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Foot-and-mouth disease: Global status and Indian perspective

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ABSTRACT

Foot-and-mouth disease (FMD) is a highly contagious and transboundary viral disease of domesticated and wild cloven-hoofed animals. Wide prevalence of the disease in Asia and Africa associated with huge economic loss to the livestock farming and industry has increased the concern worldwide. The disease is a major threat to cattle, buffalo (both milk and meat) and pig production in endemic countries and therefore considered to cause food insecurity, both locally and globally. Currently, 6 serotypes of FMD virus (O, A, Asia-1, SAT-1,-2, and -3) are circulating globally, and serotype C has not been recorded since 1995. In India, the disease is caused by serotypes O, A and Asia-1, of which serotype O is responsible for most of the outbreaks. Emergence and re-emergence of FMD virus genotypes/lineages has been detected in serotypes. Serotype A viruses have been continuously emerging in the nature necessitating frequent replacement of the vaccine strains. The knowledge generated in epidemiology, diagnosis and surveillance of the disease in the country has been instrumental in formulation and implementation of FMD Control Programme through regular 6 monthly vaccination with the aim to create disease free zones in India. The control programme, in operation since X Plan, has resulted in progressive and substantial reduction in occurrence of the disease and DIVA reactors/converters in vaccinated areas. The present review summarizes the disease, the causative agent, and epidemiology of FMD in India and the world.

Key words: DIVA reactors, FMD control programme, FMD surveillance, FMDV serotypes, Global transmission

Foot-and-mouth disease (FMD), a clinically acute, contagious viral disease of domesticated ruminants, pigs, camelids and more than 70 wildlife species including elephant, is of transboundary nature posing threat to global food security, and causes severe economic loss to livestock farmers and industry (Fenner 1993). The causative agent, FMD virus (FMDV) is a member of the genus *Aphthovirus,* in the family *Picornaviridae*. The virus exists in 7 immunologically distinct serotypes: A, O, C, Southern African territories (SAT)-1, 2, 3 and Asia-1, and within each serotype there are a substantial number of strains showing variable degree of genetic and antigenic diversities. Clinically, the disease is characterised by fever, lameness and vesicular lesions on the mouth, tongue, feet, snout and teats of infected animals (Alexandersen *et al.* 2003b). Unvaccinated FMDV-infected cattle and pigs usually develop obvious signs of the disease but in sheep and goats, diagnosis is more difficult because the clinical manifestation of the disease is often mild (Callens *et al.* 1998, Barnett and Cox 1999*,* and Viuff *et al.* 2002). High mortality is sometimes observed among young animals and is often attributed to affect the myocardium of heart (Barker *et al.* 1993). Though the disease causes low mortality in adult animals, morbidity is very high, causing weight loss, decrease in milk production and loss of draught power resulting in huge economic loss. An asymptomatic FMDV persistent infection (carrier state) can be established in ruminants beyond 28 days post-infection to several years, irrespective of vaccination status (Salt 1993) and such animals act as nucleus of infection in herd(s). Due

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to the difficulties in effectively controlling the disease, extreme contagiousness with wide host range, and its economic impact; FMD ranks first among 'Risk Group 4' animal pathogen.

FMDV genome organisation: The positive sense viral RNA consists of a single open reading frame (ORF), flanked by 2 highly structured un-translated regions (UTRs) at the 5' and 3' ends of the genome (Belsham 1993, Lin and Flint 2000). A small viral protein VPg is covalently linked to 5' end of the RNA molecule (Grubman 1980, Sangar *et al.* 1977). 5' UTR at its most 5' end contains a highly structured 'S' fragment of about 360 nucleotides residues and is predicted to form hairpin structure (Witwer *et al.* 2001). 'S' fragment prevents the digestion of viral genome in infected cells by host cell exonuclease (Mason *et al.* 2003). 'S' fragment is followed by an internal poly-ribocytidylate (poly C) tract of variable length (usually 100-400 residues) (Costa-Giomi *et al.* 1984). Following the 3' end of poly C tract there is a series of RNA pseudoknot structures of unknown function. Downstream of the pseudoknots (PK) there is a short hairpin loop structure termed as *cis*-acting replication element (*cre*) and this element includes a conserved motif, AAACA in the loop region. The *cre* region precedes the internal ribosome entry site (IRES), a complex highly structured element of about 440 residues, which is responsible for the internal initiation of protein synthesis in a cap-independent fashion (Martinez-Salas 1999).

The 3' UTR of the FMDV genome is also highly structured, consisting of 100 nucleotides that contains 2 stem loop structures (SLI and SLII) and a genetically encoded poly A tract (Chatterjee *et al.* 1976). There is extensive evidence of interaction between the 3´ UTR of picornaviruses and several viral and host proteins (Agol *et al.* 1999).

The viral ORF is divided into 4 regions (L, P1, P2 and P3) and encodes for a single polyprotein, which is cleaved by viral proteases (L,2A and 3C) (Ryan *et al.* 1989) to yield 4 structural and 8 non-structural proteins (NSPs). Each of these NSPs, as well as some of the precursor polypeptides, are involved in functions relevant to the virus life cycle in infected cells (Belsham 1993 and Porter 1993).

The L region contains two in-frame functional AUG initiation codons, which result in the generation of 2 overlapping L proteins, Lab and Lb (Beck *et al.* 1983, Rieder *et al.* 1993). The L protease (L^{pro}) is a member of the papain-like cystein proteases, which acts both intra and intermolecularly (Guarne *et al.* 1998). Both Lab and Lb catalyse their proteolytic excision at L/P1 junction of the polyprotein (Strebel and Beck 1986) and also initiate the cleavage of host translation initiation factor eIF-4G, which results in the shut-off of host cap-dependent mRNA translation (Devaney *et al.* 1988). It has been reported that L^{pro} blocks the innate immune response to FMDV infection in susceptible animals by inhibition of host IFN-α/β mRNA translation (de Los Santos *et al.* 2006).

The P1 region encodes the structural proteins VP1 (1D), VP2 (1B), VP3 (1C), and VP4 (1A). Although the 2A region was considered as a part of the P2 region, the 16-amino-acid peptide 2A catalyses in *cis* the excision of P1-2A from 2B (Ryan *et al.* 1991). It has also been proposed that 2A peptide prevents the formation of the peptide bond at the 2A/2B junction through the ribosome slippage mechanism during the polyprotein synthesis (Donnelly *et al.* 2001). The processing of the FMDV P1-2A precursor to 1AB, 1C and 1D is achieved by the 3C protease (Vakharia *et al.* 1987 and Ryan *et al.* 1989).

The P2 region encodes 2 viral non-structural (NS) proteins (2B and 2C). The function of P2 region of FMDV is poorly understood. The polypeptide 2C and its precursor 2BC are associated with cell membranes and induce vesicle proliferation (Bienz *et al.* 1990). Transport of proteins from endoplasmic reticulum to Golgi apparatus is reportedly blocked by the FMDV 2BC protein (Moffat *et al.* 2007). P3 is composed of 4 non-structural proteins, 3A, 3B, $3C^{pro}$ and $3D^{pol}$. 3A is the membrane anchored protein and plays a role in the pathogenesis of FMDV (Pacheco *et al.* 2003), due to changes of the amino acids in 3A in various strains and serotypes of FMDV produced attenuated viruses in cattle (Sagedahl *et al.* 1987, Beard and Mason 2000). FMDV contains 3 tandem, non-identical copies of the 3B protein (VPg) (Falk *et al.* 1992). The VPg protein is covalently linked to the 5' end of the viral RNA and participates in the initiation of viral RNA synthesis (Nayak *et al.* 2005,2006). 3C protein is a serine-protease responsible for most of the cleavage of individual proteins from the viral polyprotein (Ryan *et al.* 1989). The 3D protein is the viral RNA-dependent RNA polymerase and thought to recognise and interact with both positive and negative strands of FMDV RNA (Belsham 2005).

With respect to the Indian FMDV strains, a few changes were detected in the genome. Two different lineages (V-A and VI) of serotype Asia-1 virus were detected with 3 and 4 pseudoknots (PK) respectively (Mohapatra *et al.* 2008). The discrepancy in the number of PKs is due to deletion of 43 nucleotides in 5'-UTR large fragment of lineage V-A viruses. Mutagenesis studies carried out with infectious clone of FMDV O1K showed that the pyrimidine rich sequence preceding the FMDV start codon was most sensitive in the conversion of single pyrimidine residue to purine decreased the protein translation efficiency drastically. However, lineage VI-B Asia-1 viruses exhibit a group-specific transversion ($U_{695}\rightarrow G$) within the pyrimidine tract without any impact on viral multiplication (Biswas *et al.* 2005, Mohapatra *et al.* 2008). Similar to Asia-1 viruses, a block deletion of at least 45 nucleotides was observed in 5'-UTR large fragment of serotype A viruses isolated in India. However, unlike serotype Asia-1, deletion in type A isolates were not genotype/lineage specific (Subramaniam *et al.* 2011). With respect to the number of PKs, most of the

serotype A field isolates showed to have at least 2 PKs (Subramaniam *et al.* 2011). In type A Indian viruses, a lineage specific (lineage VIIg) transversion (T_{712}→A) similar to Asia-1 FMDV was detected within the pyrimidine tract (Mohapatra *et al.* 2009, Subramaniam *et al.* 2011). However, as in Asia-1 serotype, this substitution had no role on the infectivity titre of type A viruses. Therefore, it was speculated that either a minimum numbers of pyrimidine residues or a core motif (CTTT) rather than a complete pyrimidine is essential for efficient viral translation (Mohapatra *et al.* 2009).

