Introduction
Chapter 1

Introduction

Foot and Mouth Disease (FMD) is an extremely contagious viral disease of domestic animals viz. cattle, buffalo, goat and sheep. It is well known for its economic consequences due to restriction on the trade in livestock and livestock products. Though rarely fatal in adult animals it ranks first in the Office International des Epizooties (OIE; World Organization of Animal Health) list A diseases (OIE: Manual of standards, 2001) owing to nearly cent percent morbidity, rapid spread, severe decrease in livestock production, calf mortality. There is up to 25% loss in livestock productivity (Rowlands, 2003) due to reduced growth rate, decreased milk production and crippled agricultural draught power. Wide host range, ability of the virus to infect animals with a small dose, rapid rate of virus replication, high level of viral excretion and multiple modes of transmission aggravate the FMD outbreak scenario (Pattnaik et al., 2012). Furthermore, prolonged convalescence, short term immunity with no interserotypic cross protection and establishment of carrier status complicates the control and eradication of this devastating disease.

The causative agent Foot and mouth disease virus (FMDV) is prototype member of the Aphthovirus genus of the family Picornaviridae (Cooper et al., 1977). The genome consists of a single-stranded positive-sense linear RNA molecule of ~8.5 Kb in size which has poly (A) tail at 3’ terminus and carries a small Vp2 protein at its 5’ end. The genome contains a single open reading frame (ORF) that has two alternative translation initiation sites, located 84 nucleotides apart in the leader proteinase gene (L) (Sangar et al., 1987). Functionally, the genome can be categorized into three main regions: 5’ untranslated region (5’UTR), coding
region (ORF) (subdivided into L/P1, P2 and P3) and 3’ untranslated region (3’ UTR). The 5’ untranslated region (UTR) contains a short fragment called S-fragment, a poly (C) tract, followed by a Large (L) fragment of over 700 bases. The L fragment contains a number of highly conserved secondary structures that include tandemly repeated pseudoknots (PKs), a cis-acting replication element (cre) and internal ribosome entry site (IRES). IRES is a stretch of about 440 residues which serves for the internal initiation of viral protein translation in a cap-independent fashion (Martinez-Salas, 1993). The remaining polyprotein undergoes primary cleavage to give P1, P2 and P3. The P1 region codes for capsid proteins. Capsid consists of 60 copies each of four structural proteins VP1, VP2, VP3 and VP4 coded by 1D, 1B, 1C and 1A genes, respectively. VP1, VP2 and VP3 form external components whereas VP4 is entirely internal (Acharya et al., 1989). The external component of capsid carries cellular receptor attachment sites and immunologically important antigenic sites including RGD motif important in term of replication of the virus. The P2 region of polyprotein is processed into 2A, 2B and 2C proteins. Preliminary studies have suggested involvement of 2B and 2C proteins in FMDV replication. P3 region is processed into 3A, 3B, 3C and 3D proteins. Role of 3A in host range determination and virulence has been established (Nunez et al., 2001). Three non-identical copies of 3B protein incorporate into the viral genome. 3C performs majority of the viral polyprotein processing. 3D acts as the RNA dependent RNA polymerase. The 3’-UTR is of variable length and along with poly (A) tail may play important role in viral replication (Lopez de quinto et al., 2002).

The virus exists in the form of seven different serotypes; O, A, C, Asia1 and South African Territories 1 (SAT1), SAT2 and SAT3. A large number of subtypes have evolved within each serotype (Domingo et al., 2003; Knowles and Samuel, 2003), and within each
subtype there are a substantial number of strains showing a variable degree of antigenic diversity and they often co-circulate in a particular geographical location (Brooksby, 1982; Mateu et al., 1988). As a practice new virus isolates are characterized by genomic nucleotide sequencing and defined antigenic behavior. FMDV is endemic in India, the disease occurs throughout the year in all parts of the country and is attributed to three serotypes viz. O, A and Asia1 since 1996. Serotype O is responsible for majority of outbreaks (75-80%), followed by A and Asia1, and outbreaks due to different serotypes occur in the same area at the same time in endemic situation (Hemadri et al. 2000, 2002). Though precise reasons behind higher prevalence of this serotype compared to other serotypes is still unknown, it is probable that factors related to its poor antigenicity as indicated by its more payloads in multivalent vaccine preparations (Doel T.R., 2003) and its adaptability in diverse environmental conditions could play a role.

