis of forty-nine serotype O isolates from 13 states revealed similar epidemiological situation that of the previous years, where PanAsia II strains dominated followed by its parent strain (PanAsia I). There were wide spread outbreaks due to PanAsia II lineage was noticed in the southern states of Tamil Nadu, Karnataka and Kerala. In 2007 outbreaks in Bannerghata Zoological Park in Karnataka and Thrisoor Zoological Park in Kerala, due to PanAsia II virus with mortality in Mithun and Wild Gaur was observed confirming their wider host range. Reappearance of 'Ind2001' strain was noticed in Northern India indicating its presence in the population.

Year 2008-2009

Phylogenetic analysis of forty Serotype O isolates drawn from West Bengal, Karnataka, Kerala, Bihar, Maharashtra, Gujarat, Assam, Meghalaya, Uttar Pradesh, Punjab, Nagaland, Andhra Pradesh, Rajasthan and Orissa revealed presence of many sub-lineages, thus indicating the complex epidemiological situation and genetic diversity of the strains/isolates. In this period, 'Ind2001' strain dominated in major parts of Northern India and in some of the Southern states (Andhra Pradesh, Karnataka and Kerala). PanAsia II strain, which dominated serotype O outbreaks during 2007-08 was restricted to only few states (Orissa, Assam and Maharashtra), possibly due to infection acquired immunity and extensive vaccination. The parental PanAsia strain (PanAsia I) is still detectable in Bihar, West Bengal and Orissa. More than one genetic group was detected in some of the states of Orissa (both PanAsia I and II) and Bihar (PanAsia I and Ind2001). Interestingly, two isolates of 'Ind2001' strain recovered from the state of Gujarat were found to lack an amino acid at VP1 ?G-?H loop region, indicating genetic plurality in 'Ind2001' lineage. Nevertheless, it is heartening to note that the current vaccine strain (IND R2/75) still offers protection against these deletants, as evident from 2D-MNT results.



Neighbor-Joining tree depicting phylogenetic relationship at 1D genomic region among Serotype O isolates collected during 2007-09

MOLECULAR EPIDEMIOLOGY OF SEROTYPE A FMD VIRUS IN INDIA

J. K. Mohapatra, S. Pawar and P. Rameshkumar
PD on FMD, Mukteswar

Among all serotypes prevalent in India, type A virus population is found to be genetically and antigenically most heterogeneous in nature. VP1 coding (1D) region based molecular phylogeny has established circulation of four genotypes {showing more than 15% nucleotide (nt) divergence among them at 1D region} of type A so far in India. A phenomenon of genotype/ lineage turnover where periodically newer genotypes surface and older genotypes disappear has been observed for type A. Since 2001, genotype VII has been exclusively responsible for all the field outbreaks and has outcompeted all other genotypes. Within the currently circulating genotype VII, a divergent and unique lineage emerged in late part of 2002, which showed an amino acid (aa) deletion at 59th position of VP3 (VIIb-VP359 deletion group) and dominated the field outbreak scenario in 2002-03. Ever since then sporadic outbreaks due to this lineage has been documented. Recently during 2007, there is once again an upsurge in incidence of outbreaks due to this lineage. This single aa deletion is at an antigenically critical position in structural protein VP3, which is considered to be a major evolutionary jump probably due to immune selection. After its resurgence in 2007, VP359 deletion group continues to dominate the field outbreak scenario even in 2008 and 2009. 1D region based phylogeny has also revealed that this deletion group is genetically diverging with time giving rise to three lineages (VIIb, VIIf & VIIg). For type A genotypes, poor antigenic cross-relatedness has been observed between the genotypes. To worsen the situation, this deletion group was found antigenically quite heterogeneous in the sense some isolates exhibit closer antigenic relationship with genotype VI candidate vaccine strains where as others show closer relationship with genotype VII candidate strains. Nonstructural protein coding region (L, 3A and 3C) analysis has revealed the genetic distinctness of this deletion group, where they cluster away from rest of the genotype VII isolates.