In serotype O Indian isolates, the majority of recent isolates of dominant lineages circulating in India were with either 1 or 2 PKs, so the role of lesser number of PKs in giving fitness advantage to the viruses cannot be ruled out. During a recent study, a serotype O isolate with no PK was found. The number of PKs was predicted by using software (pknotsRG tool), if the prediction limits of the software is considered totally genuine, probably the FMDV natural isolate can exist without a pseudoknot (unpublished data). Therefore, the role of PK on FMDV replication needs to be determined.

Due to circulation/co-circulation of multiple lineages of different FMDV serotypes, genetic recombination events may be possible between the genome of related strains of FMDV. Evidence of recombination in capsid coding region was reported earlier in Indian serotype A isolate (Tosh *et al.* 2002a). However, for Asia-1 viruses recombination events was reported in the non-capsid protein coding region (Mohapatra *et al.* 2008). Recently, recombination events on both structural and non-structural protein coding regions were detected in serotype O viruses (unpublished data).

Cell recognition by FMDV: The first requirement for FMDV infection is the interaction of the virus with the host cell receptors. Integrin was the first molecule identified as a primary receptor for FMDV (Berinstein *et al.* 1995, Jackson *et al.* 1997). The integrin receptor recognition site includes a highly conserved arg-gly-asp (RGD) triplet on the G-H loop of the VP1 capsid protein (McKenna *et al.* 1995, Rieder *et al.* 1996). Despite being the part of important antigenic site, the RGD triplet is highly conserved amongst all the strains of FMDV, which probably reflects its requirements for the *in vivo* interaction with the cell receptors (Duque and Baxt 2003, Neff *et al.* 1998).

Infectious FMDV can enter the cultured cells using αvβ1, αvβ3, αvβ6 and αvβ8, as receptors (Berinstein *et al.* 1995, Jackson *et al.* 2000, 2002, 2004). The αvβ6 integrin receptor is expressed constitutively at high levels on the surfaces of epithelial cells at sites where infectious lesions occur during a natural infection in the cattle, but not at the sites where lesions are not normally formed (Monaghan *et al.* 2005). In infected animal tissues, αvβ6 integrin receptor is distributed on the surface of those epithelial cells also expressing FMDV antigen both in the tongue and coronary bands (O'Donnell *et al.* 2009). Thus αvβ6 is the major receptor that determines the tropism of FMDV. During cell culture, FMDV can acquire the capacity to initiate infection via heparan sulphate (Jackson *et al.* 1996), by replacement of amino acid at a shallow depression on the virion surface, at the junction of 3 major capsid proteins VP1, VP2 and VP3. The replacement of histidine residue to arginine at position 56 in VP3 protein of cell culture adapted virus enhances the interaction with heparan sulphate (HS) (Jackson *et al.* 1996, Fry *et al.* 1999, Fry *et al.* 2005). The ability of the cell culture adapted virus to bind HS was also reported for serotypes C (Baranowski *et al.* 2000), serotype A (Fry *et al.* 2005) and SAT-1 viruses (Maree *et al.* 2010). However, both field and cell culture adapted Indian viruses belonging to Asia-1 serotypes have amino acid arginine residue at position 56 in VP3 protein (unpublished data). It was reported that upon multiple passages of FMDV in cell culture, the RGD motif can become dispensable, this is associated with the use of alternative receptors for cell entry (Baranowski *et al.* 2001a, Baranowski *et al.* 2001b). Antibody-complexed virus can infect cells via Fc receptor-mediated adsorption (Baxt and Mason 1995). An alternative cell-binding site was also reported for genetically engineered virus harbouring lys-gly-glu (KGE) sequence instead of the RGD triplet in swine (Zhao *et al.* 2003).

FMD transmission and pathogenesis: The most important features of the epidemiology of the disease are extremely rapid replication and transmission of the virus. The common mechanism of spread of the disease is by direct contact which may occur by mechanical transfer of virus from infected to susceptible animals through damaged skin or intact mucosae or by deposit of droplets or droplet nuclei (aerosols) in the respiratory tract of recipient animals (Alexandersen *et al.* 2003a). Contact with virus from infected animals can also occur via fomites, for example by people contaminated at the time of shearing, de-worming, lambing and blood sampling during FMD epidemics. By air-borne exposure a minimum 10 $TCID_{50}$ of virus is sufficient to infect a ruminant experimentally. However, pigs are relatively resistant to aerosol exposure (Alexandersen *et al.* 2002). Infected pigs are a significant source of FMDV for long distance aerosol spread , as pigs release largest quantities of air-borne virus (Alexandersen *et al.* 2002). Ruminants excrete lower titres of virus in their breath but are highly susceptible to infection by the respiratory route through air-borne transmission. Pigs usually become infected either by eating FMDV-contaminated food or by direct contact with infected animals. Sheep and goat are highly susceptible to virus infection by aerosol. As clinical diagnosis is often difficult in sheep, the infection can be unnoticed and therefore sheep can play a major role in the spread of disease as has been widely implicated in the prolific spread during the 2001 outbreak in the United Kingdom.

The incubation period of FMD is highly variable, 2-14

days, and depends on the strain and dose of virus, the route of transmission, the animal species and the husbandry conditions (Alexandersen and Mowat 2005). Charleston *et al.* (2011) found that period of infectiousness in cattle is only 1.7 days and animals are not infectious until 0.5 days after the appearance of clinical signs.

The epithelial cells of the dorsal soft palate, the roof of the pharynx and part of the tonsil are thought to play a special role in the primary infection (Alexandersen *et al.* 2003b). This concept was described for cattle, but is only indirectly suggested in other host species. Recent studies reported that subsequent to aerosol inoculation, the FMDV infection is initiated at the epithelia of mucosal associated lymphoid tissue of the nasopharynx in cattle (Pacheco *et al.* 2010a, Arzt *et al.* 2010, 2011b). Shortly after initial infection ('pre-viremic' phase), virus replication is detected within pulmonary alveolar septa and as viraemia approached, FMDV is replicated more prominently within the pulmonary pneumocytes with a significant decrease in virus load within pharyngeal tissues (Arzt *et al.* 2011b). Other studies have similarly implicated roles for nasopharynx and lungs as the primary sites of natural infection in cattle (Burrows *et al.* 1981, Brown *et al.* 1996). In pigs, palatine tonsil or lungs are shown to be the primary site of infection following oral or aerosol inoculation, respectively (Terpstra 1972).

Early replication is followed by a viraemic phase of 3-5 days and during this time, the virus spreads to epithelial tissues of secondary sites of replication via the bloodstream. The secondary sites of replication include cornified stratified sqaumous epithelia of the oral cavity and skin (hairy and non-hairy parts) including the feet and mammary teats (Alexandersen *et al*. 2003b). Less commonly affected epithelial lesion sites include external genitalia and rumen. During this secondary stage, lesions which are observed initially as a blanched area subsequently develop into vesicles that cause lesions at the mouth, feet and teats (Seibold 1963, Alexandersen and Mowat 2005, Arzt *et al.* 2009). There is often secondary bacterial infection which delays healing of the lesions. Myocardial infection, when it does occur, is typically during the viremic phase in young pigs, small ruminants, and wildlife (Arzt *et al.* 2011a). Clearance of viraemia and viral tissue load is achieved by the induction of an effective immune response, and is characterised by the generation of virus specific antibody and may be dependent on the interaction of virus-antibody complex with phagocytic cells of reticuloendothelial system (McCullough *et al.* 1986,1988, 1992).

Carrier state in FMD: In ruminants, an asymptomatic, persistent infection can be established following clinical recovery, irrespective of vaccination status. This state has a significant impact on control and eradication programs (Perry and Rich 2007). Any animal from which FMDV can be recovered in oropharyngeal scrapings for a period greater than 28 days post-challenge is considered as a carrier animal (Sutmoller *et al.* 1968). The nasopharynx, particularly the dorsal soft palate was suggested to be the site of predilection for FMDV persistence in cattle (Burrows 1966, Donn *et al.* 1994, Zhang and Kitching 2001). Juleff *et al*. (2008) demonstrated depots of FMDV antigens in lymph nodes of cattle during persistent infection which are likely involved in maintaining long term immunity. However, the potential role of these depots in long term shedding of infectious virus remains uncertain. In sheep, tonsil is shown as the site of persistence of the virus (Burrows 1968, Ryan *et al.* 2008).

