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The Blue Cross Book

For the Veterinary Profession

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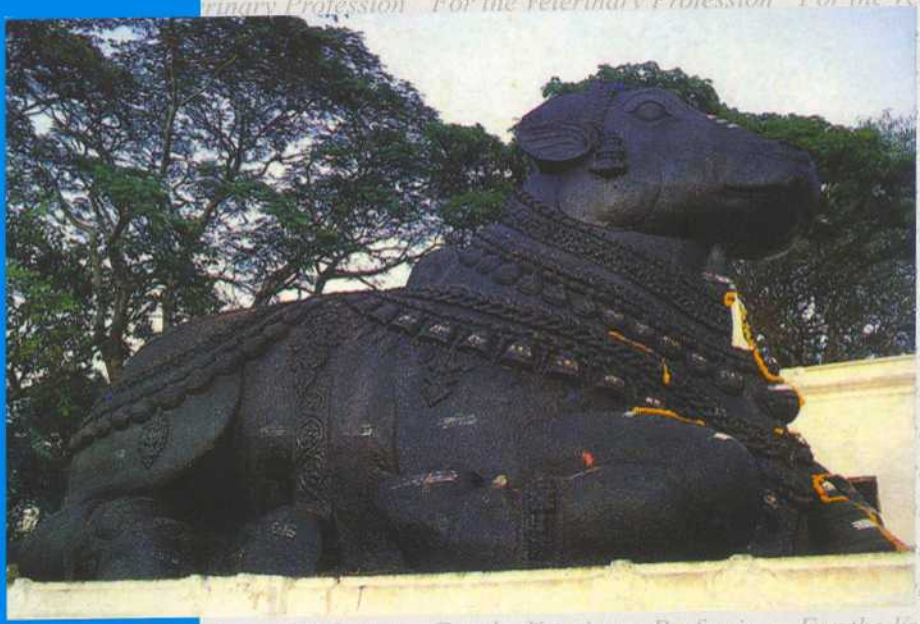

COMPENDIUM OF FOOT-AND-MOUTH DISEASE

ISSN 0972-4680

intervet

Dear Readers,

"We are glad to mention here that the valuable suggestions and technical guidance on the publication of this Foot-and-Mouth Disease compendium issue was kindly provided by -

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We are thankful to you all for your kind help."

- Editor

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Preface :

PREFACE

Dr. Lino Camponovo
Managing Director
Intervet India Pvt. Ltd.

Dear Reader,

It gives me immense pleasure to release Foot-and-Mouth Disease Compendium issue of *'The Blue Cross Book'*–24.

This is a well known fact that Foot-and-Mouth Disease (FMD) is a very economically important disease in different regions of the world with widely divergent magnitudes. It is a major problem in obtaining general understanding of the Asian and Global problem as well. As a result, this will have significant effects on international trade patterns and consequently, countries that do not have FMD-free status continue to suffer a severe handicap in terms of access to international markets.

This compendium, with quite a good number review and technical articles, attempts to provide the reader the facts on the various interacting issues. It will also help to find out the required answers in improving the management of such situations in future. As there are no simple solutions, there are different opinions which are reflected by the views of different authors.

The first and last of the total six chapters, comprising this edition, contain mainly review articles on FMD as it occurs in the Asian region.The other sections include both, papers intended to provide basic information as well as papers covering 'topical' problems such as vaccination and 'carriers' and their role in the epidemiology of the disease and the environmental implications.

This volume will be of value to veterinarians and also other animal health professionals, particularly, persons involved in farming and those concerned with the impacts of animal disease on farmers and their livelihoods.

I wish to extend my warm thanks to all the authors and to the people who contributed to the publication of this issue, which will become hopefully a reference issue on FMD.

To the editorial board members, I would like to express my sincere thanks, not only for agreeing to undertake the task of designing the contents of this book , but also for the mammoth task of reading and in cases, editing quite a large number of articles presented here.

Yours sincerely,

Dr. Lino Camponovo

"Dr. Lino Camponovo is a Swiss and studied veterinary medicine in Switzerland at the Universities of Fribourg and Zurich from 1974 to 1979 and later promoted as Dr.Med.Vet., from the Faculty of Veterinary Medicine of the University of Zurich.

His first professional experience was in rural practice in various capacities, working in different parts of Switzerland. He has then worked as an assistant in Internal Medicine and Surgery at the Faculty of Veterinary Medicine at the University of Zurich and finally as Head – Unit of Cattle Surgery. Also he was a lecturer in diseases of small ruminants at the Faculty of Veterinary Medicine of the University of Zurich and was a Vice Chairman of the local board of medical examination for veterinary surgeons in Zurich. Later on he had his own private veterinary practice. In 1997, he joined Veterinaria AG, Zurich Switzerland as Marketing Manager for productive animals and horses.

From 2000 until 2004, he was the Managing Director at Veterinaria AG, which is the Swiss Intervet subsidiary. There he had also been the Chairman of Board of Directors.

He has started his career in Intervet India as the Director, Sales & Marketing from January 2005 and currently he is the Managing Director of Intervet India, since July 2005.

His private hobbies include a licensed instructorship in mountaineering and ski touring with the organization "Youth and Sports" in Switzerland."

On behalf of the Editorial Board members, I welcome you Lino, as our new Patron of ***'The Blue Cross Book'.*** -Editor

Overview of Foot-and-Mouth Disease : Past and Present

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The Foot-and-Mouth Disease (FMD) is one of the most devastating disease of cloven-footed animals (primarily in Artiodactyla) caused by a group of seven antigenically different serotypes of extremely contagious, serologically independent apthoviruses and has a great potential for causing severe economic loss to both livestock and agricultural production. Though the disease was described as an epidemic in 1514, the agent responsible was first identified in 1897 (Loeffler, 1897). FMD causes significant financial losses (Perry *et al.*, 1999). There are direct losses due to deaths in young animals, loss of milk and meat and a decrease in productive performance. The cost incurred for control or eradication is quite high. Besides, there are major indirect losses due to the imposition of trade restrictions (Anonymous, 2001 and Rweyemamu & Leforban, 1999). In India alone, the direct losses due to FMD are estimated to the tune of Rs 60 million per year (Venkataramanan *et al.*, 2005).

History of the Disease :

The FMD has been around for many centuries. The history of research in FMD falls into several distinct areas (Brown, 2003). Although acceptable evidences exists that many of the great plagues of animals such as rabies and rinderpest existed for some thousands of years, FMD appears to be of recent origin (Henderson, 1978). The first written description of FMD probably dates back to 1514, when Fracastorius, Professor of Philosophy at University of Padua in Northern Italy described a similar disease of cattle in Italy (Fracastorius, 1546). This description was taken to be that of FMD by 'aficionados' of FMD where veterinary historians familiar with rinderpest believe that it described rinderpest (Fleming, 1871). In 1883, British losses were estimated at $5,000,000. In 1890, 431,000 cattle, 230,868 sheep and goats, and 153,808 swine were infected in Germany. The United States has experienced nine outbreaks of the Disease - the first in 1870 and the last in 1929. In all instances but two, the disease was eradicated within a few months. Almost 400 years subsequent to first reported description of FMD, Loeffler & Frosch (1897) demonstrated that a filterable agent (virus) caused FMD. This was the first demonstration that a disease of animals was caused by a filterable agent and ushered in the era of virology. The search for experimental laboratory animals, culminated in the demonstration by Wladmann and Pape of the susceptibility of guinea pig in 1920 and the sucking mouse by Skinner in 1951 (Brown, 2003). Subsequently, it was shown that the causative agent, FMD virus (FMDV), consists of a single-stranded, plus-sense RNA genome of approximately 8,500 bases surrounded by four structural proteins to form an icosahedral capsid (Rueckert, 1996).

Prevalence of the Disease :

The FMD, after World War II, was widely distributed throughout the world. In 1996, endemic areas were Asia, Africa, and parts of South America. Most European countries have been recognised as free from the disease. Countries belonging to the European Union have stopped FMD vaccination. North and Central America, Australia, New Zealand, Japan, and the British Isles have been free of FMD for many years.

The FMD is endemic in several South American countries (Astudillo *et al.*, 1997), most of Africa and the Middle East (type O and A), in Central and South-East Asia, the Indian subcontinent and probably in China (type O, A and Asia1). Some notable recent FMD outbreaks include Taiwan (1997-2001), Japan (2000), Korea (2000), South-Africa (2000), UK-France-The Netherlands (2001) which have all along been free until recently when they experienced FMD type O PanAsia strain outbreaks (Ryan, 2001).