MOLECULAR EPIDEMIOLOGY OF SEROTYPE ASIA 1 FMD VIRUS IN INDIA

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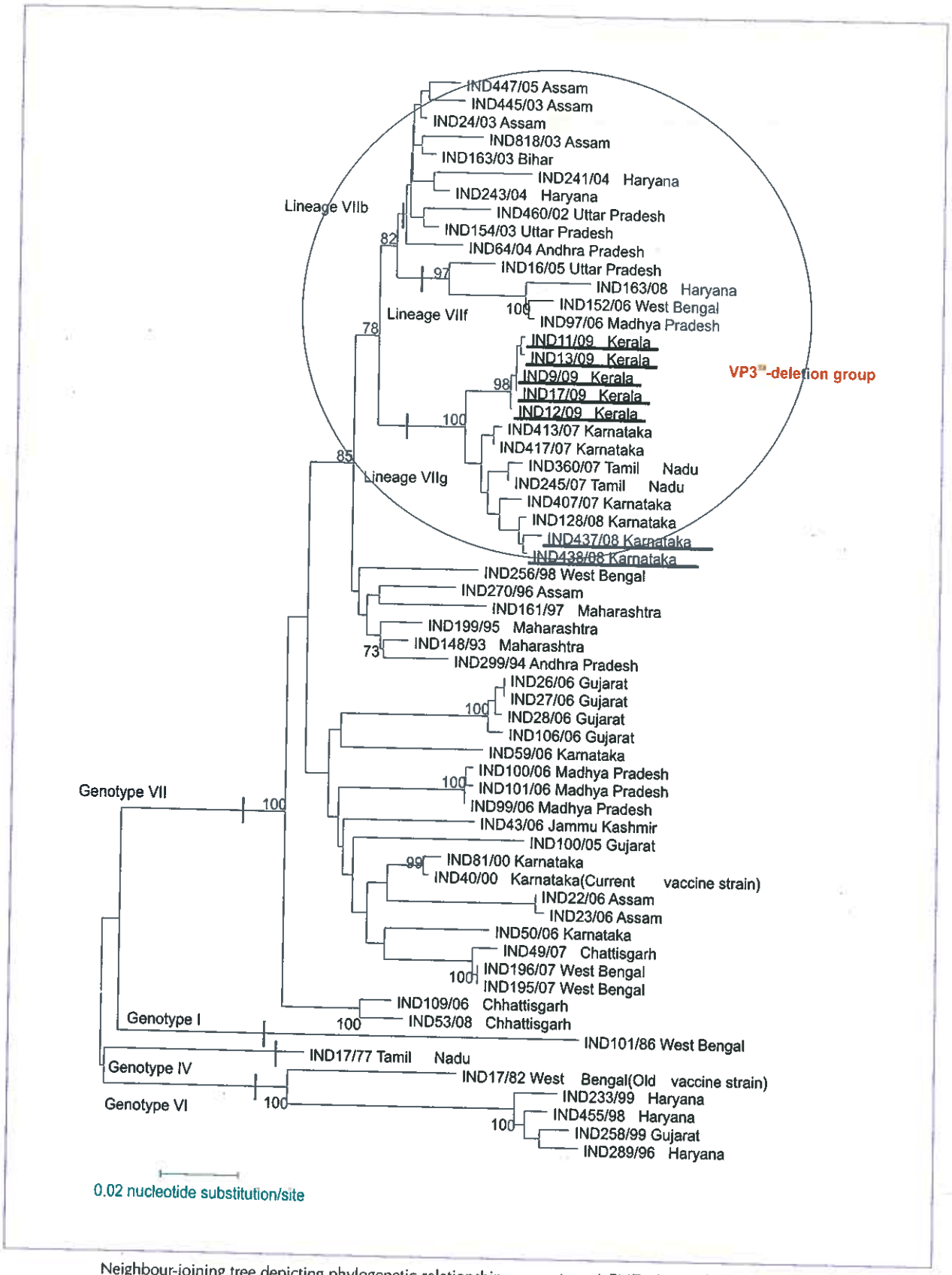
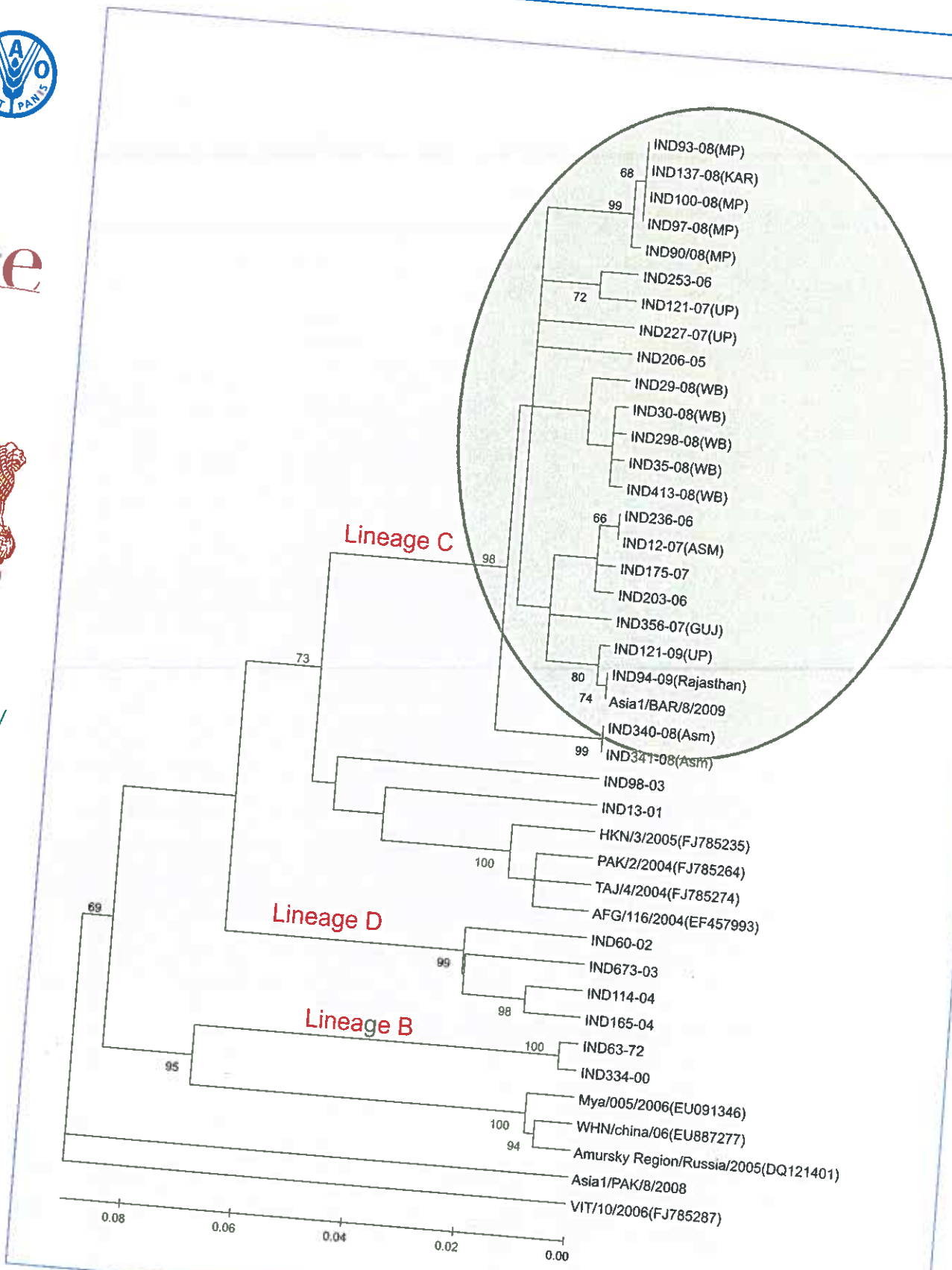
The Indian FMDV Asia 1 field isolates collected during 1985 to 2002 was clustered in a single genotype with two different genetic lineages (Lineages B and C). The lineage B which included the vaccine strain, IND 63/72 had 210 amino acid in VP1 and this lineage never appeared after the year 2000. The lineage C which circulated prominently during the period 1993 to 2001 has an extra amino acid at position 44 of VP1. A novel divergent genetic lineage (lineage D) with-in 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. The isolates of lineage C reemerged again in 2005 following the exclusive dominance of lineage D in the period between 2002 and 2004 and in recent times Lineage C is behind all the FMDV Asia1 outbreaks in the country. The isolates of lineage C and D had amino acid substitutions at twenty two and nineteen different positions respectively, from in-use vaccine strain. Irrespective of their genetic divergence, antigenic characteristics of FMDV Asia1 field isolates is stable and the in-use vaccine strain (IND 63/72) provide sufficient antigenic coverage to the circulating FMDV Asia1 field isolates.