More than 50% of FMD-recovered ruminants may become carriers of the virus. However, pigs usually clear the virus within 3 to 4 weeks of infection and do not become carriers (Alexandersen *et al.* 2003b, Parida *et al.* 2007) with an exception of a single report which showed pigs as carriers (Mezencio *et al.* 1999). The duration of carrier status varies between the species, the maximum reported duration of the carrier state in African buffalo, cattle, sheep and goats are 5 years, 3.5 years, 9 months and 4 months, respectively (Burrows 1968, Hedger 1968, Brooksby 1982, Condy *et al.* 1985, Salt 1993, Alexandersen *et al.* 2003b). However, the majority of cattle and sheep appear to lose their carrier status within a relatively short period of time. A meta-analysis of persistence studies indicated that carrier cattle cleared infection @0.115/month (Tenzin *et al*. 2008).The mechanisms for the establishment and maintenance of the carrier state are not well understood, since persistence can occur in both vaccinated and non-vaccinated cattle (Doel *et al.* 1994). The risk of carrier cattle or sheep transmitting virus to uninfected animals is generally believed to be extremely low (Kitching 2002). The only direct evidence of transmission of virus from a carrier to a susceptible animal is that of transmission from African buffalo to cattle during the outbreaks in Zimbabwe in 1989 and 1991 (Dawe *et al.* 1994a). In addition, there is experimental evidence of transmission of the virus by sexual contact from infected buffalo carrying a small amount of virus in oesophageal-pharyngeal (OP) fluids to uninfected cattle (Dawe *et al.* 1994b).

Immune response against FMDV: Upon infection, FMDV elicits rapid humoral and cellular immune responses in susceptible animals, which induce an efficient protection against re-infection with homologous and antigenically related viruses (McCullough *et al*. 1992, Salt 1993). Protection against FMDV is generally correlated with high levels of neutralizing antibodies in serum. The neutralizing antibody response is directed to the well characterised B cell epitopes located on the 3 external capsid proteins (Sobrino *et al.* 2001). IgM is the first serum neutralizing antibody that appears as early as 3-4 days following infection or vaccination, with a peak response at approximately 10-14 days post-infection, and then the response declines (Sobrino *et al.* 2001, Golde *et al.* 2008). The IgM response is followed by IgA and then IgG, which is first detectable 4-7 days after

infection or vaccination and then becomes the major neutralizing antibody by 2 weeks following vaccination (Salt *et al.* 1996). In both vaccinated and infected cattle, the IgG1 antibody response was reported to be higher than IgG2 (Salt 1993). Although neutralizing antibody often provide protection against FMD, it was also seen that hyper immune sera raised to mutant FMDV antigen with minimal neutralizing activity *in vitro* offered passive protection against wild type FMDV challenge (Dunn *et al.* 1998). Therefore, it is purposed that, non-neutralizing antibody may also play a preposed role in the clearance of FMDV from infected animal by macrophage mediated phagocytosis of opsonised virus-antibody complex (McCullough *et al.* 1986, 1988, 1992).

In contrast to the serotype restricted B-cell mediated humoral immunity, the T-cell response to FMDV in animals is shown to be cross reactive between FMDV serotypes, which is of interest for vaccine design (Collen *et al.* 1998, Blanco *et al.* 2001, Parida *et al.* 2006b and Cox *et al.* 2010). In cattle and pig, B-cell activation and antibody production are associated with a lymphoproliferative response mainly mediated by CD4+ T-cells (Sobrino *et al.* 2001). These helper T-cells recognise a number of epitopes located both in structural and non-structural proteins of FMDV (Collen *et al.* 1989 and Blanco *et al.* 2001). For the production of anti-FMDV antibodies, CD4+ T-cells assists in the maintenance of appropriate microenvironment required for a synergistic immune response (Sobrino *et al.* 2001). The role of CD8+ T cell mediated cytotoxic T-cell response to FMDV infection is unclear and largely remains unexplored. This may be partly due to the unavailability of a reliable assay of cell killing for highly cytolytic FMDV or due to down regulation of MHC class-I molecules on the surface of infected epithelial cells (Sanz-Parra *et al.* 1998). There is a single report of FMDV specific MHC-I restricted proliferation response in the CD8+ enriched fraction collected from the blood of FMDV infected cattle (Childerstone *et al.* 1999). Nevertheless, the levels of proliferation were low, variable and completely absent in a significant proportion of the animals tested. However, using a sensitive IFN-γ restimulation ELISpot assay, the FMDV specific MHC-I restricted CD8+ T cells response was detected in cattle (Guzman *et al.* 2008).

Diagnosis: The accurate diagnosis of infection with FMDV is of great importance for both control and eradication campaigns in FMD endemic areas and as a supportive measure to maintain disease free zones. It is also important to differentially diagnose the disease from other vesicular diseases such as, swine vesicular disease, vesicular stomatitis, and vesicular exanthema of swine. In addition, FMDV infected sheep and goats are difficult to diagnose clinically. The suspected cases showing symptoms are usually confirmed by laboratory diagnosis either by detecting the viral antigen or antibody.

Virus detection: The OIE Terrestrial Manual (OIE 2009) describes 4 methods for the detection of FMDV. These tests are virus isolation (VI), antigen detection ELISA (Ag-ELISA), complement fixation test (CFT) and nucleic acid recognition (NAR) methods.

Virus isolation is currently the only *in vitro* method for the detection of live virus in the clinical samples. FMDV can be grown on a variety of cell culture systems of bovine (Snowdon 1966), ovine (Hess *et al.* 1963) and porcine (Bachrach *et al.* 1955) origin. The cytopathic effect (CPE) usually develops within 48 h. If no CPE is detected, the cells are frozen and thawed and the lysate is used to inoculate fresh cultures and examined for CPE after 48 h. The earlier used CFT has been substituted by more sensitive antigen capture ELISA (Ag-ELISA) for antigen detection and serotyping of the virus (Roeder and Le Blanc Smith 1987, Ferris and Dawson 1988, Bhattacharya *et al.* 1996). A pen side test based on the principle of Ag-ELISA and chromatography technology was developed for diagnosis of FMD (Reid *et al.* 2001, Ferris *et al.* 2001,2009 and 2010).

Various reverse transcription-polymerase chain reaction (RT-PCR) procedures were developed for detection of FMDV RNA: conventional RT-PCR (Rodriguez *et al.* 1992, Pattnaik *et al.* 1997), RT-PCR ELISA (Callens *et al.* 1998), nested RT-PCR (Moss and Haas 1999), real-time RT-PCR (Reid *et al.* 2002, Moonen *et al.* 2003), portable real time RT-PCR (Donaldson *et al.* 2001, Hearps *et al.* 2002) and automated RT-PCR (Reid *et al.* 2003). Other nucleic acid detection methods were also developed for FMDV detection, the nucleic acid sequence based amplification (NASBA) test (Collins *et al.* 2002, Lau *et al.* 2008) and the RT loop-mediated amplification (LAMP) test (Dukes *et al.* 2006). Lineage differentiating RT-PCR for serotype A (Mohapatra *et al.* 2007) and Asia-1(Mohapatra *et al.* 2006) and multiplex-PCR (Mohapatra *et al.* 2011b) were also developed, and are in use in India for detection of circulating Indian strains. In India, a double antibody sandwich ELISA (Bhattacharya *et al.* 1996) is routinely used for identification of FMDV serotypes. RT-PCR (Pattnaik *et al.* 1997, Mohapatra *et al.* 2006, Mohapatra *et al.* 2007) is applied on ELISA negative samples. If the disease is reported late, diagnosis is done by a liquid-phase blocking ELISA.

Antibody detection: Detection of antibody against structural protein of FMDV: Virus neutralization test (VNT), liquid-phase blocking ELISA (LPBE) and solid phase competitive ELISA (SPCE) are 3 serological tests prescribed (OIE 2009), for detection of antibody against the structural proteins of FMDV. These tests are used as serotype specific serological tests. VNT is labour intensive, requires sensitive cell lines, live FMDV and containment laboratory facility. The advantages of ELISA over VNT are that the test is rapid, can use inactivated antigens, and requires smaller volumes of post-vaccination sera, which are often available in limited quantities (Paton *et al.* 2005). LPBE based antibody detection system is quicker, more reproducible, less variable and the result correlates well with VNT (Hamblin *et al.* 1986,

Hamblin *et al.* 1987, Pattnaik and Venkataramanan 1994). However, the LPBE was criticised for the specificity and variable stability of inactivated antigen used in the test (Mackay *et al.* 2001). The SPCE was been developed to overcome this problem, which has higher specificity than LPBE (Mackay *et al.* 2001, Paiba *et al.* 2004). In India an indigenously developed LPBE is used extensively to monitor post vaccination antibody response.

Detection of antibody against non-structural protein of FMDV: Detection of FMD-specific structural antibody can be useful for diagnosis, but this requires the absence of any history of vaccination, as vaccination with purified vaccine elicits antibodies only against structural proteins (SPs). FMDV infection elicits antibodies against both SP and NSPs. Therefore, an ELISA that measure antibodies to FMDV NSPs can be used for differentiating infection from vaccinated animals (DIVA). The detection of antibodies to the NSP 3ABC of FMDV was shown to be a sensitive and specific DIVA test when combined with the confirmatory immunoblotting test (EITB) against 5 bioengineered FMDV NSPs 3A, 3B, 2C, 3D and 3ABC (Bergmann *et al.* 2000, OIE 2009). However, this OIE-index test for NSP serology is only available from PANAFTOSA, Brazil to the South American laboratories. A number of 3ABC ELISA test kits have recently become available and their sensitivity and specificity were compared to one another and to the OIE-index screening method at an international workshop in Brescia in 2004 (Brocchi *et al.* 2006). It was concluded in the workshop that 2 tests performed comparably to the OIE-index method of which the Ceditest (currently known as PrioCHECK® FMDV NS) is the only one available as a commercial kit (Paton *et al.* 2006). In addition to 3ABC ELISAs, a variety of NSP tests based on the detection of antibodies to the recombinant 2B (Inoue *et al.* 2006), 2C (Mezencio *et al.* 1998), 3AB (Mohapatra *et al.* 2011a), 3D (Sorensen *et al.* 1998) were developed. Moreover, multiplex profiling assays were also evaluated (Mackay *et al.* 1998, Perkins *et al*. 2006, Perkins *et al*. 2007). In India, an indigenously produced 3AB/3ABC-ELISA kit is extensively used to monitor virus circulation and clearance in vaccinated areas. A multiple NSP ELISA using FMDV 2C, 3AB, 3ABC and 3D antigens was also developed in the country for monitoring of disease free zones.