Out of the 3 serotypes prevalent in India during the last decade (O, A and Asia 1), FMD type O only accounts for more than 85% of the outbreaks. It is clear from the data available (Anonymous, 1984-1985) that type O was the most prevalent during serotype during the last three decades in the country. During the last 9 years (1995-2003) type-O was responsible for about 85.5% of the confirmed outbreaks. The region-wise distribution of type O for the same period showed that in eastern and southern India it accounted for 86% and 92% outbreaks, followed by 84% in northern India and about 72% in western India. The disease due to serotype Asia 1 came next in the rate of incidence. During the period 1971-2002, FMD due to type Asia 1 was recorded in 18% of the confirmed outbreaks. During the last 9 years (1995-2003) Asia 1 accounted for 8.4% of the outbreaks in the country as a whole. The highest incidence of Asia 1 during this period was in the western parts of the country. Occurrence of disease due to type A was 11.5% during the period 1971-2002. During the last 9 years (1995-2003) disease due to type A was recorded only in 6.1% of the total outbreaks recorded in the country (Venkataramanan *et al.*, 2005).

In India, presently FMD virus serotypes O, Asia-1 and A are prevalent. Type C which was prevalent earlier has not been detected after 1995. Details of the serotypes involved are shown in table:

Table: Details of the Serotypes

Period	Serotypes			
	O	Asia 1	A	C
1971-1980	2,422	808	638	317
1981-1990	3,285	1,728	1,006	529
1991-1994	2,806	671	372	62
1995-2003	7,600	750	535	0
Total	16,113	3,957	2,551	908

Ref: Table adopted from Venkataramanan *et al.* (2005)

In India, FMD is endemic and occurs throughout the year. The disease out breaks due to serotypes O, A and Asia 1 are being reported every year, of which type O accounts for more than 85% of the outbreaks. Analysis of field strains of serotype O in the past has revealed genetic heterogeneity (Pattnaik *et al.,* 1998) and dominance of one of the genetic groups in causing disease outbreaks (Hemadri *et al.,* 2000).

Host Range :

The major host species for FMD are domestic cattle, buffaloes, sheep, goats and pigs. The African buffalo (*Syncerus caffer*) is considered to be the definitive host for the serotypes SAT 1 - 3. Natural infection with FMDV has been reported in several mammal species within the zoological families viz. Bovidae, Cervidae, Suidae, Tayasuidae, Camelidae, Giraffidae, Erinaceidae, Muridae, Elephantidae, Tapiridae and Ursidae (Federer, 1969 and Hedger, 1981).

The experimental infections resulting in typical lesions have been demonstrated in porcupines *(Hystrix galeata and Hystrix cristata)*, guinea pigs *(Cavia porcellus)* and wild rabbits (*Oryctolagus cuniculus*) (Hedger, 1981 and Thomson *et al.,* 2001). These animals may play a role in the epidemiology of FMD (Schaftenaar, 2001).

Carrier Status :

The carrier state of FMDV is an inapparent persistent infection, in which the intermittent recovery of virus from the orophyrynx is the sole means of detection. Persistent infection in cattle and buffalo, and to a lesser extent in sheep and goat, is a common sequel to both clinical and subclinical foot-and-mouth disease. It also commonly occurs in vaccinated animals following contact with FMDV (Salt, 1993).

The carrier cattle are defined as animals from which FMDV is intermittently recovered in probang fluid after 28 days following infection. FMDV RNA fragments have been detected in probang fluid samples after infectious virus can no longer be isolated (Rossi *et al.,* 1988).

Transmission :

The FMDV infection is often airborne and the virus may enter via the respiratory tract. FMDV can also enter the new host through abrasions of the epithelium of mouth, the skin of feet and udder. The peak of infectivity is just prior to or during the development of lesions. Infectivity is much reduced 3-4 days after the lesions develop. Some virus strains are host adapted. Although pigs are major producers of virus aerosols, cattle produce several times more viruses. Cattle are probably the main source of environmental FMD contamination.

The FMD is very infectious and the virus can be transmitted in many ways. Diseased animals excrete the virus in enormous quantities. The most common way of dissemination is by an infected live animal and contamination animal products. Indirect transmission can be by people, vehicles, equipment, hay or bedding contaminated with faeces or urine of diseased animals. Massive animal movements of all species, as a result of intensive animal husbandry practices are especially hazardous. Over the years, illegal activities have often been attributed to introductions of FMD into non-infected countries, such as the importation of infected meat and feeding of non-heat treated

swill to pigs, and the illegal trans-boundary movement of animals. Recently, the danger of spread of FMD by animal movement was clearly illustrated by a shipment of sheep from the UK that disseminated the virus to other animals in a rest-station in western France.

FMDV may be disseminated through direct contact with infected animals during transport, markets, shows, fairs, etc. and through indirect contacts such as farmers, veterinarians, inseminators, contaminated food, trucks used for the transport of livestock, etc. Other mechanisms involve the exposure of livestock to contaminated products such as meat, offal and milk. Calves drinking contaminated milk will become infected by this route (Donaldson, 1979). Milk trucks have also been implicated as an important source of virus spread (Sellers *et al.,* 1971). Pigs, consuming swill containing contaminated meat, organs and offal are particularly at risk. Secondary spread involving feeding of pigs with contaminated skimmed milk was involved in long distance spread to at least two location in 1967-68 in UK (Anon, 1969).

The FMD virus can be introduced into a free area by the following means :

- Direct or indirect contact with infected animals.
- Spread of aerosol from infected animals (requires proper humidity and temperature). A person in contact with infected animals can have sufficient FMDV in his or her respiratory tract for 24 hours to serve as a source of infection for susceptible animals.
- Feeding contaminated meat, milk, blood, glands, bones, cheese, etc.
- Contact with contaminated objects (hands, footwear, and clothing).
- Artificial insemination.
- Contaminated biologicals such as hormones (extraction procedure may not inactivate the virus).
- Vehicles used for transport of materials from one farm to the other Some strains of FMDV seem to have a predilection for certain species. There have been strains that affect pigs but not cattle. In South America, mature cattle have had clinical signs of FMD, when sheep in an adjacent pasture were normal.

Clinical Signs :

In sheep and goat, signs of FMD are less readily differentiated from those of the more common conditions. High infectivity and short incubation period cause a characteristic pattern of spread within the population unit. In cattle, the early clinical signs are much more definitive or suggestive than in pigs. In Dairy herd, several cows may suddenly show a dramatic drop in milk yield, go off feed, run high fever, and a little later start salivating profusely, saliva running from their mouth (slavering). The most common symptoms in cattle are slobbering and smacking the lips, shivering, tender feet with sores and blisters, raised body temperature, reduced milk yield and sore teats (Radostits *et al.,* 2000). Within a matter of hours, vesicles develop on the upper surface of the tongue, the dental pads, lips and muzzle. At first, they appear as whitish blisters 1cm to 2 cm in diameter, but these rapidly increase in size and may coalesce to cover large areas of the buccal mucosa and tongue. Rupture and leakage of fluid leaves the necrotic epithelium loose and its detachment reveals obvious areas of reddened erosions which are highly susceptible to bacterial infection (Adlakha, 2003).

Impact :

In India, the disease is endemic and occurs throughout the country and more than 5000 outbreaks are reported every year. All cloven footed animals viz. cattle, buffalo, sheep, goat, pigs, elephants and other ruminants are susceptible to FMD. The number of domestic animals alone susceptible to FMD are 518 million comprising cattle 222 million, buffaloes 95 million, sheep 59 million, goats 124 million, and pigs 18 million. Besides this, a large number of wild ungulates are also susceptible to the disease (Venkataramanan *et al.*, 2005).

Control and Prevention :

The strategies for control or eradication of disease from any geographical area are governed by a large number of factors. In countries where disease occurs as occasional epizootics, slaughter of all infected and in-contact animals is carried out by adopting stamping out policy as is the case in many FMD-free countries. However, in countries where the disease is enzootic like in many developing countries, the eradication of disease by slaughter is seldom feasible and vaccination is carried out to bring the incidence of disease to a level from where stamping out or eradication can be carried out. Control of FMD is usually achieved by mass vaccination of all susceptible livestock repeatedly at regular intervals till the disease incidence comes down to negligible levels. Several countries in Western Europe followed this strategy from early 1950s and successfully eradicated the disease, and from 1992 stopped the vaccinations altogether. These countries now follow stamping out method, in which all the affected and incontact animals are killed and disposed off in an outbreak to quickly control the disease. Though very expensive, this strategy has been used very widely in the developed countries now. In developing countries where the disease is endemic, repeated vaccination along with other control measures will be the best option to build up the herd immunity, which in turn, will eliminate the circulating virus from the population and bring down the incidence (Venkataramanan *et al.*, 2005).