Neighbour-joining tree depicting phylogenetic relationship among type A FMD virus isolates at 1D region. Isolates sequenced during the reported year are underlined



Neighbour-joining tree depicting phylogenetic relationship among type Asia1 FMD virus isolates at 1D region

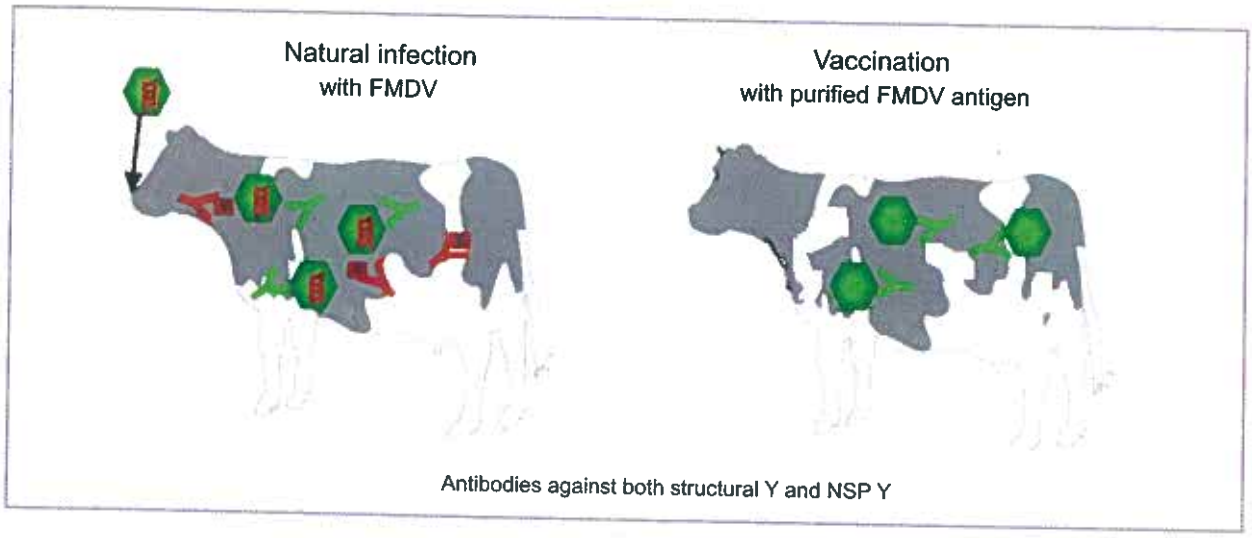
RECOMBINANT 3AB3 NSP-ELISA: A HANDY TECHNIQUE FOR DIFFERENTIATION OF FOOT-AND-MOUTH DISEASE VIRUS INFECTED FROM VACCINATED ANIMALS (DIVA)

J. K. Mohapatra and L.K. Pandey
PD on FMD, Mukteswar

Background concept

Foot-and-Mouth Disease (FMD) is a highly contagious O.I.E. list A disease of farm livestock with serious economic implications. FMD continues to be a challenge as far as international trade of livestock products is concerned leading to colossal loss to the country's economy. India poses a complex epidemiological scenario for FMD owing to a large population of susceptible domestic livestock viz. cattle, buffalo, sheep, goat, yak, pig, mithun and camel (~ 483 million), varied geo-eco-meteorological environment, cocirculation of multiple serotypes and sharing of vast porous international border with FMD endemic countries. In such a scenario, the priority is to contain and bring down disease incidence rate as the first stepping stone in realizing the ultimate vision of an FMD free India. To achieve this goal, control measures through intensive vaccination have been adopted in the country since 2003 as an ambitious project to create disease free zones. Formulation and effective implementation of control policies warrants a constant surveillance of the circulating field viruses and study of disease prevalence.

Through different schemes launched by Government of India an ever increasing number of districts are gradually covered under systematic vaccination campaign against FMD. Serological tests like liquid phase blocking ELISA (LPBE) detect antibodies to the structural capsid proteins of the virus and are effective tools in postvaccinal seromonitoring. Antibodies to the capsid proteins are induced by both vaccination and infection. It is not possible, therefore, to differentiate animals that have been infected from those that have been vaccinated but not infected based on the detection of antibodies to the structural proteins alone. Differentiation of these two categories of animals is important during serological surveillance to detect evidence of infection and circulation of virus, as a follow up to intensive vaccination campaign, for import/export serology and for assessing success of various control policies. The



currently used FMD vaccines consist of purified preparations of inactivated virions and therefore induce antibodies almost exclusively to the structural proteins of the virus, provided the vaccine batch is significantly free from non structural proteins (NSPs). But viral replication during infection results in the production of an array of NSPs, of which some are immunogenic.