Mucosal antibody detection: Parida *et al.* (2006a) studied the oropharyngeal IgA responses in FMDV-vaccinated and infected cattle and demonstrated that parenteral administration of conventional FMD vaccine does not elicit any IgA antibody in saliva. In contrast, challenge with live virus elicits a strong local IgA response, including vaccinated animals that have become persistently infected. Pacheco *et al.* (2010b) reported that parenterally vaccinated pigs do not elicit mucosal antibody whereas vaccinated and subsequently infected pigs produce high levels of mucosal antibody, as seen in cattle. Thus the IgA test has considerable potential for the detection of persistently infected cattle following the application of a vaccinate-to-live policy (Parida *et al.* 2006a, Biswal *et al.* 2008).

FMD vaccine and protection: Currently the FMD vaccine is produced by infecting baby hamster kidney-21 (BHK-21) cells with virulent FMD virus, followed by chemical inactivation by binary ethylenimine (BEI) and purification by ultrafiltration. Some vaccine manufacturers use industrial scale chromatography to purify whole virus particles from NSPs. As inactivated FMDV is poorly immunogenic, effective formulation of FMD inactivated vaccines requires the use of adjuvants to enhance the antigenicity of the vaccine. Two types of adjuvants are used in general, aluminium hydroxide gel ($Al(OH)_3$) supplemented with saponin and oil emulsion adjuvant (Doel 1999). Oil-emulsion vaccines are widely used for the immunization of pigs, sheep, goats and cattle, whereas $Al(OH)_3$/saponin adjuvant is mainly used for cattle. In endemic countries, FMD vaccine formulation contains more than 1 serotype of virus depending upon the epidemiological situation of the particular country. The inactivated FMD vaccine induces a relatively short duration of protection which lasts for 4-6 months. Boosting of the immune response by repeated vaccination, as done in endemic countries, dramatically increases both the magnitude and duration of neutralising antibody responses (Doel 2003, Parida 2009). Emergency vaccines, which are normally used in FMD free countries during the incidence of an outbreak, are of higher potency (≥ 6 protective dose 50 (PD_{50})) than the conventional vaccines (≥ 3 PD_{50}). Higher antigen dose ensures rapid protective immunity and better cross production within serotype (Cox and Barnett 2009). There has been good documentary evidence that emergency vaccines, as either an oil or aqueous formulation confer rapid and protective immunity in appropriate target species within 4 to 5 days of vaccination (Barnett and Carabin 2002). This is attributed to induction of combined innate and early adaptive immune responses (Barnett *et al.* 2002, Rigden *et al.* 2003, Barnard *et al.* 2005).

Efficacy of vaccination against FMD is affected by the lack of cross protection between serotypes, as well as incomplete protection between subtypes (Mattion *et al.* 2004). Therefore, it is necessary to match the vaccine strains with the circulating filed strains (Doel 1999). Currently, the methods of vaccine strain selection are mainly based on the serological approaches (Paton *et al.* 2005). The relationship between field isolates of FMDV and vaccine strains are usually expressed as 'r' values (Doel 2003), calculated between the viruses using pools of antisera prepared against vaccine strains and other candidate strains to be matched. The antigenic similarities between the vaccine strain and field isolates can either be calculated by using a neutralization test (Rweyemamu *et al.* 1978) or an ELISA based approach (Kitching *et al.* 1988). The advantages of ELISA over a virus neutralisation test (VNT) are that the test is rapid, can use

inactivated antigens and requires smaller volumes of post-vaccination sera, which are available in limited quantities (Paton *et al.* 2005). If VNT is used to determine the antigenic relationship an 'r' value of 0.3 or higher indicates a close antigenic match between vaccine strain and the field isolate (Rweyemamu 1984). While in ELISA based approach (LPBE), an 'r' value of 0.4-1.0 indicated a close match between vaccine strain and field isolate (Kitching *et al.* 1988). If the r value is less than the prescribed value, the field isolates need to be examined against alternative vaccine candidates and there may be a need to identify and select another vaccine strain (Paton *et al.* 2005). Another approach for determining the antigenic relationship and selection of vaccine strain could be by comparison of capsid coding gene (P1) sequences of both vaccine strain and field isolates, which would provide the genetic information regarding the epitopes playing a role in vaccine-induced protection (Parida *et al.* 2009). Presently, this approach is being investigated in various FMD laboratories (Reeve *et al.* 2010). However, caution must be taken when extrapolating between nucleotide or deduced amino acid differences and antigenic homology, since the impacts of specific amino acid changes on antigenicity are completely not well determined (Paton *et al.* 2005).

There are various concerns associated with the use of conventional FMD vaccine such as duration of immunity, thermal stability, spectrum of protection, bio-containment and efficient distinction between infected and vaccinated animals. Therefore, many approaches were made for development of an alternative safe and effective vaccine (Rodriguez and Grubman 2009). Based on the information concerning FMDV capsid structure and knowledge about the immunological importance of G-H loop, several strategies were used to develop subunit vaccines. These include use of VP1 protein produced by recombinant DNA technology (Kleid *et al.* 1981), VP1 derived peptides (Strohmaier *et al.* 1982, Venkataramanan *et al.* 1994), use of live vector expressing VP1 fusion proteins (Kit *et al.* 1991, Kitson *et al.* 1991), DNA vaccine expressing VP1 epitopes alone (Wong *et al.* 2000), and co-administration of DNA vaccine expressing VP1 with DNA encoding IL-2 (Wong *et al.* 2002). However, till now none of these vaccines elicit the same level of protection as the current, conventional inactivated whole virus vaccine (Doel 2005). The lesser amount of protection offered by the subunit vaccine may be due to the limited number of antigenic sites and/or T-cell epitopes, which are unable to induce significant protection (Rodriguez and Grubman 2009). An alternative approach to protein and peptide vaccines is the production of the empty capsid which involves expression of the regions of FMDV genome (P1-2A-3C) that are essential for synthesis, processing and assembly of viral structural proteins to form the empty capsid. The empty capsid based vaccines which are highly immunogenic could easily be used for DIVA purposes (Grubman and Mason 2002). FMDV empty capsid based vaccines were produced using baculovirus (Li *et al.* 2008), adenovirus (Mayr *et al.* 1999, Mayr *et al.* 2001), herpesvirus (D'Antuono *et al.* 2010) some of them were tested in targeted species (Mayr *et al.* 2001, Pena *et al.* 2008, Li *et al.* 2011) . One of these molecular vaccines, based on an adenovirus vector has undergone advanced development and licensure process (Rodriguez and Gay 2011). In India, a trivalent (O, A and Asia-1) inactivated and oil adjuvanted vaccine at 3 PD_{50}/dose is being used for control of FMD. There are 4 FMD vaccine manufacturers in the country to meet domestic demand.

Global distribution of FMDV serotypes and topotypes

Various FMDV serotypes are not uniformly distributed across the world, the virus infection is primarily maintained within 3 continental epidemiological clusters Asia, Africa

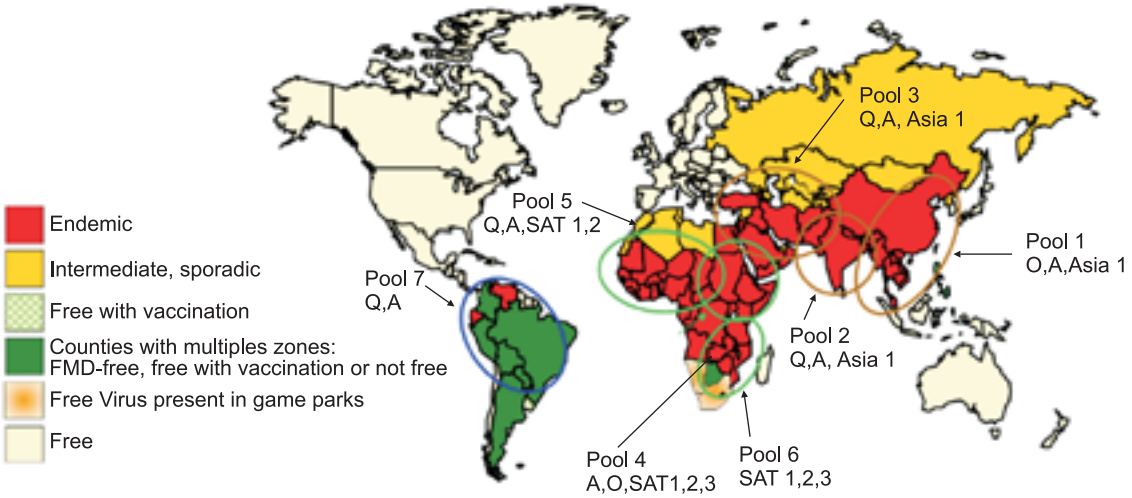

Fig. 1 Conjectured foot and mouth disease status in 2010 with regional foot and mouth disease virus pools and predominant virus serotypes (Modified by Paton *et al.* 2009)

