Introduction
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This section of the thesis deals with the importance of livestock to the mankind, diseases which affects the livestock in common etc. The section explains the foot and mouth disease in detail. The symptoms of the disease in cattle and other affected animals are explained. The section addresses the issues of economic losses caused due to the disease in the global and national levels. It also throws light on control measures and the different vaccination strategies followed. Further the rationale of the present study is discussed in this section.

Livestock play a vital role in the agricultural economy of the world. They include cattle, goats, sheep, pigs, poultry, horses, camels, yaks, and llamas. An estimated 600 million people, including 150 million landless poor, own livestock in the world (Delgado et al., 1999; Thornton et al., 2000, 2002). The contribution of livestock goes beyond direct food production to include multipurpose uses, such as skins, fibre, fertilizer and fuel, as well as capital accumulation. Furthermore, livestock are closely linked to the social and cultural lives of several million resource-poor farmers for whom animal ownership ensures sustainable farming and economic stability.

As the mankind is highly dependable on the livestock population, any Livestock diseases are a major concern. The important Livestock diseases that have the potential, of serious socio-economic or public health consequences include the following:

- foot and mouth disease
- Swine vesicular disease
- Vesicular stomatitis
- Rinderpest
Amongst the above listed diseases, foot and mouth disease (FMD) has been ranked as the highest priority disease for control and eradication by Office International des Epizooties (OIE). The disease is one of the most contagious animal diseases posing a major threat to the livestock sector. Foot and mouth disease affects extensive areas worldwide. Foot and mouth disease is recognized as a significant epidemic disease threatening the cattle industry since the sixteenth century and even today it is a major global animal health problem. The history of FMD may be traced to era of Hieronymus Fracastorius, a monk who described a disease outbreak in 1546 A.D. that occurred in cattle near Verona, Italy (Fracastorius, 1546). Almost 400 years later, in 1897, Friedrich Loffler and Paul Frosch demonstrated that a filterable agent is responsible for FMD. This was the first demonstration that a disease of animal was caused by a filterable agent and ushered in the era of virology. Foot and Mouth Disease cause huge losses in terms of livestock productivity. Besides causing direct losses to livestock economy it also causes indirect losses in terms of severe trade restrictions, impact which may be higher than direct losses. The disease affects cloven footed animals such as cattle, swine, buffalos, sheep and goats as well as 70 species of wild animals including deer, camels, llamas, antelopes and elephants (Fenner et al., 1993).

1.1 The causative agent of foot and mouth disease

The Foot and mouth disease is caused by a single stranded positive sense RNA virus (8.5 kb), which belongs to genus *Aphthovirus* of the family *Picornaviridae*
(Franki et al., 1991). The mature virion which is an icosahedron is 30nm in diameter (Ruekert and Wimmer, 1984). The single stranded positive sense RNA genome is packed in a capsid composed of 60 copies of the four structural proteins, VP1 (213 aa), VP2 (218aa), VP3 (220aa) and VP4 (85aa) (Acharya et al., 1989). Amongst the four structural proteins first three (i.e., VP1, VP2 and VP3) are partly exposed on capsid surface while VP4 is buried internally and is myristylated at the N terminus (Madshus et al., 1984). The virus has seven antigenically distinct serotypes viz., O, A, C, Asial, SAT 1, 2 and 3 (Cooper et al., 1978) with over 65 subtypes (Pereira, 1977). This antigenic plurality of the foot and mouth disease virus arises due to the quasi species nature (Domingo et al., 1985).

1.2 Symptoms of foot and mouth disease

The Infection to the susceptible animals with Foot and Mouth Disease Virus (FMDV) can lead to appearance of vesicles on the feet, in and around oral cavity, and also on mammary glands of the female animals. The severity of the clinical symptoms varies with the strain of the virus, exposure dose, age and breed of the animal. In cattle, the disease in the early stage is characterized by a rise in temperature. The animal becomes dull, and shows a sudden drop in milk yield. There is quivering of the lips and uneasy movement of the lower jaw, with frothy saliva around the lips that drips to the ground (Fig.1). Blisters begin to develop, usually within a few hours, most frequently on the upper surface of the tongue (Fig.2). At first the blisters are seen as small raised areas, whitish in colour and containing fluid, they quickly increase in size covering half the surface of the tongue. Later, the blisters burst and collapse, leaving the tongue epithelia loose and wrinkled, with a
dead appearance. The blisters develop on the feet about the same time as in the mouth (Fig. 3). Most commonly they occur at the bulbs of the heels, at the front of the cleft of the hoof, and in the cleft itself (Fig. 4).