In India, a national FMD control programme covering 54 districts spread over 8 states has been launched in 10th year Plan for creating FMD-free zones. Approx. 32 million cattle and buffaloes in these target districts are vaccinated twice a year. This programme will be model for further expansion of this activity to cover larger areas. In addition to this, vaccination is also carried out in different parts of the country, which covers a large number of animals (about 40 millions) but may not be focussed to all animals in a particular area. There has been a considerable increase in the vaccination of animals against FMD during the last three years and this trend is expected to increase further to cover more and more animals (Venkataramanan *et al.*, 2005).

Vaccines :

Two recent reviews on FMD (Sobrino *et al.*, 2001) and FMD vaccines (Brown, 1999) provide excellent account on disease situation and vaccines. Another review by Venkataramanan *et al.* (2005) gives detailed account of FMD situation in India, impact and strategies to be adopted for FMD control and eradication. Preparation of FMD conventional

vaccines requires high-containment facilities, as virus is grown in high quantities in tissue culture. FMD viruses included in conventional vaccines are chemically inactivated (Barteling & Vreeswijk, 1991). The inactivant formalin used commercially, was sometimes associated with insufficient inactivation and residual live virus (Beck & Strohmaier, 1987). This has not been reported with the use of binary ethyleneimine as an inactivant. Before formulation, it is important that virus cultures are well purified. This is also essential to be able to differentiate vaccinated from infected animals. The effectiveness of inactivated FMD vaccines depends on the addition of good adjuvants. $Al(OH)_3$/saponin (for ruminants) and incomplete oil-based formulations (for pigs and cattle) (Doel, 1999) have been widely used. Vaccine formulations frequently include viruses of different serotypes. One time vaccination does not prevent infection of ruminants with FMDV (Salt, 1993) and repeated vaccinations at regular intervals are required. The establishment of the carrier state is independent of the immune status of the animal at the time of exposure to infection, i.e., pre-existing immunity to FMD in the form of circulating antibody does not prevent animals becoming carriers. Transmission of clinical disease from carrier to susceptible cattle has never been demonstrated under experimental conditions. However, there is growing circumstantial evidence from Africa that transmission can occur from carrier cattle and buffalo to susceptible cattle with which they have close contact (Thomson, 1997). Many countries or international organisations such as the EU hold a strategic reserve (a bank) of FMD concentrated antigens stored in liquid nitrogen, which can be rapidly formulated onto vaccine. Exceptionally a bank can consist of ready formulated into vaccines. Unlike conventional vaccines, emergency vaccines are often of higher potency (= 6 protective dose 50 (PD50)) to ensure both rapid protective immunity (4 days) (Salt *et al.,* 1998 and Cox *et al.,* 1999) and greater cross-specificity.

Live attenuated strains, e.g. classical attenuated FMDV strains, obtained by the adaptation and further passages of virulent viruses in non-susceptible hosts (Sagedahl *et al.,* 1987) such as rabbits, are used as vaccines in China and some CIS-countries. The use of such vaccines is not without danger. The frequent reversion of attenuated viruses to virulent forms is well known (Mason *et al.,* 1997). Moreover, viral strains attenuated for a given host may be virulent for other natural hosts (Sagedahl *et al.,* 1987). New attenuated virus vaccines have been designated by genetic - engineering (Chinsangaram *et al.,* 1998). Chimeric viruses with parts of poliovirus and FMDV induced protection in natural hosts. A careful study of the safety, stability and pathogenicity of new recombinant vaccines is necessary before they can be considered for field trials.

Subunit Vaccines :

Animals immunised with the capsid protein VP1 induced neutralising antibodies and partial protection (Bachrach *et al.*, 1975 and Meloen & Barteling, 1986). The isolated VP1 has not exactly the same 3- dimensional structure as VP1 integrated in the capsid and this is probably the cause of the lower immunogenicity. Production of empty capsids in recombinant vectors for vaccine purposes has been studied by different groups. Empty capsids retain most

of the immunogenic and antigenic properties of viral particles. However, further work is required to improve the efficiency of empty capsid formation (Grubman *et al.,* 1993).

Peptide Vaccines :

The antigenic structure of the FMD virus has been studied in great detail. This has allowed the design of vaccines based on synthetic peptides corresponding to B cell epitopes identified on the viral capsid (Brown, 1988). The immunogenicity of these peptide constructs in a number of host species was lower as compared to conventional vaccines. Taboga *et al.* (1997) reported that different peptides induced low and partial protection to viral infection in cattle. However, in a great proportion of the lesions of the unprotected animals, FMDV mutants were detected. The use of mixtures of peptides covering different antigenic variants may decrease the chances of selection of escape mutants. Also the inclusion of immunodominant T cell epitopes in FMDV structural and non-structural proteins (Blanco *et al.,* 2000) could result in the design of safer and more effective anti-FMD peptide vaccines.

Expression of viral proteins in replicating vectors for a number of viruses, including some of the picornaviridae family, it has been reported that live vaccines induces a more efficient protective immunity than inactivated antigens (Usherwood & Nash, 1995). One strategy to achieve this goal is to present foreign antigens in a replicative form expressed from recombinant viral vectors. Sanz-Parra *et al.* (1999a, b) showed that recombinant adenovirus expressing P1 confers partial protection against FMDV challenge in cattle and pigs. The protection observed with this vector vaccine is likely to be mediated mainly by cellular immune response (Sanz-Parra *et al.,* 1999b) as it was elicited in the absence of detectable anti-viral antibodies. This is in clear contrast to observations with inactivated vaccines.

DNA Vaccines :

Ward *et al.* (1997) recently reported the induction of protection against FMD in swine immunised with a DNA vaccine containing a attenuated FMDV full-length infectious clone. The effectiveness of DNA vaccines may be increased by co-expression of FMDV immunogens and cytokines relevant to the induction of protective immunity (Lai & Bennett, 1998).

Major Constraints and Recommendation :

Major constraints for the control of FMD in India are lack of national coordinating agency, lack of national coordination in organizations dealing with research, epidemiological studies and field control, besides large population of susceptible livestock and wild life. Other major constraints include inadequate disease reporting system, availability of vaccine, cold chain maintenance, large scale unrestricted livestock movements throughout the country and unaffordable cost of the vaccine for the farmers. The farmers also don't realise the importance of vaccination and thus extensive extension programme is needed urgently to educate the farmers regarding cost effectiveness of FMD vaccination.

Future strategies of our country for control and eradication of FMD should include economic impact analysis for FMD, establishment of a national authority coordinating of all activities

pertaining to FMD, active involvement of the private sector in the planning and implementation of control programs, developing effective FMD surveillance and reporting systems, expanding diagnostic capabilities for virus identification and serotyping, implementation of mass vaccination programs as the primary method for reducing the incidence of disease to low levels and implementation of other control methods for individual outbreaks as appropriate.

In the last two decades several vaccinating countries have gradually moved from vaccination to a non-vaccination strategy supplemented with emergency vaccination. Whilst these countries rely primarily on slaughter, movement restrictions and zoosanitary measures for control, emergency vaccination will offer supportive measures in case an outbreak becomes more extensive. This emergency vaccination can be done as a ring vaccination or as region-country based protective vaccination at a certain distance around the FMD infected area to protect the animals or as a suppressive vaccination in the very close proximity of and towards the FMD herd for damping down the virus excretion and spread. In both cases, the vaccinated animals can be left to live and to enter the internal country food chain or can be culled and destroyed. For controlling a serious outbreak, vaccination has to be supplemented with strict controls of animals and humans movement.