and South America, which can be further grouped into seven major virus pools (Paton *et al.* 2009) (Fig. 1). Each of these virus pool contains at least 3 serotypes of virus, and because virus circulation is mainly within these regional reservoirs, strains have evolved which are specific to the region and that often (in type A and SAT viruses) require tailored vaccines (Paton *et al.* 2009). Only 4 serotypes (O, A, C and Asia-1) have been recorded in Asia and Middle East, while 6 out of the 7 serotypes (O, A, C, SAT-1,-2 and -3) have been circulating in Africa and only 3 serotypes (O, A and C) in South America. Globally last outbreak due to FMDV type C was recorded in 1996. With the advent of molecular biology techniques, it is now possible to divide FMDV into different genotypes/lineages/sub-lineages based on the genetic distances in the VP-1/P1 coding region (Pattnaik *et al.* 1998, Hemadri *et al.* 2000, Samuel and Knowles 2001, Tosh *et al.* 2002b and Knowles and Samuel 2003). Geographically restricted genotypes were named as topotypes, as they usually circulate in the defined geographic region (Knowles and Samuel 2003). Eleven topotypes were assigned for serotype 'O', while 3 topotypes were defined for serotypes A and C, and all the Asia-1 viruses were considered into a single unnamed topotype. In Africa, 9, 14 and 5 topotypes were defined for SAT-1, SAT-2 and SAT-3 viruses respectively (Knowles *et al.* 2010).

Europe: With respect to the epidemiological patterns of FMD, entire Europe is grouped into 2 categories, countries recognised by OIE as free of FMD without vaccination, and countries not recognised by OIE as free of FMD without vaccination because of being at risk of incursion of FMD from neighbouring regions. Almost all European countries, west of Russian federation and the Balkan countries of Bosnia, Macedonia and Serbia-Montenegro are free of FMD in Europe. Although the trans-caucasus countries are mainly free of FMD but are bordering endemically affected parts of Iran and Turkey. In Turkey, FMD is being reported throughout the year in the east and south-east of Anataolia, while western Anataolia only experience periodic incursion of disease due to animal movement from the east and south-eastern parts of the country (Leforban and Gerbier 2002, Rweyemamu *et al.* 2008 and Valarcher *et al.* 2008).

North America, Central America and Caribbean: North America is free from FMD and last reported outbreak was seen during 1952 and 1953 (Bachrach 1968). North America was able to control FMD due to 'stamping out' policy (Sutmoller *et al.* 2003). Central America and the Caribbean have never reported outbreaks of FMD and have remained free of the disease up to now.

South America: FMD was first recognised in South America in 1870, in the Province of Buenos Aries, Argentina, in the central region of Chile, in Uruguay and southern Brazilian states, due to importation of livestock from Europe (Correa Melo *et al.* 2002, Sutmoller and Casas Olascoaga 2003). FMD spread further into central western Brazilian States and was recorded in Peru, Bolivia and Paraguay during the first half of 20th Century and in Columbia during 1950s and in Ecuador during 1961. Since then, FMD has become endemic in South America. Three distinct epidemiological situations could be observed in South America: the FMD free countries/zones (Chile, Uraba in Colombia, the Guyanas, eastern and western Brazil states and the Atlantic coast in Columbia), the FMD endemic regions (the Andean areas, northern and north-eastern Brazil), and epidemic area in the Southern Cone (Saraiva 2004).

In South America, FMD spreads mainly with the trade of bovines. The knowledge of time-space behaviour of the disease, also known in South America as FMD ecosystems is useful in planning and executing the control/eradication strategy. The concept of ecosystems (primary endemic, secondary and sporadic) is based on a series of variables such as cow/calf ratio, number of milking cows per herd, existence of other susceptible species and average size of herd. Besides, the Omega 1 index, which is based on time/space distribution of disease for each country/region on a 10-year time series is also a variable for the consideration of FMD ecosystems (Saraiva 2004). The Hemispheric Foot-and-Mouth Disease Eradication Plan (PHEFA), launched in late 1980s, was instrumental in the application of the FMD ecosystems concept (Rweyemamu *et al.* 2008).

FMDV serotypes A, O and C were recorded in South America. FMDV type C was prevalent in Argentina, Bolivia, Brazil, Paraguay, Peru and Uruguay between 1972 and 1995. While type C is rare in the Andean region, type O and A are quite common. Since 2003, type O has occurred frequently in Ecuador. In Venezuela, type A FMD virus is widespread in the country, especially close to the border with Colombia. In Venezuela, serotype O virus is also present along with type A (Saraiva 2003).

To control the disease, authority in Argentina, Uruguay and Brazil decided to implement measures such as vaccination of the whole population in three consecutive rounds, stringent animal movement control, controlled culling of in-contact herds and serological sampling to evaluate the presence of viral activity. As a part of the project to achieve freedom from FMD, serological studies by 3D (VIA) and 3ABC-ELISA/EITB were conducted by several countries in South America. This served as a support to South American countries in designing their FMD control/freedom strategies (Correa Melo *et al.* 2002). As of now, Chile, Guyana, Patagonia, Southern and Central Western zones in Peru are free from FMD without vaccination (Correa Melo and Lopez 2002).

Africa: Depending on the distribution of FMDV serotypes, topotypes, disease prevalence data and various factors such as animal movement patterns, impact of wildlife and farming system, various epidemiological clusters were proposed for FMDV distribution in Africa. These epidemiological clusters are Southern African Development Community (SADC),

Angola, East African Community, Coastal West Africa, Soudan/Sahel, North Africa/Meghreb, and Inter-governmental Authority on Development (IGAD) (Rweyemamu *et al*. 2008). South-SADC countries (Swaziland, Lesotho, South Africa, Botswana and Nambia), meet the conditions of the OIE for zonal/country freedom from FMD without vaccination. However, in some of these countries there are isolated wildlife areas, where the African buffaloes are known to be asymptomatically infected with FMD virus serotypes SAT-1, -2 and -3. Therefore, the wildlife areas are separated from livestock through a system of game-proof fencing and vigorous surveillance. The north-SADC cluster, consists of Zimbabwe, Zambia, Mozambique, Malawi and southern Tanzania. Though, in these countries FMD was in control through intensive vaccination and animal movement control during the 1970s and 80s, currently serotypes SAT-1,-2 and -3 are circulating in the north SADC cluster (Rweyemamu *et al.* 2008). The primary source of infection seems to be from African wild buffaloes, which are capable of maintaining silent infection of SAT serotypes (Vosloo *et al.* 2002b). In Angola, serotypes exotic to Southern Africa occurred regularly up to 1975 when official reporting seems to have ceased. Therefore, there is little information about the true incidence of FMD in this cluster (Rweyemamu *et al.* 2008).

The epidemiology of FMD in the West and Central Africa has not been deeply studied. In the Central Africa, serotypes O, A, SAT-1 and SAT-2 were responsible for most outbreaks of FMD. In West Africa most outbreaks were caused by serotype A, followed by serotypes SAT-2, SAT-1 and O respectively. East African Community (EAC) consists of the countries Tanzania, Uganda, Kenya, Rwanda and Burundi. These countries perhaps promote the most complicated FMD situation in the world. Serotypes O, A, C, SAT-1 and SAT-2 are endemic in the EAC cluster. This appears to be the only region in the world where serotype C was found in recent times (Vosloo *et al.* 2002a).

The Soudan/Sahel cluster consists of Western Sudan, Niger, Chad, Burkina Faso, Mali, Northern Nigeria and Senegal. This cluster serves as an important disease corridor, linking the IGAD cluster with West Africa and West Africa with North Africa. In North Africa, FMD has not been reported since 1999, through routine preventive vaccination and other measures. However, in Libya and Egypt sporadic cases of FMD were reported byKnowles *et al.* (2007) and Rweyemamu *et al.* (2008). FMD is endemic in the IGAD cluster comprising Sudan, Eritrea, Ethiopia, Somalia, Northern Kenya and Northern Uganda. Habiela *et al.* (2010) reported that long-distance animal movement and within country circulation are the main factors for the prevalence of O, A and SAT-2 serotypes in Sudan. Molecular characterization of viruses recovered from FMD outbreaks in Ethiopia during 1981-2007, revealed the emergence and re-emergence of serotypes O and SAT-1 (Ayelet *et al.* 2009). Therefore, continuing emergence of FMD viruses in Ethiopia may affect the spread and consequent control strategy of FMD in Africa continent.

Asia: Based on the distribution of FMD virus serotypes, disease prevalence and proximity to FMD endemic neighbouring country, the following epidemiological clusters can be described for prevalence of FMD in Asia.

Middle East: FMD was recorded in almost all countries of the Middle East on numerous occasions in the past; Iran, Iraq and Syria can now endemic to FMD. Molecular characterisation of FMD viruses at World Reference Laboratory for FMD, revealed a link between the virus strains from Afghanistan, Pakistan, Saudi Arabia, Iran and Turkey, suggesting that FMD possibly spreads from South-Central Asia westward through the trade of ruminants. Therefore, viruses of serotype Asia-I are now constantly present, together with the serotypes O and A. From Africa, both SAT-1 and SAT-2 serotypes invaded from Ethiopia, Somalia and Sudan into Middle East primarily by trade. Therefore, sustainable FMD control in the Middle East depends on the success of control programme in Far East, Central and South Asia and also in Africa (Aidaros 2002).