FMD DIVA strategy based on anti-NSP seroconversion

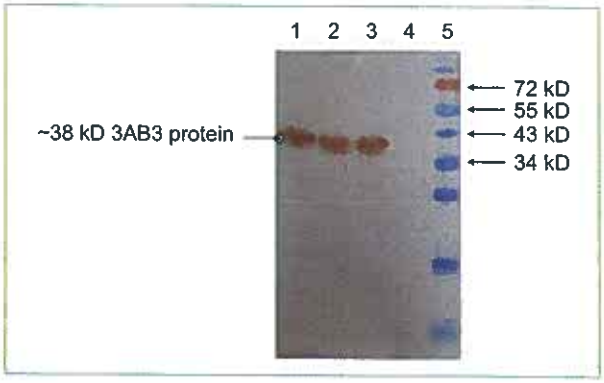
Differentiation of infection from vaccination based on the detection of the antibodies to NSPs, in particular 3ABC or 3AB has been established to be a single reliable indicator of FMD virus infection or exposure as per the OIE guidelines. Besides, this NSP based antibody screening can also be useful for detection of subclinical infection particularly in caprine and ovine species and for retrospective diagnosis in case of delayed reporting of outbreaks or sample deterioration collected from active phase of outbreaks. These properties make detection systems based on antibodies to nonstructural proteins ideal for large scale sero-surveillance. The usefulness of this diagnostic test is more in the context of herd based surveillance rather than for judging individual animal infection status as OIE recommends Electroimmuno transfer blot (EITB) assay using a panel of NSPs as a confirmatory test for the latter purpose.

Hence a sensitive, accurate and user-friendly test amenable to mass scale sero-surveillance which can differentiate infected from vaccinated animals after the launch of FMD control programme was quite imperative. Though such test has limited application in the presence context when only 55 districts are under routine vaccination, in future it can be exploited to its full potential when the entire country would be covered with regular vaccination bringing down the disease incidence significantly. As the country so far was not having any indigenous test kit for DIVA, development of an infection-vaccination discriminatory assay was the need of the hour for making the country self sufficient and prepared to undertake surveillance for vaccinated areas and trade as per the World Animal Health body, OIE's norms.

Taking all the above mentioned lacunae and requirements into account, a recombinant nonstructural protein (3AB3) based indirect ELISA for differentiation of FMD virus infected from vaccinated animals was developed at Central FMD Laboratory, Project Directorate on FMD, Mukteswar (ICAR) with top priority.

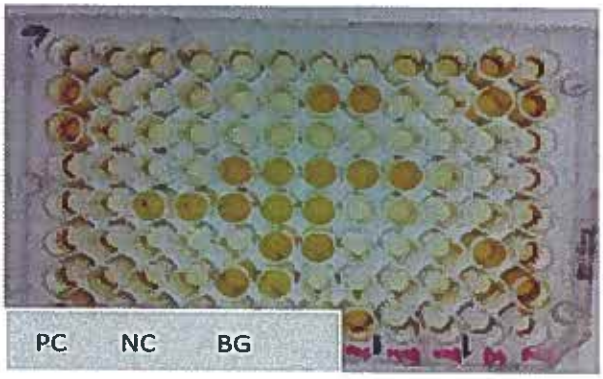
Development and performance of r3AB3 DIVA-FMD Kit

The 3AB3 protein of FMD virus serotype Asia 1 was expressed in prokaryotic system as a ~38kD fusion protein and was subsequently purified by metal affinity chromatography to be used in the final indirect ELISA format of DIVA Kit.



Western blot analysis of expressed 3AB3 fusion protein with anti-His HRP conjugate

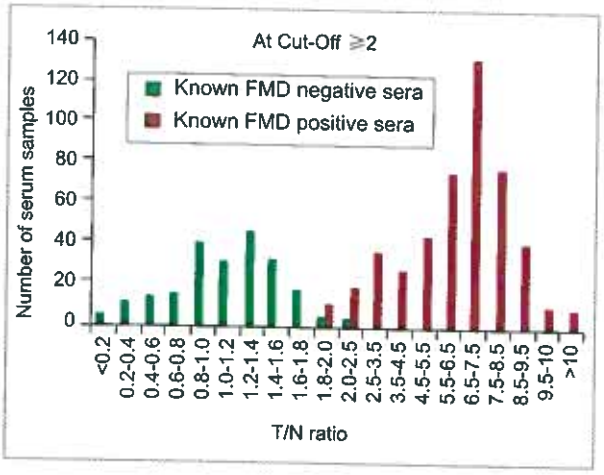
The optimised indirect ELISA assay was



found to work in a serotype independent manner and was convenient to perform as 45 bovine serum samples in duplicate can be accommodated on a single 96-well immunoassay plate.