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Foot-and-Mouth Disease in the North Eastern States of India

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The Foot-and-Mouth disease (FMD), a highly communicable viral disease of cloven-footed animals both domesticated and wild animals continues to be a global problem. The OIE (Office International des Epizooties) has placed the FMD on the top of the list A diseases that have the potential for very serious and rapid spread, irrespective of national border that are of serious socio-economic or public health consequences and are of major importance in the international trade of animals. The disease is considered to be one of the major hurdles in the growth of livestock industry of our country. The total annual loss due to milk and non-milk losses was estimated to be about Rs. 3463 crores annually. The average annual loss per animal due to the disease was calculated to be about Rs. 125. The loss is the highest (Rs. 854/- per animal) in cross bred cows followed by buffloes (Rs.250/- per animal) and in working bullocks it is about Rs.166/- per animal annually (Saxena,1994 a and Saxena, 1994 b). Besides, indirect losses due to the disease is enormous. The disease brings a restriction on world trade as the milk & milk products and meat & meat products of our country which are not accepted by the countries, free from this disease. Hence, the control of the disease in one of the top priorities, not only to reduce the economic losses but also to provide better health coverage for the high yielding cross bred animals, introduced through the massive livestock improvement programmes almost all over the country and also to protect the valuable wild animals, susceptible to this disease.

Epidemiology of FMD in Assam and NE States :
To study the epidemiology of FMD in Assam and other North Eastern (NE) states, the Indian Council of Agricultural Research (ICAR), New Delhi has set up a project called the All India Coordinated Research Project for Epidemiological studies on the Foot-and-Mouth disease (AICRP on FMD) during April 1973 at the Department of Microbiology, College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati. The work of the AICRP on FMD, Guwahati centre was reviewed by a mid-term appraisal committee during 1988 and the centre has been upgraded to a Regional Research Centre.

The centre has gathered valuable information, particularly on the prevalence of FMD virus types in the NE states, incidence of the disease, host susceptibility and seasonal prevalence, etc. In this article, an attempt has been made to review the works being carried out by the AICRP on FMD, Guwahati center, since January 1974 to March 2004.

FMD Outbreaks :
During January 1974 to March 2004, the centre

has recorded and studied a total of 1699 outbreaks of FMD in Assam and other NE States. The highest number of outbreaks (1330) was in Assam followed by 115 outbreaks in Meghalaya and 104 outbreaks in Sikkim . The number of outbreaks recorded and studied during the period are given in Table-1.

Table-I. FMD outbreaks recorded and studied in NE states including Sikkim during 1974-2004.

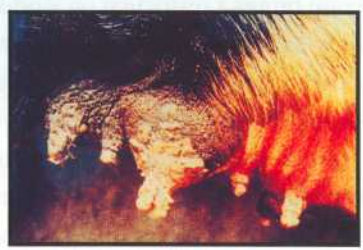
Name of the states	No. of FMD outbreaks recorded and studied
Arunachal Pradesh	43
Assam	1330
Manipur	25
Mizoram	26
Meghalaya	115
Nagaland	22
Sikkim	104
Tripura	34

FMD Virus Types :

Out of the 1699 outbreaks of FMD studied by the centre, the FMD virus types could be identified in 1222 (71.92%) samples of the outbreaks. From the remaining 477 (28.08%) outbreaks, the virus types could not be detected due to poor quality of samples.

FMD virus types O, A, Asia 1& C were found to be involved in the FMD outbreaks. The virus type 'O' was predominant and involved in 799 (65.38%) outbreaks. The other virus types viz. A, Asia 1& C were found in 214 (14.55 %), 142 (13.39%) and 67 (6.45%) outbreaks, respectively (Table-II). The incidence and distribution of FMD virus types in Assam and other NE states have been reported by different workers (Dutta *et al.*, 1984,Das *et al.*, 1988 and Sarma *et al.*, 1999).

During the last few years a change in incidence of FMD virus types was observed. The virus type C was not detected since 1993.The virus type O although is predominant and involved in 65.38% of the FMD outbreaks, but the incidence of FMD virus type 'A' has increased over type 'O' in 1999-2000, 2000-2001 and 2002-2003 in the FMD outbreaks studied in the NE states.



Vesicular lesions in the teats of a sow affected with FMD

Table- II. Prevalence of FMD virus types in NE states including Sikkim during 1974-2004.

FMD virus types	No. of FMD outbreaks with the virus type involved
O	799 (65.38)
A	214 (14.55)
Asia 1	142 (13.39)
C	67 (6.48)

Figures in the parenthesis, indicate per cent of the outbreak

FMD in Different Species :

FMD outbreaks in different species of animals during 1974-2004 in the NE states are shown in Table 3. The highest number (1591) of outbreaks were recorded in cattle, followed by 53 and 22 number of outbreaks in pigs and buffaloes, respectively. The outbreaks of FMD have also recorded in goat, mithun, deer, yak and elephants. occurrence of FMD in different species of animals in the NE states has been reported (Sarma *et al.,* 1989). Verma & Sarma (1997) reported series of FMD outbreaks in mithun (*Bos gaurus*) in 57 villages of Arunachal Pradesh during the period between June and February 1995. A total of 6239 mithun have been affected and 818 of the affected mithun died during the course of the outbreaks. The occurrence of the disease in elephant (*Elephas maximus*) of the Kaziranga National park, Assam has been reported by Sarma et al., 1994. The cattle were found to be the sources of infection for most of the FMD outbreaks, recorded in the semi-domesticated and wild animals. The involvement of more than one species in some of the FMD outbreaks has also been observed.

Table- III. FMD Outbreaks in Different Species of Animals of NE states during 1974-2004

Species of animal	No. of FMD outbreaks
Cattle	1591
Pig	53
Buffalo	22
Goat	9
Mithun	12
Deer	4
Yak	5
Elephant	3

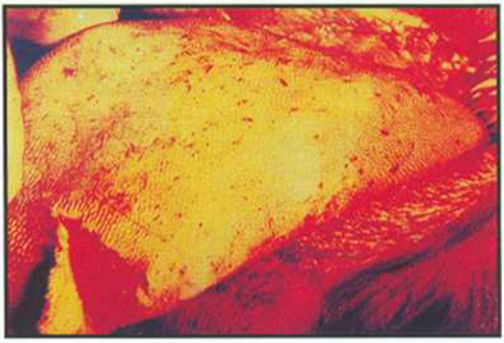
The FMD in Vaccinated and Unvaccinated Cattle :

The occurrence of FMD in vaccinated and unvaccinated cattle was studied during 1990 to March 2004. Out of the 781 outbreaks, 670(85.79%) were observed in unvaccinated cattle and only 111(14.21%) were in cattle, vaccinated against FMD in the NE states. The severity of the lesions and duration of the outbreaks were also more in unvaccinated cattle as compared to the vaccinated cattle. The regular vaccination against FMD has beneficial affect, as the duration of outbreak and severity of infection are less (Sarma & Hazarika, 1996).

FMD virus type 'O' was involved in all the FMD outbreaks recorded in pigs. The high mortality of the piglet recorded in the two outbreaks was mostly due to occurrence of the disease in the unvaccinated animals. The exposure of the unimmunized pregnant animals to FMD virus along with the stress involved have caused the heavy mortality (Sarma et al., 1994).

Table IV. The details of the animals affected and died in the FMD outbreaks in organised pig farms in Assam

Category of Pig	Number of Pigs affected / dies			
	Network project (Total No. of Pigs 252)		Base pig breeding farm (Total No. of Pigs 165)	
	Affected	**Died**	**Affected**	**Died**
Sow	44	Nil	36	Nil
Boar	17	Nil	8	Nil
Gilt	14	Nil	20	Nil
Piglet	108	102	57	57



Ruptured vesicle in tongue of a cow affected with FMD

FMD Outbreaks in Different Districts of Assam

FMD outbreaks recorded and studied by the centre in different district of Assam are shown in Table-V. Out of the 1330 outbreaks of FMD, studied in Assam during the period the highest number of outbreaks was in the Kamrup district (563) followed by Nagaon, Goalpara, Cachar and Darrang districts.The highest number of FMD in the Kamrup district is possibly due to high concentration of cross bred animals and greater mobility of man and animals in the district.

Most of the districts are connected by the river Brahmaputra, which may play an important role for spreading the disease during flood. Besides, some of the districts are connected with the neighouring states and there are migration of animals and human beings which also play role in spreading the disease.