South-East and East Asia: With respect to South-east Asia, FMD is endemic in Cambodia, Laos, Malaysia, Myanmar, the Philippines, Thailand and Vietnam. However, three countries (Brunei, Indonesia and Singapore) are recognised as free from FMD without vaccination by OIE. East Malaysia and parts of the Philippines (Mindanao, Visayas, Palawan and Masbate) are also recognized internationally as being free of FMD without vaccination. Serotypes O, A and Asia-1 are the only 3 serotypes endemic in the region and type O is the most common circulating serotype with 3 distinct topotypes, South-east Asia (SEA), Middle east-South Asia (ME-SA) and Cathay (pig-adapted) (Gleeson 2002). Phylogenetic analysis revealed how different introductions of viruses may be sourced from different countries and similar viruses can consequently be found in various countries in South-East Asia. A phylogenetic analysis showed that Malaysian isolates belonging to the O/Mya-98 lineage were interleaved with those from neighbouring countries in close groups (Abdul-Hamid *et al.* 2011).

In East Asia, Japan, Republic of Korea and Taiwan Province of China are recognised as countries free of FMD without vaccination. The last outbreaks of FMD in Japan and Republic of Korea were during 2000 and 2002 respectively (Sakamoto and Yoshida 2002). However, in 2010 FMD virus type O (lineage Mya-98) outbreaks were reported in Japan and Republic of Korea (Akashi 2010, Yoon *et al.* 2011).

In China, the FMD virus serotypes O and Asia-1 and A are prevalent. The Cathay topotype of FMD virus serotype O appears to be endemic in Swine production systems of Southern China. FMD viruses regularly move into China from Myanmar and Laos through cattle trade into Yunnan

(Rweyemamu *et al.* 2008).

Control and eradication of the disease was facilitated in the regions through inter-country participation, as the disease is of transboundary nature. South-east Asia—Foot and Mouth Disease (SEA-FMD) campaign was launched in 1997 to coordinate sub-regional control of FMD. In 2010, China joined the campaign and it was renamed as South-East Asia and China Foot and Mouth Disease (SEAC-FMD) campaign. The main strategy is the implementation of a progressive zoning approach to ensure effective use of limited resources from the donors and national governments. The campaign has established Epidemiology Network (EpiNet) and a Laboratory Network (LabNet) to provide technical support to enhance member countries' capacity for effective surveillance and diagnosis.

Central Asia: With respect to Central Asia, countries which form the core are Kazakhstan, Kyrgystan, Tajikistan, Turkmenistan and Uzbekistan that were kept FMD free in the past by virtue of the USSR border disease control programme. However, since the transition to independence, FMDV serotype O and Asia-1 have become widespread due to increased cross border trade and contiguous livestock farming system. Most of the times the source of FMD infection to Central Asia, is from Afghanistan and China. In the absence of information and molecular phylogenetic study, little epidemiological information can be deduced from unsystematic reporting in Central Asia (Rweyemamu *et al.* 2008).

South Asia: India and Pakistan are the key countries for the progressive control of FMD in South Asia (Rweyemamu *et al.* 2008). Jamal *et al.* (2010) found that serotype O is most prevalent followed by Asia-1 and A in Pakistan. The authors also reported that higher number of outbreaks of the disease was noted between January and March, due to the movement of livestock during the religious festival *Eidul Azha*. With respect to FMD distribution in Sri Lanka, Bhutan, Nepal, Bangladesh and Maldives, very little is known.

Epidemiology of FMD and its control in India

Livestock sector is an important contributor in Indian economy–its overall contribution to the agricultural GDP is 28-32% and to national GDP is 4 to 6%; and it employs 8-10% country's labour force. India has largest population in the world with 528 million domesticated animals; highest buffalo population in the world (105.3 million), second highest in cattle (199 million) and goats (140.5 million), and third in sheep (71.5 million). Such a big population is under great risk of FMD due to unrestricted movements of animals throughout the country, limited systematic vaccinations, and inapparent infection in small ruminants which probably act as reservoir of the virus. Direct loss of ₹ 20,000 crore/annum was reported by Venkataramanan *et al.* (2006). Eighty per cent of the total direct loss caused by FMD is due to drop in milk production (Mathew and and Menon 2008). The other economic losses caused by the disease are due to massive expenditures by the government sector on FMD control, added cost on treatment, low productivity (meat, wool etc.) and loss of draught power.

In India, FMD remains endemic and was first officially documented in 1864 during extensive outbreaks in many parts of the country (Government of India 1868). However, research on FMD by Indian Council of Agricultural Research (ICAR) dates back to 1929. Epidemiological studies on FMD in India was initiated by ICAR in the form of an "All India Co-ordinated Research Project (AICRP) for virus typing" in 1968 with a central laboratory at Mukteshwar and 3 regional centres. Subsequently, the scope of the project was expanded in 1971 to "AICRP for Epidemiological studies on FMD" with addition of 4 more regional centres. The AICRP was expanded to Project Directorate on Foot and Mouth Disease (PDFMD), which is the premier institute for FMD research in the country under ICAR, in 2001. Currently the project directorate has 8 regional centres and 15 network units covering the length and breadth of the country. The PDFMD has developed scientific and technical expertise in both conventional and cutting edge areas of FMD research for use in the country. Further, PDFMD also acts as the FAO Reference Centre for South-Asia, in the field of FMD diagnosis, epidemiology and research. O, A and Asia-1serotypes of the FMD virus are currently prevalent in India and the disease is reported throughout the year. Among the serotypes, type O is the most prevalent one and accounts for 83-93% of the outbreaks followed by Asia 1 (3-10%) and A (3-6.5%). Serotype C has not been reported in the country since 1995. Different genotypes and lineages of the 3 serotypes were detected in the country. Serotype O, FMD virus dominates the FMD outbreak scenario in India, and on molecular studies all the serotype O isolates were grouped into 7 sub-lineages namely Branch A, B and C-I, C-II, Pan Asia I, Pan Asia II and 'Ind2001' under Middle East-South Asia (ME-SA) topotype (Fig. 2). Currently 3 lineages, viz. 'Ind2001', Pan Asia I and Pan Asia II, are responsible for type O outbreaks in the country. The Pan Asia II lineage emerged in 2003 and since then it is causing outbreaks along with parent Pan Asia I viruses. Lineage 'Ind2001' first identified in the year 2001, re-merged in 2008 and is co-circulating along with Pan Asia lineages since then. Though the current serotype O vaccine strain (IND R2/1975) is not genetically identical to the circulating field strains, it continues to antigenically cover the outbreak strains. Serotype A is the most diverse among all the 7 serotypes of FMDV. Throughout the world, 26 serotypes have been identified till now, however in India, 4 genotypes [genotypes I (2), IV (10), VI (16) and VII (18)] were identified (Fig.3) (Mohapatra *et al.* 2011c). Genotypes 2 (Euro–South Asian topotype) and 10 (Asia topotype) of serotype A were recorded before 1990 and no longer exist in India. Endemic co-circulation of 2 genotypes (16 and 18) with dominance of