**Figure 1: Excessive salivation observed in the affected animal**

**Figure 2: Lesions on the tongue of the affected animal**
The animal experiences severe pain in the feet area and lies down constantly. The lameness usually gets worse, until the animal can only hobble when moving on hard or uneven surfaces. The blisters may also develop on the teats (Fig. 5) and the animal resents attempt of milking.

In young animals, the virus can lead to inflammation of the muscular walls of the heart which leads to death by myocarditis. In sheep goats and pigs the disease is generally mild and can be difficult to distinguish from other diseases such as Swine

The incubation time between infection and clinical signs of disease, is two to 14 days. The animal can begin shedding virus before it shows clinical signs. In infected animals, the virus multiplies to such an extent that their expired air is virtually a cloud of virus. Though the mortality is low, morbidity is high as affected animals take months to recover. Even after recovery, they do not normally regain lost weight and produces very less milk.

1.3 Transmission of foot and mouth disease

The foot and mouth disease virus gets transmitted through aerosol to the oral cavity of the affected animals. From the oral cavity, the infectious particle enters oropharynx and reaches the lung tissue. The virus passes readily in to blood stream from the lungs (Sutmoller and McVicar, 1976, 1981) leading to viraemia. The virus is then distributed throughout the body via blood, to reach epithelium of the feet and the udder where secondary virus multiplication results in formation of lesions. (Hyslop,
Diseased animals excrete the virus in enormous quantities in urine, milk, semen, faeces (Sutmoller et al., 2003). Animals pick up the virus either by direct contact with an infected animal or by contact with foodstuffs which have been contaminated by such an animal.

The route of introduction of FMDV in the 2001 outbreak of the disease in the United Kingdom was through feeding contaminated meat product scraps to pigs. (BBC News, February 2001). The disease can also be spread by inanimate objects like vehicles used for transporting affected animals, clothing or bedding, mud/soil of the manger in which the animal is housed, utensils used for milking etc. Massive animal movements of all species, sporting events (FMD technical report, Texas University) and cattle fairs (Indian express Dec 10th 2009) are some of the other ways the disease spreads.

1.4 Epidemiology of foot and mouth disease

The foot and mouth disease is currently enzootic in all continents except Australia and North America (Brown et al., 1992). Available data on the occurrence of different FMD virus serotypes across the globe indicate seven distinct pools which includes Pool 1-South East Asia (serotypes O, A, Asia), Pool 2-South Asia (serotypes O, A, Asia), Pool 3-Afghanistan, Pakistan (serotypes O, A, Asia), Pool 4-Eastern Africa (A, O, SAT 1,2,3), Pool 5 north east Africa (A, O, SAT 1,2), Pool 6 South Africa (SAT 1,2,3), Pool 7 South America (serotype O, A) (Fig.6). Some regions like Africa, Southern and South-East Asia and Central Asia are continuously infected and re-infected with all serotypes of the FMD virus. Outbreaks of the disease have occurred in every livestock-containing region of the world with the exception of New Zealand (Grubman et al., 2004).

The important FMDV out breaks which have occurred in the world include:
1960s to 1980s – in European countries


1993 – Italy

1993-Russia

1994, 1995, 2000-Greece

1996- Albania, Macedonia, and Yugoslavia

2001- United Kingdom

In India, the disease is endemic and outbreaks occur throughout the year and in all parts of the country. Majority of outbreaks are caused by FMDV serotypes O, A and Asia1. Serotype O was responsible for 80% of the confirmed outbreaks/cases in the past decade, whereas Asia1 and A caused 12% and 8%, respectively
(www.indiaveterinarycommunity.com). Geographical region-wise assessment of prevalence rate of foot and mouth disease in India is depicted in the Fig. 7. The data suggests that highest prevalence of the disease is found in the eastern regions of India.

1.5 Economical impacts of foot and mouth disease

Foot and mouth disease causes significant distress and suffering to animals and impacts on the farmer’s livelihood. The economic losses caused by the disease are mainly due to losses in milk production and reduction in working capacity of animals (Bandyopadhyay, 2003; Venkataramanan et al., 2006)

**Figure 7: Pie chart depicting the prevalence percentage of foot and mouth disease in India**

(43% in Eastern region, 31.5% in Southern region, 11.6% in North-eastern region, 5% Central region, 4.4% Western region and 4% in Northern region).