Table -V. FMD outbreaks in different district of Assam during 1974-2004

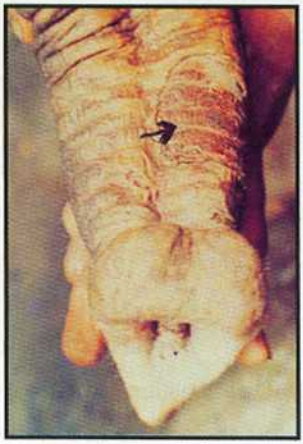
Sl. No.	District	No. of outbreaks
1	Kamrup	563
2	Nalbari	40
3	Barpeta	57
4	Goalpara	75
5	Bongaigaon	4
6	Dhubri	23
7	Kokrajhar	25
8	Darrang	74
9	Marigaon	27
10	Nagaon	95
11	Sonitpur	16
12	Golaghat	21
13	Jorhat	42
14	Lakhimpur	59
15	Dhemaji	21
16	Sibsagar	34
17	Dibrugarh	31
18	Tinsukia	11
19	Karbi Anglong	10
20	North Cachar	7
21	Cachar	75
22	Karimganj	14
23	Hailakandi	6

FMD in Wild and Semi-domesticated Animals :

The occurrence of FMD in different species of wild and semi-domesticated animals of the NE states has been reported by Barman *et al.*, 1999. During January 1974 to March 2004 the AICRP on FMD, Guwahati centre has recorded and studied a total of 24 outbreaks of FMD in different species of wild and semi-domesticated animals. The highest number (12) of outbreaks was studied in mithun where a morbidity and mortality of were recorded 22.9% and 6.5%, respectively. The Yak, which is an important semi-domesticated animals, prevalent mostly in Arunachal Pradesh and Sikkim, also suffered from the disease. The five outbreaks of FMD in the yak, studied by the centre the morbidity was found to be 24.51%. The outbreaks of FMD have also studied in spotted and samber deers in the Assam State Zoo and morbidity was recorded in spotted and samber deers 18.75% and 35.57% respectively. The occurrence of FMD have also been studied in wild buffaloes and elephant of the Kaziranga National Park. Although the cattle have been identified as important sources of infection for most of these wild and semi-domesticated animals, but the role of these animals in maintaining virus in the nature has to be studied. Besides, studies on the antibody response of these animals against FMD vaccination and the possible ways of controlling the disease in these susceptible animals species have to be undertaken.

Table VI : FMD outbreaks in wild and semi-domesticated animals in NE states during 1974-2004

Species of animal affected	No. of outbreaks studied	Morbidity percentage	Mortality percentage	FMD virus types identified			
				O	A	C	Asia-1
Mithun *(Bos gaurus)*	12	22.9	6.5	6	2	1	-
Yak *(Bos mutus)*	5	24.51	-	4	-	-	-
Elephant *(Elephas maximums)*	3	14.28	-	2	-	-	-
Samber deer *(Cervus unicorn)*	3	35.57	-	2	-	-	-
Spotted deer *(Axis axis)*	1	18.75	-	-	-	-	-
Wild buffalo *(bos taurus)*	1	4.47	-	-	1	-	-



FMD in Elephant : Ruptured vesicle on the trunk

FMD affected Mithun : Showing drooling of Saliva

Besides the above works on the epidemiological aspects of the disease, the AICRP on FMD Guwahati centre has undertaken studies on the effect of simultaneous vaccination of FMD with other vaccines, particulary FMD and swine fever vaccines in pigs, FMD and haemorrhagic septicaemia vaccines in cattle and FMD and enterotoxaemia vaccines in goat. Some of the works have already been published (Barman *et al.*, 2004 and Gogoi *et al.*, 2004). The protective antibody response of cattle vaccinated with commercially available oil adjuvant FMD vaccine with or without the use of immunomodulators has also been studied (Saikia *et al,*. 2004).The antigenic characterization of the FMD virus type O isolates from the FMD outbreaks in the NE states has also been studied (Sarma & Sarma 2005).

Foot-and-Mouth disease in the NE states is enzootic. The disease occurs regularly almost throughout the year and different species of animals both domesticated and wild animals. The mapping of the disease in NE states and calculation of economic loss due to the disease are important to carry out. A monitoring of the disease in the NE states in important because of a number of porous internationl borders. The control strategy of the disease in the NE states is important to undertaken because of large number susceptible livestock population and wild animals.

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Present Status of Foot-and-Mouth Disease in West Bengal with reference to General Concept of the Disease

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Foot-and-Mouth disease (FMD) remains the most feared and major scourge of cloven-footed domestic and wild animals like cattle, buffalo, pig, goat, sheep, deer, gayal, elephant and kangaroo, as well. The morbidity of the disease is extremely high (may be up to 100%) but mortality is low, generally below 5% but may reach up to 50% when the virus invades the heart muscles of younger animals. The disease is characterized by the formation of vesicles / blisters along with high rise of temperature (may be up to 105°F), followed by dullness, anorexia, salivation and drooling as well as smacking of lips. Vesicles appear on the tongue, gum, oral cavity, hooves, interdigital space, teats, udder, nostrils and sometimes in the ear and inner canthas of eyes. The vesicles subsequently rupture, leading to ulceration at all the sites, complicated by secondary bacterial infection resulting in lameness and mastitis and reduced feed intake with high rate of morbidity. Pregnant animals may abort and significant mortality takes place in young animals. The disease might be further complicated by concurrent invasion of Pasturella sp. causing hemorrhagic septicemia, thus increasing the rate of morbidity and mortality.

The virus exhibits its pathologic effect on some vital hormone glands, which control the metabolic functions in the body. Thus disordered function of the gland may lead to panting, restlessness, and reduced breeding capacity and draught power of bulls. Moreover, infection on udder and teat may progress to mastitis resulting into permanent milk loss. Thus, infected animals remain severely debilitated for a considerable period of time accompanied by permanent loss (~25%) of productivity. High rate of morbidity results in huge economic losses due to reduced animal productivity in terms of milk, meat, wool, hide and the draught power. FMD is the major disease constrains to international trades in livestock and animal products due to the embargo imposed by the FMD free countries like North and Central America, Australia, New Zealand, Caribbean Islands and several parts of Europe.

In India, the disease is endemic and occurs in all parts of the country throughout the year. Among the seven distinct serotypes, O, A, C and Asia-1 types are the causal agents for FMD outbreaks in West Bengal as well as in India up to 1995. Type C virus has not been recorded since 1996. Type "O" FMD virus (FMDV) is mainly responsible for most outbreaks (~85-90%) followed by types Asia-1, A. While studying the disease situation of the rest of the country including the neighboring states, the identical picture observed in the prevalence of different sero-types of the virus.

The infected animals are the major source of disease transmission. The FMD virus spread mainly through air or direct contact of infected animals, feed or fodder, utensils, transport

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vehicles, attendants etc. Cattle fair and markets also play major roles for spreading of the disease due to congregation of animals. The virus is excreted from the infected animal through saliva, nasal discharge, exhaled breath, milk, urine, semen, feces and vaginal discharges. In favourable day like wind and fogging winter day, it can spread up to 10 km from the place of origin and infect the animal within the radius. Pigs are known to excrete 3000 times more virus particles than cattle and hence play a major role as an "amplifier host" in spreading the disease.

Environments - External Habitats:

FMD virus has been recorded in a wide variety of temperate, sub-tropical and tropical climates. The virus may rapidly multiply and spread (causing a disease outbreak) wherever susceptible animals in close contact are exposed to the virus. Cloven-footed mammals (the main host species) exist in virtually all areas and so occasional outbreaks caused by introduction of the virus through import may occur in almost any habitat type. However, for a virus to become freely circulating, it must be able to exist in the environment, either inside or outside the host, for long enough to infect a susceptible host. Many viruses survive for only a short time outside their hosts, however FMD virus is one that may show a longer survival in the external environment. FMD virus is killed by prolonged exposure to sunlight (due to drying and heating) and rapidly by acids (such as citric acid, 0.2%, w/v, solution), and strong alkalis (such as washing soda, sodium carbonate, 4%, w/v, solution). However, it has been reported to remain infective for over 2.5 years in carrier cattle, for at least 5 years in African buffalo, for up to 9 months in sheep and goats, 2 months in carrier deer, 1 year in infected premises and in hides, 15 weeks on wood, hay and straw, 10-12 weeks on infected feed or clothing, 8 weeks in fragments of infected skin in winter, and 4 weeks on hair and soil particles.

Any habitat type with conditions that allow contact of susceptible species with any of the above (infected or carrier animals, animal products, other objects containing / carrying virus) within the indicated time scale will allow the virus to continue to circulate. The disease would thus become endemic.

Ways of FMD Virus Transmission:

FMD generally introduced by -

- The importation of infected susceptible species of livestock

- The importation of infected or contaminated meat, other livestock products (including semen for artificial insemination), hay, straw, dried grass, packing materials etc.