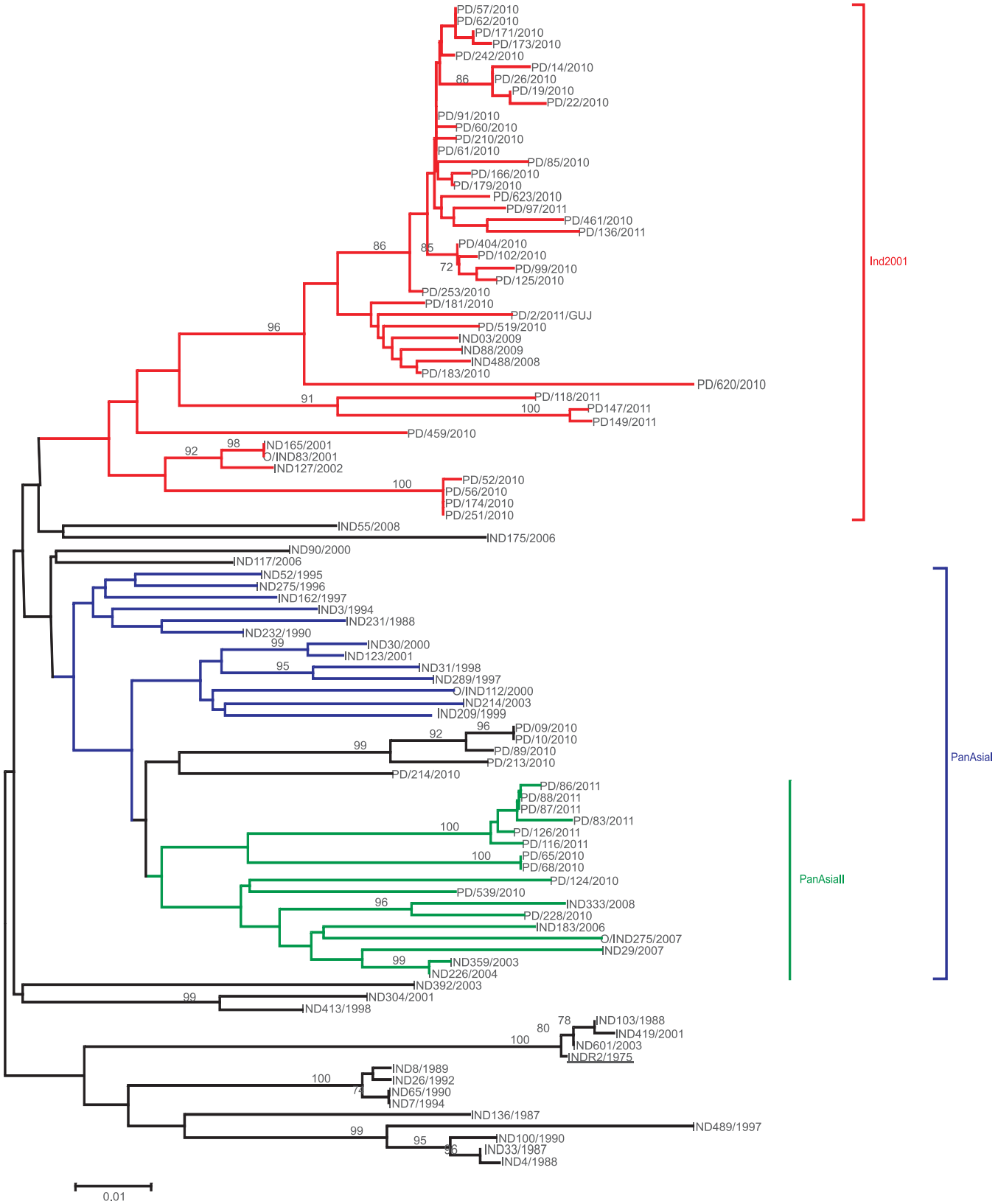

Fig. 2 Phylogenetic tree depicting genetic relationship among serotype O isolates at VP1 coding region.

Currently used vaccine strain is underlined. The isolates from different states could be placed in 3 major lineages designated 'Ind2001' and Pan Asia. Within parent Pan Asia lineage, a divergent Pan Asia II sub-lineage emerged in 2003.

genotype 18 was observed in the country, while other 2 (2 and 10) genotypes have not been detected since 1990. The isolates belonging to genotypes 16 and 18 are divergent both genetically and antigenically. The current serotype A vaccine strain is from genotype 18 and is most appropriate in covering the circulating filed strains. The earlier vaccine strains were from genotype IV (10) and VI (16). In serotype Asia-1 three prominent lineages (lineages B, C and D) are in circulation in India (Sanyal *et al.* 2010) (Fig. 4). The lineage B which includes the vaccine strain IND 63/1972 has 210 amino acids in VP1 and this lineage never appeared after the year 2000. The lineage C, which was prominently circulating in India during the period 1993 to 2001, has an extra amino acid at position 44 of VP1. Lineage D, within the lineage C appeared in 2001 and it outnumbered the parent lineage in terms of field outbreaks. Lineage C has been responsible for all Asia-1 outbreaks in the country since 2005. The serotype Asia1 vaccine strain IND 63/1972 currently in use for vaccine production is continuing to provide optimum antigenic coverage for the circulating field strains.

Control of FMD is significant for protecting the livestock industries and for improving livelihoods and income generation in the developing countries, where FMD is endemic. Progressive risk reduction of FMD can help in progressive market access of livestock commodities from developing countries. In India, progressive control pathway (PCP) for the control and eradication of the disease was adopted (Rweyemamu *et al*. 2008). The FMD control programme (FMDCP) was launched in India in X Plan (based on epidemiological data acquired over more than 35 years) in 54 districts selected in 8 states of the country covering 30 million cattle and buffalo. In these regularly vaccinated areas, there has been progressive build up of herd immunity and substantial decline in the occurrence of the disease, severity of clinical disease and NSP reactors/converters since 2006-07 (Table 1) and 2003-04 (Table 2). With example of the states like Punjab and Haryana where only a few sporadic cases of FMD could be recorded due to impact of regular vaccination and building up of herd immunity. With the implementation of FMDCP, surveillance was intensified for antibody against structural and non structural proteins of FMDV to a clear picture of the disease in terms of herd immunity, virus circulation and clearance from time to time. For differentiation of infected animals from vaccinated ones, a panel of recombinant NSP of FMDV were utilized for development of profiling immunoassay. Some of the vaccines used in India are having very low levels of NSP that do not elicit prolonged immune response even after multiple vaccinations (Mohapatra *et al.* 2011a), however for exclusion of occasional false positive cases, a multiple DIVA ELISA using 3AB3, 3ABC, 2C and 3D has been developed. Detailed investigation in Haryana (FMDCP area) using serum samples collected periodically has revealed decline in 3AB3 DIVA reactors from 31.94% before implementation of regular vaccinations to 12% after eight rounds of vaccinations, indicating reduction in DIVA converters. Due to success of the FMDCP in 54 districts, additional 167 districts (another 80-90 million cattle and buffalo) have been included under

Table 1. Number of confirmed FMD outbreaks/cases in different geographical region of the country during 2006-07 to 2010-11

Year	South Z	North Z	Central Z	West Z	East Z	North-East Z	Total
2006-07	224	7	23	29	431	64	778
2007-08	445	20	35	31	258	88	877
2008-09	64	18	33	16	66	43	240
2009 -10	59	55	20	24	365	75	598
2010-11	51	9	29	18	29	40	176
Total	843	109	140	118	1149	310	2669

Z, Zone

Table 2. FMD outbreaks/cases in FMDCP districts

Year	Uttar Pradesh	Haryana	Punjab	Maharashtra	Gujarat	Andhra Pradesh	Tamil Nadu	Kerala	Andaman Nicobar	Lakshadweep	Dadra and Nagar Haveli
2003-04	42	60	4	29	12	37	….	63	….	….	….
2004-05	3	1	….	1	1	….	….	1	….	….	….
2005-06	2	….	….	3	1	….	….	….	1	….	….
2006-07	1	1	….	….	1	….	….	….	….	….	….
2007-08	….	….	….	1	1	….	….	….	….	….	….
2008-09	….	….	….	…..	2	….	….	….	….	….	….
2009-10	15	1	…..	6	0	3	….	….	….	….	….

…., Disease not reported/ Data not available

Fig. 3 Phylogenetic tree depicting genetic relationship among serotype A isolates at VP1 coding region.

The isolates from India were placed in four genotypes (2, 10, 16 and 18) showing more than 15% nt. divergence among them. Isolates of genotype 18 exclusively dominates type A outbreaks in the country since 2001 and currently used vaccine strain belonged to this genotype.

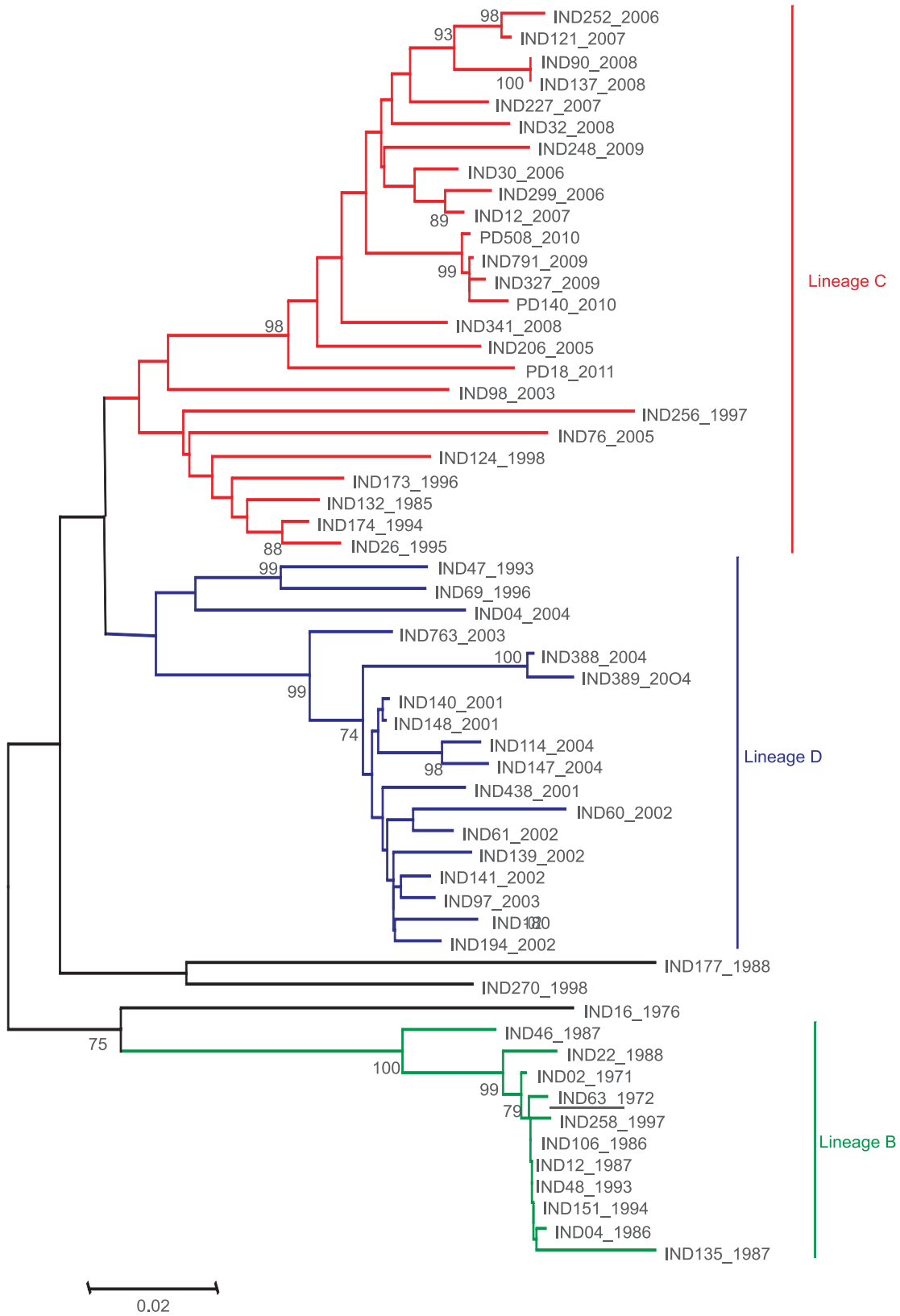

Fig. 4 Phylogenetic tree depicting genetic relationship among serotype Asia1 isolates at VP1 coding region.