Among the RNA viruses FMDV exists in highly diverse genetic and antigenic forms. An error-prone replication by FMDV RNA polymerase enzyme, which lacks proof-reading activity, gives rise to a dynamic population of non-identical but closely related mutant and recombinant viral genomes called quasispecies (Domingo et al., 1992). From quasispecies population, antigenic variants are selected either in the presence or absence of immune pressure (Holguin et. al., 1997; Manoj kumar et al., 2004). Immune pressure may induce positive selection at antigenically critical sites helping in further diversification and adaptation of the virus (Tosh et. al., 2003). Recombination further contributes to the genetic heterogeneity by providing an opportunity to quickly pool useful mutations from different genomes together and is more frequent in the region coding for non-structural proteins (King, 1988).

The most challenging aspect of FMD is its control, which has become more troublesome due to the complex epidemiological scenario prevailing in the subcontinent arising from lack of
proper vaccination campaign, unrestricted animal movement, no control strategies at international borders and large population of susceptible animals. In India, vaccination is the sole and most effective mode of control. To successfully implement vaccination, thorough understanding of the molecular basis of pathogenesis and epidemiology of the disease is definitely a prerequisite.

Vaccine matching (antigenic relationship) is routinely carried out by two dimensional virus neutralization test (2D-VNT) using viruses isolated in cell culture to establish the appropriateness and antigenic relevance of the vaccine strains in relation to the circulating field strains. Conventionally, virus isolation is done by serial propagation of homogenate of clinical tissue material in cultured cells. The standard approach shown to have a diminished ability to recover infectious FMD, where virus could be isolated only from 10-30% of outbreak derived clinical materials in BHK-21 cell line (Annual Report PDFMD 2012-13). Such reduced sensitivity could be ascribed to deterioration of clinical materials by the time they are received at the central laboratory making them unfit for virus isolation in cell culture in many occasions. Factors affecting the quality of the field samples may be linked to delay in disease reporting and transportation of infected tissue material from the field to the referral laboratory, large distance between point of collection and laboratory, high ambient temperature and interrupted cold chain during storage and shipment, unavailability of dry ice, and inadequate infrastructure for preservation at the local laboratories. Nonavailability of cell culture isolates hinders further characterization of outbreak strains including antigenic profiling, vaccine matching and genome sequence analysis which is imperative in effective implementation of vaccination based control programmes in the country.

Nucleotide sequence characterization of RNA viruses has become an important tool for epidemiological surveillance by determining evolutionary relationships among strains and
keeping track of the origin and dissemination of viruses in the field. To study each and every outbreak comprehensively, an alternative system of virus isolation with higher efficiency than the standard cell culture technique which could circumvent to a maximum extent the negative effects of the hostile factors related to climate, geography, logistics and infrastructure on the stability and infectivity of the capsid is a primary requirement. For effective implementation of FMD control in the country, the strains circulating and causing disease outbreaks need to be matched with in-use vaccine strains. Hence there is a need to develop an efficient method, which either alone or in combination with the conventional cell culture isolation method should be able to increase the rate of virus isolation.

Keeping all these in view, this work entitled “Development and application of a method to rescue infective Foot and Mouth Disease Virus in eukaryotic system and their molecular characterization” was undertaken with the following objectives:

OBJECTIVES:

1. Optimization of transfection method for recovery of infectious FMD Virus from different field outbreaks where no virus could be isolated in cell culture (from preserved RNA samples).

2. Rescue of infectious FMD virus from difficult (minute in quantity; shipped at undesirable pH and temperature) clinical materials.

3. To understand genetic and antigenic characteristics of rescued FMD viruses in comparison with its native counterpart, isolated by conventional cell culture passage.