- Movement of people and their clothing, vehicles etc. from infected areas.

- Airborne movement of virus.

- Possibly by movement of birds, stray dogs, carrying infected materials from one place to another.

Chronic Problems occurred as a result of FMD Virus infection:

Persistent foot Infection: probably due to secondary infection (usually bacterial) and chronic damage of the tissues of the foot (including the hoof)

Prolonged Enteritis - persistent diarrhoea: probably due to secondary infection (usually bacterial) and chronic damage to the tissues lining the gut system.

Pneumonia: probably secondary to infection (usually bacterial) of lesions in tissue of the

upper respiratory system.

Permanent udder damage - reduced milk yield: probably due to secondary infection (usually bacterial) and chronic damage to the milk-producing tissues.

Diabetes mellitus: abnormally high blood sugar levels, probably due to damage to pancreatic (insulin-producing) tissue.

Chronic "Poor-Doing" - specific syndrome with panting (heat intolerance), anemia and long hair coat: probably due in part to damage to endocrine (hormone-producing) tissues and possibly heart muscle tissue.

Infertility : probably caused by various chronic tissue damage.

General poor condition and reduced growth rate : probably caused by various chronic tissue damages.

Table : District wise FMD virus type distribution during 1999-2003 in West Bengal. (From Reported Outbreaks)

Sl. No.	District	No. of Outbreak	Type Virus Involved				NVD (No Virus Detected)
			O	A	C	Asia1	
1.	Kolkata	3	2	1	-	-	-
2.	North 24 Parganas	69	57	2	-	4	6
3.	South 24 Parganas	45	32	1	-	3	9
4.	Howrah	48	39	2	-	2	5
5.	Hooghly	79	50	-	-	10	19
6.	Burdwan	54	42	1	-	3	8
7.	Nadia	102	75	3	-	3	21
8.	Midnapur East	16	9	-	-	1	6
9.	Midnapur West	82	58	1	-	10	13
10.	Bankura	26	16	3	-	2	5
11.	Birbhum	30	20	-	-	4	6
12.	Purulia	44	25	-	-	5	14
13.	Murshidabad	26	15	3	-	2	6
14.	Malda	23	19	2	-	-	2
15.	Dakshin Dinajpur	19	13	1	-	1	4
16.	Uttar Dinajpur	2	2	-	-	-	-
17.	Darjeeling	3	3	-	-	-	-
18.	Jalpaiguri	30	19	3	-	-	8
19.	Coochbehar	5	3	-	-	-	2
	Total	**706**	**499**	**23**	**-**	**50**	**134**
	Percentage	**100.00**	**70.68**	**3.26**	**-**	**7.08**	**18.98**

Though most often clinical signs are indicative of FMD, confirmatory laboratory diagnosis is essential to differentiate from other vesicular diseases like vesicular stomatitis (VS), swine vesicular disease (SVD) etc. For implementing an effective control measure prompt diagnosis is of utmost important. Diagnosis of viral agents is carried out by serological test like Enzyme-linked Immunosorbent Assay (ELISA). Presently, an indirect sandwich ELISA is used for confirmation of the disease as well as for virus serotyping directly from the field materials of the outbreak areas. For the detection of antibody to the structural proteins of the FMD virus in the serum, a " Liquid phase Blocking Sandwich ELISA" (LPBS-E) is adopted. Every serum sample must be tested for each of the four different types of virus. The test can also be used as a retrospective diagnostic technique to determine which types of virus are/have been present in an outbreak area.

Disease Control :

Although symptomatic treatment may reduce the severity of the clinical manifestation of the disease, but it does not prevent the spread of infection. In endemic countries like India, slaughter of infected animals is an impractical proposition due to economical and social bindings.

Following disease control measures are followed in outbreak areas of West Bengal to check the spread of the virus:

- Identification of affected animals
- Isolation / segregation of those animals
- Control of movements of the affected animals
- Restriction of movements of the Veterinarian / Para-veterinarian staffs and attendants
- Healthy one should be treated first before the affected one
- Strict hygienic measures along with ample use of 4% (w/v) washing soda solution

In West Bengal control of FMD is primarily possible through proper vaccination programme, application and monitoring of which definitely rely on the epidemiological surveillance of the disease. Vaccination against FMD in the state is in vogue for the last twenty years and is still continuing. But in proportion to the population, vaccination was undertaken in only meager number of useful, productive, crossbred and exotic animals due to paucity of fund and at the same time non-availability of vaccines. Previously, aqueous/gel polyvalent vaccines were used in the FMD control programme. But due to short-lived immunity, the oil adjuvant polyvalent vaccines have replaced gel vaccines, which are expected to have a better grip on the disease.

Foot-and-Mouth Disease Outbreaks in South Andaman

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Andaman-Nicobar island is located in the bay of Bengal at 92^0 - 94^0 East longitudes and 6^0 - 14^0 North latitude. Though there are about 556 islands only 38 islands are inhabited. There are two districts i.e. Andaman and Nicober in the islands and total area of Andaman & Nicobar is 8249 Sq km and about 90% of the area is covered by forest. South Andaman is one of two Sub-divisions of Andaman districts. In south Andaman, there are two tahasils i.e. Ferrargunj and Portblair with an area of 1085 and 2021 Sq km, respectively. The area of Portblair municipality is 16.64 Sq km. Foot-and-Mouth Disease (FMD) outbreaks occurred in the part of Ferrargunj and Portblair tahasils and Portblair municipality only.

Under the directorate of Animal Husbandry and Veterinary Services of Andaman and Nicobar administration there are a good number of veterinary hospitals, dispensaries and sub-dispensaries, disease diagnostic laboratories, artificial insemination centers, and farms these are located in different corners of the districts. Regarding the animal husbandry practice, the animal keepers are mostly poor and marginal farmers and improved husbandry practice was not observed during this investigation. Very few animals were stall-fed and most of the animals were used to roam freely in search of food and fodder. In urban areas animals were used to move and graze in seashore and roadside area and also in the vicinity of the market.

History of FMD Outbreaks in Andaman :
The island is said to be free from FMD for the last two decades. Before 1980, there was a report of outbreak in Andaman due to transportation of animal for meat purpose from Kolkata. Although an incidence of the said disease was reported in Dollygunj farm in South Andaman during 1988 and it was investigated that the cause of outbreak was the entry of some new crossed-breed animals from the mainland of Kerala. The spread of the outbreak was restricted to Dollygunj area due to immediate ring vaccination measures adopted for containment of the FMD. Since then, it was not reported elsewhere in Andaman and Nicober islands till the Tsunami in December 2004.

Identification of First Case of the Present Outbreaks :
It was revealed at the meeting with all the veterinary surgeons of South Andaman in Junglighat veterinary hospital on 21.04.05 that the FMD first occurred in the area of Delanipur, Anarkali and South Point under Portblair municipality.

Methods and Investigation :
The Epidemiological data during investigation were collected by retrospective household survey of the affected areas in Portblair municipality and parts of South Andaman.

- Location
- Onset of illness (specific date)
- Animal affected with age, breed and sex
- Susceptible population at risk
- Number of death and affected
- Sign and symptoms exhibited by the affected animals
- Type of feed and fodder used by the owner
- Vaccination status
- Last occurrence of illness by the same type of disease in the area
- Entry of new animals in house or locality, if any
- Stall fed or grazing outside (movement of animals)

Distribution of the Disease Incidence :

The disease spread rapidly in different places under Portbalir municipality and Portblair tahasils within a radius of 10 km form the village of Anarkali. The places affected were

Raptured vesicle in the mouth of cow, affected with FMD

FMD affected Cattle : Showing drooling of Saliva

Anarkali, Delanipur, Bunyadabad, Premnagar, Lillipur, Abedeen village, South Point, Sadhipur, Nayagaon, Chkragaon, Austinabad, Brookshabad, Brijgang, Lambaline, and Dairyfarm.

All groups of animals like cattle buffalo, goat and pig irrespective of age, sex and breed were affected, But mostly young animals were died. In the caprine populations mild infection was noticed. The distribution of morbidity and mortality in different species are given in the Table.