DIVA ELISA plate reflecting positive results for brownish yellow colour development

The assay takes only 2 hours to perform on over night coated plates. To minimize inter-run variability, results are interpreted in terms of ratio of test serum OD and supplied negative control serum OD values (T/N ratio more than 2 as cutoff). The cutoff was decided from the frequency distribution of known FMD positive and negative serum sample absorbance values.



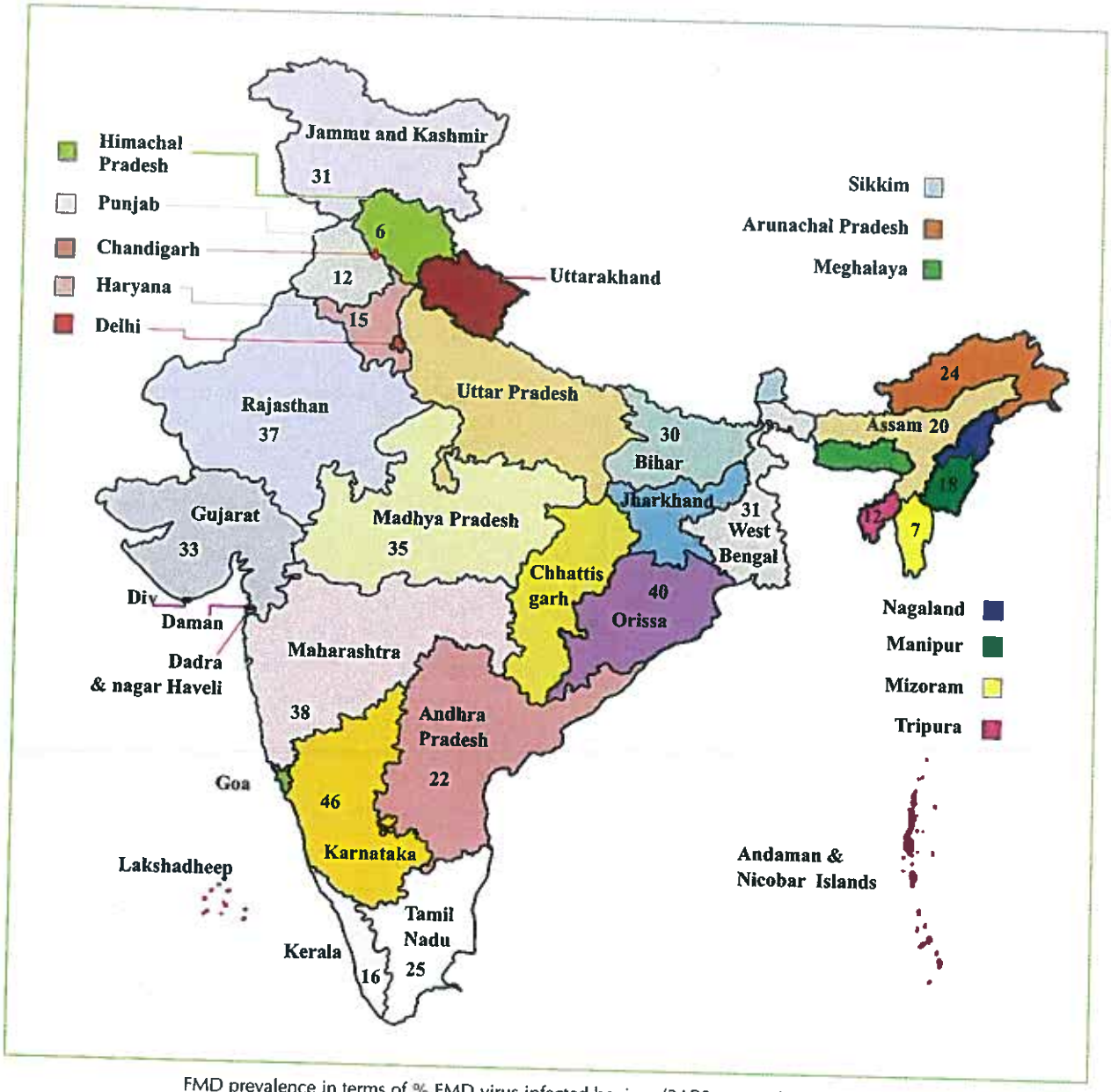
Frequency distribution of normalized test to negative control OD (T/N) ratio of serum samples from animals " with known FMD status