The isolates from India were placed in three lineages designated B, C and D. Indian vaccine strain (IND63/1972) belonged to lineage B. Lineage C is exclusively responsible for all type Asia1 outbreaks in the country since 2005.

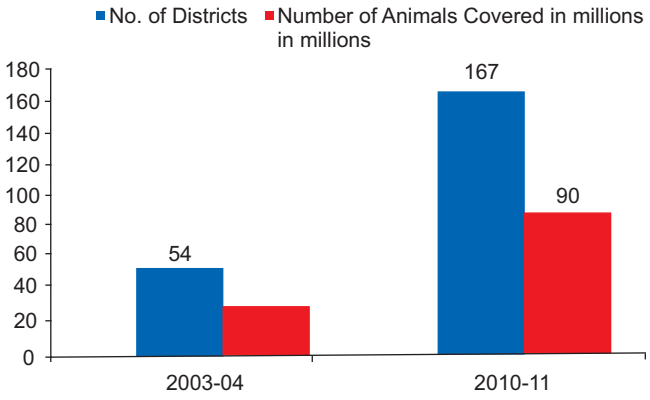

Fig. 5 Coverage of FMD-CP

the programme in 2010-11 (Fig.5) bringing total districts under FMD-CP to 221 covering states of Southern peninsula (Kerala, Tamilnadu, Puducherry, Karnataka and Andhra Pradesh), Maharashtra, Goa, Daman and Diu, Gujarat, Punjab, Haryana, Delhi, Dadra and Nagar Haveli, Andaman & Nicobar Islands, Lakshadweep and 16 districts in Uttar Pradesh (Fig. 5 and Fig. 6). Required doses of trivalent vaccine and companion diagnostics (LPBE and DIVA) are available locally. Recently, establishment of a National FMD Commission was proposed to coordinate all activities associated with FMD control programme including quality assurance of vaccines in order to effectively implement and monitor the control programme and achieve zoning of the disease (initially with vaccination) in the country in a definite time frame. Now great emphasis is given on improvements in vaccination strategies so that vaccination density and timing is made uniform to obtain the best outcome. In addition, the availability of quality vaccine as per the requirement is being ensured with public-private partnership. Research and development in the country is oriented towards obtaining quality vaccine with high potency which will cover wide antigenic spectrum and elicit longer duration of immunity (9-12 months). Use of bivalent and monovalent vaccines based on epidemiological data is under discussion for judicial use of limited resources for maximum outcome. Use of monovalent vaccines (where a particular serotype is prevalent for a long time) will reduce the cost of the operation. Additionally, due importance has been diverted to study the role of small ruminants in FMD epidemiology in the country in recent years (Ranabijuli *et al.* 2010) and inclusion of small ruminants in vaccination campaign is under consideration.

As per the current scenario, it is expected that by 2018, the disease situation in entire Southern peninsula will be under control (stage 3 of PCP) with a herd immunity of >85%. By 2025, it is anticipated that the southern peninsula will be disease free with vaccination (stage 4) when it will be monitored for the next five years. It is expected that by 2025, most parts of the country will be in stage 3, southern peninsula will be in stage 4 (free with vaccination), and parts of northern India will enter stage 5 of PCP.

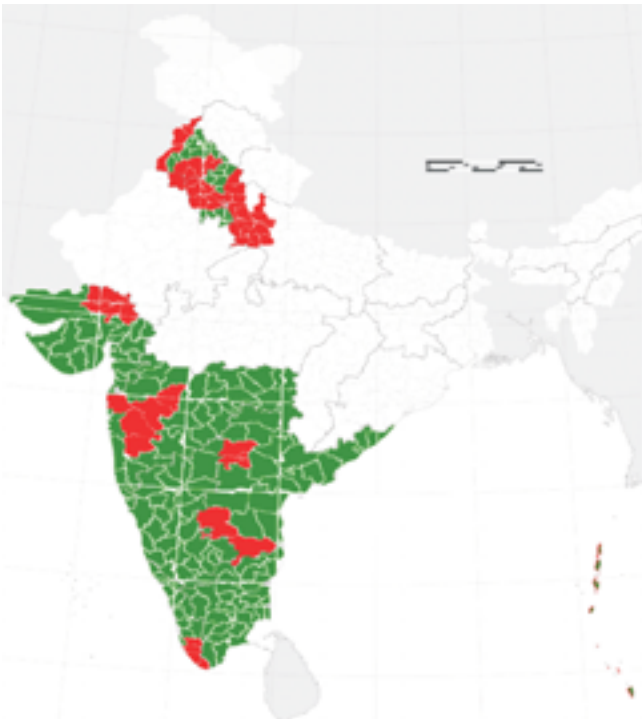

Fig. 6 Districts covered under FMD-CP.
Districts covered: Red, 2003-04; Green, 2010-11

From the above account of global distribution of FMD, it can be proposed that any coordinated regional/global strategy for FMD control should be based on the efficient epidemiological assessment of incidences and distributions of FMD. Identification of primary endemic areas (virus maintenance areas), secondary endemic areas (areas of virus propagation) and epidemic areas (areas of explosive outbreak) are equally important for the development of a sustainable programme for the progressive control of FMD in endemic settings. Ecosystem-based description of FMD in South America can be a guiding principle for the progressive control of FMD in India and other endemic countries. It is relevant to apply landscape genetics approach to elucidate mechanisms, understanding basic ecological process driving infectious disease dynamics and to understand the linkage between spatially dependent population process and the geographical distribution both within host and parasite (Biek and Real 2010).

Future researches in FMD

1. Considering the extreme contagiousness of the disease and high antigenic diversity of the virus, the research would be directed towards thorough understanding of the origin and maintenance of nuclei of virus infection, spread of the virus and differential distribution of virus serotypes in the country. An important research requirement is to establish how the disease, ecosystem condition, livestock rearing pattern and other

demographic meta-data modulate the antigenic diversity of FMDV. It is essential to study the spatial and temporal dynamics of FMDV by means of Bayesian phylogeography.

2. Early warning and immediate epidemic response require rapid diagnosis of FMDV isolates. Research work should be directed to develop animal-side diagnostics and pre-clinical diagnosis of FMD for an early and confirmatory detection of FMDV.
3. Current serological methods to select vaccine strains are cumbersome, difficult to standardize and require the generation of monovalent (vaccine-specific) bovine antisera. In future, these tests shall be replaced by rapid, gene sequence-based prediction of antigenicity and diversity/similarity. The combined application of virus gene sequence, virus structure and virus neutralization titre data would be helpful for the prediction of antigenicity of the vaccine strain.
4. Control of FMD in India through vaccination could be enhanced, if vaccines are available with improved thermal stability and longer duration of immunity. Molecular biology and biotechnological intervention to replace the unstable amino acids with a stable version in order to achieve better stability of the viral capsid structure that could result in an improved thermal stability of the existing vaccine. The stabilised capsid antigen would also able to produce a faster and stronger immune response *in vivo*.
5. By using reverse genetics techniques, quick substitution of vaccine viral capsid gene sequence with that of the field virus, would lead to the generation of robust, efficacious and customised vaccine as per the FMD outbreak situation.
6. There are also research gaps with respect to the ability to predict the performance of vaccine against FMDV infection without conducting *in-vivo* vaccine potency tests in cattle. Research work would be directed towards the development of alternate assay systems for the assessment of FMD vaccine potency to replace costly cattle challenge-protection experiments.
7. An understanding of host and viral factors for establishing FMD carrier status may lead to a strategy to cure or limit the persistent infection. Stimulation of local mucosal immune response by means of mucosal vaccination or by modulation of various cytokines may be useful in eliminating the virus. Alternatively if persistent infection cannot be cured or prevented, future research must be directed towards absolute reliable means for carrier identification.
8. Though considerable progress has been made in developing and determining the performance characteristics of different DIVA tests, in future assays based on a panel of various non-structural proteins would be required in estimating the likely prevalence of infection following the use of prophylactic vaccination. The DIVA panel test would help in post-outbreak serosurveillance strategies to facilitate rapid recovery of FMD-free status.

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