Analysis of Data :

Clinical data : On analysis of clinical records exhibited by the affected animals i.e. rise of temperature (103^0 - 104^0 F), lacremation, inapptitance, drooling of saliva,

Table. Epidemiological data of Food-and-Mouth Disease outbreaks in South Andaman

Location of outbreak		No. of animal affected (% of morbidity)					No. of animal died (% of mortality)					Susceptible Population					Sq. Km involved
Tahasil	Villages	Cattle	Buffaloes	Goats	Pigs	Total	Cattle	Buffaloes	Goats	Pigs	Total	Cattle	Buffaloes	Goats	Pigs	Total	
Port Blair Tahasil & Municipa -lity	Anarkali Delanipur South Point Sadhipur, Dairy Farm Austinabad Brookshaba & Surrounding area	554 (17.5)	105 (8.25)	673 (18.08)	216 (41.0)	1548 (17.82)	06 (0.19)	02 (0.16)	04 (0.11)	16 (8.73)	58 (0.67)	3164	1273	3722	527	8686	35
Ferrer -gunj	Cauddlegunj Ferrergunj Aniket Mthra Portmout & Surrounding area	729 (21.55)	249 (19.59)	305 (12.17)	0	1283 (17.45)	03 (0.09)	01 (0.08)	0	0	04 (0.05)	3382	1271	2506	192	7351	30

characteristics smacking sound, ulcerative lesion of tongue, lips, gums, dental pad, lameness due to ulceration on the skin of inter digital space were found. Duration of illness was recorded as 5-20 days depending on the severity of infection and management practice followed by the owner. As per clinical signs and pathgnomic lesions of affected animals, the cases were suspected for FMD. After typing of virus in the laboratory of AICRP on FMD in IAH & WB, Kolkata, it was confirmed that the disease was FMD and type 'O' virus was involved for the of disease.

Laboratory Data :

The random sera samples, which were collected from unvaccinated healthy animals, were tested in the said laboratory by liquid phase blocking ELISA technique. At the serological examination, no antibody of FMD virus was detected from 28 sera samples of the healthy animals of neighboring village of the affected places. On the otherhand 16 sera samples were detected positive to the FMD virus antibodsy against the 'O' type.

Interpretation of Data and Source of Infection :

The comprenension picture of outbreaks indicate that the infection of FMD came from outside of the South Andaman. As per information of first occurrence in the animals of Anarkali it revealed that the animals picked up the infection from seashore and coast guard area of Bunyadabad or from surrounding area through free movement of animals in search of food.

In epidemiological investigation process of the current FMD episode of Andaman and Nicober Islands several hypothetical sources were considered to be responsible for transmission of the disease. The sources that were considered responsible for transmission of the disease are follows :

- Entry of new infected animals in the islands from outside of endemic region.
- Fodder like hay straw from the mainland after Tsunami as relief materials.
- Supply of cattle feeds like blocks; concentrate feedbags, etc. as relief items.
- Transported canned meat, raw meat, and other meat product.
- Through milk and milk product.
- Persons coming from mainland for relief work at tsunami affected area.
- Cloth and garments imported from main -land as relief items.
- Floating infected carcasses of FMD en demic zone of mainland.
- Through air land and over sea from main land.

Disease Control :

The basic strategy of control measures is to reduce of the number of frequency of the outbreaks of the disease in the island. The methodologies of control strategy will be implemented adopting following approaches:

- Prevention of transmission of the disease which has already been introduced within the Island.
- Prevention of transmission disease from outside of the island i.e. from mainland.
- Evolution of control strategy periodically.

Acknowledgments :

The investigators are thankful to Dr. B. Mahato, Director of Animal Husbandry & Veterinary Services, Andaman and Nicobar Islands for providing all possible help to conduct the epidemiological investigation work. Sincere thanks are also due to Dr. Lokenathan for his constant help and participation during investigation process. Thanks are also due to Dr. P. Balakrishnan, Dr. Chaubey and all other veterinary surgeons and veterinary assistant surgeons for their sincere help to complete the investigation work.

Review of the Status of Foot-And-Mouth Disease and Approaches to Control

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Foot-and-Mouth Disease in Wild Life : An Overview

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The wildlife is becoming of increasing economic and aesthetic importance in many countries. Due to expanding human population the areas available to free living wild animals have greatly reduced as a result wild animals are coming in contact with domestic animals and the hazards of disease transmission from wild animals to domestic animals and from domestic to wild animals have increased. There has long been interest in the interrelationship between diseases in domestic stock and wild animals. Foot-and-Mouth disease (FMD) is considered as one of the most important infectious diseases not only for cloven-footed domestic animals, but also for wild animals. Nearly 70 wild species both free living and captive within the families of mammals have been found to be susceptible to either natural or experimental infection with FMD virus. Some of the important species of wild and zoo animals where natural infection of FMD has been confirmed are Impala *(Aepyceros* melampus), Black buck *(Antilope cervicapra)*, Bisonmigai *(Boselaphus tragocamelus)*, Ibex (*Capraibex* spp.), blue wild beast (*Connochaetes taurinus*), Antelope (*Hippotragus* spp.), Gemsbok (*Oryxgazell* spp.), African buffalo (*Syncerus caffer*), Spotted deer (*Axis axis*), Samber (*Cervus unicolor*), Wart hog (*Phacochoerus aethiopicus*), Wild boar (*Sus scrofa*), Giraffe (*Giraffa camelopardalis*), Indian elephant (*Elephus maximus*), Tapir (*Tapirus* spp.), Kangaroo (*Maavcropus* spp.), etc., In this article an attempt has been made to review the disease in wildlife particularly in India.

FMD in Free Living Wild Animals :

In India, an entire herd of gaur at Manikgarh in Hyderabad was perished due to FMD and in a similar epidemic hundreds of wild species died in Warangal district (Ali Salim, 1935). Occurrence of FMD in Biligiri range hills of Karnataka caused death of 15 to 20 mithun and infection has spread from cattle which come in contact with the animals of the forest due to lack of grazing ground in the nearby villages. Severe outbreaks of FMD in mithun been reported by Verma & Sarma (1997). The outbreaks were recorded in Arunachal Pradesh, India. The morbidity and mortality due to the disease in the species was as high as 42.08% and 16.5%, respectively and the source of infection was detected to be the FMD affected cattle of nearby areas. In North Eastern states of India, Barman *et al.* (1999) have reported an incidence of 22.90% and the virus serotype O was involved in majority of the outbreaks.

FMD in Yak has been reported for the first time in a village located in the Indo-Tibetan border district of Kannaur of Himachal Pradesh during July 1977 (Prasad *et al.,* 1978). In 1981, three outbreaks of FMD in Yak in Sikkim have been reported (Katiyar *et al.,* 1981). During 1974 to 1997 four outbreaks of FMD in Yak have reported from the North Eastern states, India by Barman *et al.* (1999).

FMD in Captive Wild Species :

The occurrence of FMD in nilgais (*Boselaphus tragocamelus*) at Van Vihar, Nagpur was reported in December 1972. The disease was characterized by lameness, abortion and death

of the affected animals. Necropsy lesions of the dead animals were: the cyanosis of the eye conjunctiva, pulmonary congestion and oedema and engorgement of subcutaneous veins and yellow streaks in heart giving tigerold appereance in the young. Ulcers on tongue and dental pads were also noticed and FMD virus type A was identified to be the cause of the outbreak (Mukhopadhyay *et al.,* 1975). Kar *et al.* (1983) reported outbreak of FMD in cevides and antelopes in the biological park, Bhubaneswar, Orissa. The disease was first observed in black buck and then in spotted deer and sambers. FMD virus type O was involved in the outbreak and domestic cattle grazing nearby the park were considered to be the source of infection.

During 1985, FMD was reported in nilgais and gazelles (*Gazella gezella*) in deer park, Hisar, Haryana and the spread of infection was reported to be from domestic cattle via attendants residing in the vicinity of the park (Ahuja *et al.,* 1985). Three outbreaks of the FMD, out of which, two in samber deer and one in spotted deer of the Assam State Zoo, Guwahati have been recorded (Barman *et al.,* 1999). The morbidity in samber deer was 35.57% and in spotted deer the morbidity was 18.75%. The sources of infection in the outbreaks were reported to be the feed and the attendants coming from nearby villages. Singh (1988) has reported the occurrence of FMD in male blue bull (Boselaphus tragoamelus) and male hog deer (Axis porcinus) in Patiala deer park, Punjab.