Besides, the diagnostic sensitivity (DSn) and specificity (DSp) was estimated at 95% and 98%, respectively. Although in many bovines seroconversion against 3AB3 protein was prominent 10-14 days postinfection, one month postinfection samples were found to be ideal for subjecting to this assay for retrospective diagnosis as majority of infected samples showed seroconversion at that time. Anti-3AB3 antibodies in infected animals could be detected in this kit upto 2 years postinfection. The experimentally vaccinated animals (with two different commercial vaccines available in India) revealed no detectable anti-NSP antibody titre even after 5 boosters at six monthly interval simulating field vaccination regimen, making this test suitable as a discriminatory assay even in case of multiple vaccinations. Even in animals under a regimen of six vaccinations at 15 days interval, no seroconversion against 3AB3 protein was observed. This test was taken up either by different people in the same laboratory or in 15 different laboratories across the country and the intra-run and inter-run variation was accounted to be 5% and 18%, respectively. The level of discordance between the in-house developed Kit and a commercial kit (Svanovir 3ABC Kit from Sweden) was observed to be at 6-8% and maximum of this was seen on very early samples collected within 2-3 weeks of virus exposure. This indigenously developed r3AB3 DIVA-FMD kit is first of its kind in the country and has been designed as per the OIE approved guidelines and is at least four-fold cheaper at Rs. 25,000 per 450 tests than the commercial DIVA kits available on import. Now this kit is in extensive use in the country in different laboratories with consistency in its performance.

Application

In nutshell, substantial scientific, technological and socio-economic significance are attached with this newly developed r3AB3 DIVA-FMD Kit. In India this is the first validated test kit available to carry out differentiation of infected from uninfected vaccinated animals. Development of such a kit will make the country self sufficient and save a lot of foreign exchange avoiding import of commercial DIVA kits. This assay is rapid, user friendly and fit for large scale sero-surveillance.

A total number of 21,526 random bovine serum samples collected at the rate of 100 per district from 248 districts covering 20 different states of the country were tested in DIVA ELISA in an exercise to estimate FMD prevalence in the country. This revealed 31 % of the bovine population in the country to be FMD infected upon testing of serum samples collected during 2008-2009 at a confidence level of 95%.



FMD prevalence in terms of % FMD virus infected bovines (3AB3 reactors) upon testing of random serum samples collected during 2008-2009

State-wise FMD prevalence in bovines varied from 5% in Himachal Pradesh to 46% in Karnataka. Level of virus circulation in the states differed either due to geographical factors or due to positive effect of regular vaccination. The % infected bovines in Haryana is almost half of that of the country's average indicating the effectiveness of regular FMD vaccination in disease containment and suitability of this DIVA kit in assessing the impact of regular vaccination and detecting evidence of infection in multiply vaccinated animals. This kit will definitely find extensive applications in the FMD control programme being run by the Government of India for surveying FMD virus circulation in vaccinated zones, in turn aiding in ongoing epidemiological investigations. All these factors will lead to improvisation of animal productivity and stabilization of sustainability of animal enterprises leading to a better economy.

DIAGNOSTIC KITS DEVELOPED BY THE INSTITUTE AND AVAILABLE

Diagnosis and surveillance of FMD is a major animal health activity in the country. The institute has made the country self insufficient by developing several diagnostic kits (indigenously) for FMD that are being used though out the country and have saved valuable foreign exchange. The diagnostic kits in use and always available on demand are:

FMD Diagnostic Kit - 1

1. Name of the Kit : Type-FMD
2. Application / Use : Foot and mouth disease virus (FMDV) serotyping.
3. Description / features:

The kit is based on a standard sandwich ELISA technique to determine the presence of FMDV antigens in tissue samples. The kit contains reagents and ELISA plates sufficient for testing of minimum 500 FMD suspected clinical samples.

Sandwich ELISA Kit for FMD virus serotype identification

Sandwich ELISA Kit has been developed by PD FMD, Mukteswar for serotype identification of FMD virus. This Kit is being used countrywide for FMD virusserotyping.

FMD Diagnostic Kit - 2

1. Name of the Kit : LPBE-FMD
2. Application / Use : For measuring post vaccinal antibody response to FMD Virus and seromonitoring
3. Description / features

The test is aimed at the quantification of protective antibody level in the animals following vaccination (seromonitoring). The kit contains reagents and ELISA plates sufficient for testing of minimum 1000 serum samples.