This in turn leads to losses in dairy and meats and hides industries due to reduction in the export potential. The loss of productivity also leads to expenditures on feed, medication and shelter of the affected animals (Rich and Winter-Nelson 2007). The Disease eradication costs during an outbreak add on to the economic losses. It was
estimated that around 582 million cattle, 3 billion sheeps, 146 million pigs were slaughtered during 2001 United Kingdom outbreak (FMD Economic Factsheet.UK 2002). Culling of the unproductive animals also leads to loss of important breeds of cattle and therefore affects livestock improvement practices like interbreeding or cross breeding. In addition to crippling animal industries through lost production and mortality, Foot and Mouth Disease (FMD) also leads to embargos on international trade of livestock. This alone lead to a loss of £5 billion annually in U.K during the outbreak (The Economic and Social Data Service Health and Social Consequences of the 2001 Foot and Mouth Disease Epidemic in U.K , 23 September 2009).

Beyond the farm, the impact of FMD is felt by retailers and consumers, who must endure shortages of meat, milk, and food products, or must pay much higher prices to obtain them. Although difficult to quantify, farmers and farming families would be affected emotionally and suffer stress, strain and distress. Even travel and tourism industries are affected by FMD (Rweyemanu and Leforban, 1999). During an FMD outbreak, tourists are not allowed to visit villages and rural areas. Such restrictions are necessary to prevent the transmission of the disease beyond affected areas. The FMD outbreaks in U.K in February 2001 lead to a loss of £2 billion for the tourism industry (Taylor, 2004). Even after the crisis of the outbreak, the ban on tourism will leave a negative impact on the potential tourists, which hard to recover from.

India is an agriculture based country where in Livestock is an important sub-sector contributing 25% of the output of the agricultural sector (National Accounts Statistics, 2009). India has a total livestock population of 485 million. The country ranks first with respect to cattle and buffaloes, second in goats, third in sheep production in the world. About 22.45 million people work in the livestock sector and the sector play a vital role in improving the socio-economic conditions of rural masses (DAHDF,
2009-10). But due to large population of susceptible animals (more than 470 million), no systematic vaccination programs and Unrestricted movement of animals within the country there is a huge economical loss incurred by the Indian government due to foot and mouth disease annually. From a recent survey, in India the economic loss due to this disease was to the tune of Rs. 4,000 crores in 2003 (Bandyopadhyay et al., 2003) and 15000-20000 crores in 2004 (Prabhu et al., 2004).

1.6 Control of foot and mouth disease

With the inevitability of globalisation and in order to safeguard world animal agriculture and trade, it is becoming increasingly important to control FMD (Rweyemamu and Astudillo 2002). Extreme measures are required to control FMD and if they are not effectively applied during an outbreak there is a high probability that the disease will reach epidemic proportions. The two basic approaches followed for control of FMD is vaccination and slaughtering. Control of FMD by vaccination involves the use of inactivated conventional viral vaccines for mass annual prophylactic vaccination campaigns (Most of the countries where FMD is endemic, follow this strategy). Restrictions of the movement or confinement of the infected animals with the stringent application of zoo-sanitary measures along with vaccination are also used to control an outbreak. The control by slaughtering involves culling all affected stock which has been exposed to such risk of infection that it is reasonably certain that they would develop the disease if left alive (The United Kingdom followed this strategy during 2001 outbreak). The above mentioned control strategies were followed through the outbreaks in some areas of the world to eradicate FMD. Australia being a prime example. However, FMD is still endemic in much of the developing world, including Africa, much of Asia and South America.
This endemic situation exists due to reasons like poor veterinary infrastructure, unavailability of proper diagnosis and less enthusiasm amongst the farmers to vaccinate the animals since foot and mouth disease is not a killer disease.

Broad control measures for foot and mouth disease have been taken up in India, which includes vaccination and preventing uncontrolled movement of animals. Slaughtering of infected and in contact animals is not possible in Indian scenario due to socio-economic and religious reasons. Thus vaccination and periodic updating of the vaccine strains to control of the disease is the only viable option and this has motivated the present research work.

1.7 Vaccines for Foot and mouth disease

Vaccines are employed worldwide to control FMD. The conventional vaccines currently available are inactivated and contain whole virus in a semi-purified state. These vaccines elicit mainly a strong humoral response with high titres of antiviral neutralizing antibodies against antigenic sites identified on the viral capsid (Brown, 1995; Mateu, 1994). This humoral response correlates with a solid lymph proliferative response (Saiz et al., 1992; Collen et al., 1989) and with a considerable, but short-lived protection. However the production of conventional vaccine involves high capital investment. Also, it is risky to handle active virus in large scale for vaccine preparation as there are chances of virus leakage from production units (Brown, 1992). Further, the tissue culture-based whole virus vaccines are thermo labile and require cold chain maintenance during storage and transport. Since the immunity conferred by inactivated virus was short-lived, repeated vaccination had to be carried out to prevent outbreaks, which again gets added to the vaccination cost.
Since the conventional vaccines for FMD have their drawbacks, using recombinant DNA technology, attempts have been made to develop genetically modified alternate vaccines such as protein based vaccines (Suryanarayana, et al., 1992; Bayry et al., 1999; Lewis, et al., 1991; Roosien et al., 1990; Belsham et al., 1990, 1991; Abrams, et al., 1995), DNA based vaccines (Chinsangaram, et al., 1998; Berinstein et al., 2000; Mayr et al., 2001) and the edible vaccines. These alternative vaccines are safe, potent, provide long term protection from the virus and are cost effective. The vaccines are heat stable and thus eliminate the need for cold storage. They also produce humoral and cellular immune responses like their conventional counterparts.