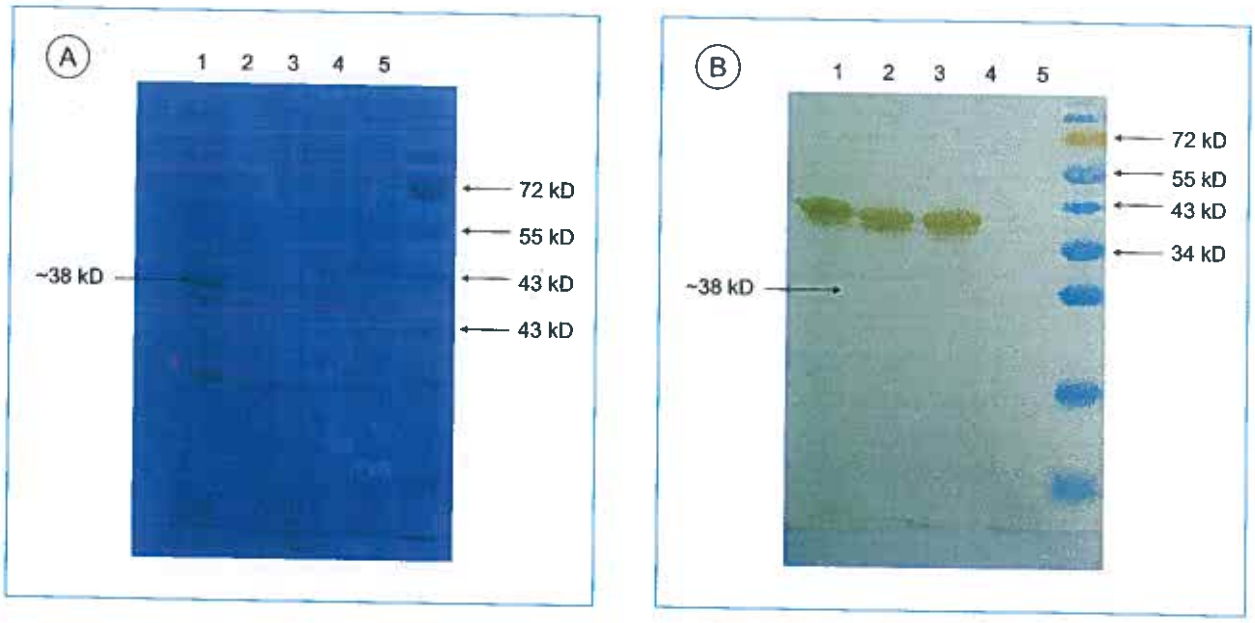
Liquid Phase Blocking ELISA Kit for FMD Seromonitoring

Antibodies against the structural proteins play an important role in protecting the animals against FMD. The LPBE Kit developed at PF FMD, Mukteswar is successfully used in seromonitoring to detect protective antibody level following vaccination. It has facilitated the FMD Control Programme in India.

FMD Diagnostic Kit - 3

1. Name of the kit : DIVA FMD indirect
2. Application / Use : For differentiating vaccinated animals from FMDV-infected animals
3. Description/Features

Indirect immunoassay for differentiating FMD infected from vaccinated animals. The kit contains recombinant antigen coated microtitre plates, Bovine and ovine anti-IgG HRPO conjugate, positive and negative control serum and test protocol.



Recombinant 3AB3 antigen for DIVA Kit

FMD Diagnostic Kit - 4

1. Name of the Kit : mPCR-FMD
2. Application / Use : Foot and mouth disease virus (FMDV) serotyping.
3. Description/Features

PCR based serotyping kit in the multiplex format. The Kit contains a set of serotype-specific oligonucleotide primers, positive and negative controls, PCR tubes, RNase free water and a manual. The Kit is available in two formats one with PCR reagents and the other without that.

FMD Diagnostic Kit - 5

1. Name of the Kit : Lineage-FMD
2. Application / Use : Differentiation of genetic lineages of FMD Virus serotypes A and Asia 1
3. Description/Features

PCR based genetic lineage differentiating kit. The Kit contains a set of lineage-specific oligonucleotide primers, positive and negative controls, PCR tubes, RNase free water and a manual. The Kit is available in two formats one with PCR reagents and the other without that.

Diagnostic Kits under development:

1. Real-time multiplex PCR.
2. Animal side test/Chromatographic Immunoassay

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