The edible vaccines are a very attractive alternative to the conventional vaccines. The concept is based on transfer of the viral genes under a strong promoter into a selected plant system. Once the gene gets integrated into the plant genome, it gets expressed along with the plant proteins systemically (Charmi et al., 2011). The vaccine is engineered to contain the antigen, but bears no genes that would enable to form the whole pathogen thus there is no way of establishing infection, assuring its safety. This method is user friendly and requires less monitoring by medical personnel. The technology requires no fermentation or cumbersome downstream processing and can be easily scaled up (www.molecularfarming.com). Another advantage of edible vaccines is mass immunization of the animals can be undertaken by mixing the vaccine along with the regular feed (Khandelwal et al., 2003). The Edible vaccines elicit mucosal immune response which is very effective in combating diseases where route of infection is through mucosal surface (Bae et al., 2003). These results cannot be obtained by either injection or by oral administration of the recombinant protein vaccine (Ogra et al., 2001).
The antigens in case of edible vaccines are delivered through bioencapsulation; where in the outer tough wall of the plant cells protects the antigen from gastric secretions (Kong et al., 2001). The antigen breaks down in the intestine and is taken up by the M cells present on the intestinal lining that overlie the payer’s patches. The antigen then reaches the gut associated lymphoid tissue (GALT), passes on to macrophages and other antigen presenting cells. This results in IgE, IgG and IgA antibody responses which will neutralise the attack by the real infectious agent (De Aizpura et al., 1988).

The technique of development of edible vaccines for various diseases is well established. There are several reports demonstrating that plants can express the pathogenic antigen in higher levels in authentic form (Mason et al., 1992; Gomaz et al., 1998; Arakawa et al., 1997) and can induce immune response (Haq et al., 1995; Manson et al., 1996) when administered orally. Several plant species have been successfully used for expressing FMDV monovalent immunogens and have been tested as edible vaccines for FMDV (Carrillo et al., 1998; Dus Santos et al., 2005; Pan et al., 2008).

1.8 Rationale of the present research work

The FMDV exists as 7 antigenically distinct serotypes (A, O, C, Asia1, SAT1, 2, and 3) in several geographical areas and several antigenically distinct strains with no cross protection among serotypes (Ogra et al., 2001). Therefore the Monovalent vaccines to be used in a particular geographical area should contain the antigenic moieties corresponding to the circulating strains. Since monovalent vaccines provide protection for only one specific serotype any alternate vaccines should contain immunogens which would provide effective protection for more than one serotype. So
far the studies have been directed towards the production of major immunogen, VP1 alone (Carrillo et al., 1998) or VP1 in combination with other capsid proteins (Pan et al., 2008) of a single serotype in plants. Till now, no attempts have been made to study the immune response to a complex multivalent antigen expressed in plants. Hence the need of the hour is to develop a transgenic plant which can express multivalent FMDV antigen and thus provide protection for more than a single serotype of the foot and mouth disease virus.

In India 3 of the FMDV serotypes O, A, and Asia1 are in circulation. Amongst the three serotypes, the O and A serotypes are responsible for large number of outbreaks in our country. Therefore, in the present study a successful attempt has been made to prepare transgenic forage crop, Sunnhemp (Crotalaria juncea) expressing bivalent FMDV 1D proteins of two serotypes, O and A linked in tandem. The recombinant plant has been tested as bivalent FMD vaccine in guinea pig model.

The present investigation was envisaged with the following objectives:

- To develop binary plasmid constructs using FMDV structural protein gene 1D of two serotype “O”and”A” and transformation of Agrobacterium with these plasmids.
- To explore in planta strategies of Agrobacterium mediated gene delivery in Sunnhemp.
- To perform molecular and genetic analysis of putative transformants, and ascertain delivery integration, expression, inheritance of the introduced gene.
- To test the immune responses in laboratory animal models.