FMD in captive elephants has been reported by a number of workers in India.On 21st February, 1988 a six year old cow elephant of the Zoological garden Kolkata suffered from FMD and the virus type O has been identified, but the source of infection could not be traced (Chakraborty & Mazumder, 1990). In 1990, FMD in three adult elephants of the wildlife sanctuary, Jaldapara, West Bengal was reported. FMD virus type was also identified as the cause of the outbreak (Maity *et al.*, 1991). Occurrence of FMD in a baby elephant of the Kaziranga National Park, Assam was reported. Severe erosive lesions in foot pad, trunk and small vesicles in tongue and rima oris of the affected animal were observed. FMD virus type O was identified as the cause and the feed and water contaminated by FMD affected cattle were the sources of infection (Sarma *et al.,* 1994).

North Eastern states, in India are considered to be the hot spot of biodiversity. The states have large number of wild and semidomesticated animals, which are susceptible to FMD virus infection. Protection of the animals from the disease is not only important from the economic view point, but also for the conservation of the animal species.

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Foot-and-Mouth Disease : A Solemn Dilemma for Livestock and the Humans

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The Foot-and-Mouth disease (FMD) is well known as the solemn dilemma for the livestock industry. The disease is one of the most devasfating as it results declined productivity in cloven-footed animals. Globalization of markets means the global scope for those diseases, which were hitherto circumscribed to the specific regions and the FMD has already the status of generalized distribution with their 60 subtypes from 7 immunologically distinct types of FMD virus.

FMD has a very rare history in human beings with an incident of mild, short lived and self-limiting this disease in Great Britain. It was in 1966, when this case was recorded with the general effects and similarities with influenza with some blisters (Anonymous) were found. However an unrelated condition occurred in humans called hand, foot and mouth disease (HFMD). An outbreak with mild febrile disease first ever was identified in the year 1957 in Toronto, Canada. It was characterized by papulovesicular lesions on the skin and in the mouth. The name HFMD was first used in 1960 during a similar outbreak in Brimingham, England (Tindall & Miller, 1972). Recently the same disease has been reported in Calicut, India (Sasidharan *et al.*, 2005).

In relation with FMD and HFMD, both the diseases affect mainly young ones. Pan-Asian strains of the O-type of FMD virus and Enterovirus-71strain of HFMD, which are highly pathogenic, have recorded their wide geographical spread across the Asia. The clinical entity recorded in FMD and HFMD is also somewhat comparable with each other. Though the host specification is an obligation for FMD and HFMD but it is consequential to explore their relationship to build a sustainable future. Beside the wide scope over the issue, FMD keeps a concern of the future of livestock industry. Hence it is into consideration to make a tremendous beneficial endeavor in maintaining the success index in globalize modern practice of farming.

Severity of FMD :

The FMD is a highly contagious viral disease affects sheep, goat, cattle, pigs and deer. It is found more severely in cattle causing fever and vesicles in the mouth and on the feet. It results into lameness and decreased feed intake. General lassitude may be observed along with or without foot lesions. Abortion or death of young calves, chronic reduction in fertility and milk yield are notable. Death rates due to the FMD have exceeded 2 % in adults and 20 % in young stock. Its prolonged convalescence causes severe losses in production and health, cripples livestock industry and severely inhibits travel and tourism. It is a hurdle in retaining the internationally meat export license, economic development, and the alleviation of poverty.

About the Virus :

After special laboratory tests seven types of Immunologically distinct FMD virus is familiar, which is single stranded RNA virus belongs to the family Picronaviridae, Genus Apthovirus viz. A, O, C, SAT1, SAT2, SAT3 and Asia1. Among which sixty subtypes have been identified. Recently observed Pan-Asian strain of the O-type FMD virus seems to be highly pathogenic and rapidly spreading through out the world. It is a very stable virus. It can survive up to 1 year in the environment, 10-12 weeks on clothing and feed and 30 days on hair. It can also survive in flash pasteurization of dairy products. But sunlight, boiling and autoclaving can destroy the virus rapidly. Disinfectants do have little effect on it. However, sodium carbonate (4%), formalin (1-2%), and sodium hydroxide (1-2 %) destroy the virus within few seconds. Multiple tests are being used for their identification, which includes virus neutralization, compliment fixation tests, experimental infection, tissue culture, PCR techniques and ELISA tests. The virus survives in lymph nodes and bone marrow at neutral pH. It is destroyed in muscle when pH turns <6 i. e. after rigor mortis. It also localizes in the cardiac muscles of young ones. Depending upon the temperature and pH, it can persist in contaminated fodder and the environment for up to a month.

Spread of Virus :

The virus spread by inhalation or ingestion. The virus also spread by contaminated semen, meat and milk products. Rapid spread would be the consequence of inhalation i.e. airborne virus. Virus spread has been estimated to be as far as 62 miles. Wind, moderate temperature, overcast skies and high humidity appear to enhance windborne spread. The virus also persists at a low level in the absence of clinical disease with cycling between infected and susceptible animals. Variety of species, include elephants, coypu, rodents, capybara, hedgehogs, birds and wild ruminants may not show clinical signs, but may anchorage the virus to allow later spread of the infection to susceptible species. Goats and sheep also serve as a repository and carriers of the disease. Imported meat from infected areas appears to have been the origin of infection. Swine, as a host releases viral particles1500 times greater than that of produced by cattle. Any animal may act as a vehicle to spread the virus.

Clinical Findings :

The High fever, low milk production, anorexia, excessive salivation are the usual findings. Foot lesions usually finds on the bulb of the heel, along the coronet, and in the interdigital space. Oral lesions are less common, generally occur on the back of the dorsal surface of the tongue rather the tip. Lesions may also occur on the buccal mucosa, dental pad, lips, vulva, prepuce and ruminal mucosa. Due to the myocardial lesions sudden death may occur in young ones. Lameness and diarrhea, occasionally with blood are also the prominent findings.

Diagnosis :

The diagnosis of the FMD can be the easy task after clinical findings but the virus remains at a low level in infected and susceptible animals without any clinical symptoms. The diagnosis should be specific, rapid and accurate. In sheep and goats the lesions found less pronounced,

even the foot lesions may go unrecognizance. Death of young stock may occurs due to the acute degeneration of myocardial cell fibers, where striped appearance on the heart muscle may noticed, which termed as 'tiger heart' (Graham, 1959). The typical tattered lesion on rumen pillars may also be noticed (Cottral & Callis, 1980). The agalactia in milking sheep and goats would be the feature. In the pigs, the severe foot lesions may develop on concrete ground. High mortality in piglets recorded frequently. Blisters established on the tongue, dental pad, gums, cheek, hard and soft palate, lips, nostrils, muzzle, coronary bands, teats, udder, snout of pigs, corium of dewclaws and interdigital spaces. Clinically, much identical features may be eminent with the vesicular stomatitis, swine vesicular disease and the vesicular exanthema of swine. Some other diseases also provide the collaborative clinical features with FMD, those are rinderpest, mucosal disease, infectious bovine rhinotracheitis, bluetongue, bovine papular stomatitis, bovine mammillitis and the bovine viral diarrhoea.

The laboratory diagnosis can provide the significant aid to confirm its presence where animal inoculation test, compliment fixation test, neutralization test, agar gel diffusion precipitation test, fluorescent antibody test, ELISA and tissue culture may be the valuable one. Molecular biology is proficient to detect the genetic characterization, the physiology of a virus can be defined and the origin and movement of an outbreak can be precisely tracked. A molecular genetic test called PCR (polymerase chain reaction) may also be used in 'fingerprinting' the virus.

Trend of Treatments :

The clinical manifestations can only elucidate the symptomatic treatments. No specific treatment can be made available for FMD. Antibiotic mouth wash and the same line applications are needed on foot lesions and lesions on the mammary tissues.

Vaccine and its Limitations :

It is a known speculation about the vaccines for FMD that, the vaccinated animals are not 100% resistant to the disease and can emit the virus. The vaccination may reduce the clinical symptoms of FMD, neither it stamp out the infection nor it prevent the carrier state. Immunologically different types of FMD virus has important implications, as vaccines developed for one strain may not be effective in protecting the disease due to another strain. Hence, vaccines must be multivalent (several serotypes) in most endemic regions. In the recent trends, it is impossible to distinguish the vaccinated animals and the animals suffering from FMD. The ring vaccination around infected zones is necessary to prevent the further transmission.Vaccination against the disease also affects the FMD free status, which may be the cause for ban in international trade prospects. The vaccination of susceptible animals to FMD would require a huge financial budget and the tremendous efforts, which is not painless task Vaccination against the FMD in Western Europe and United Kingdom has been considered as a means of rapid containment of the disease and in North America vaccination against FMD is illegal.

Biosecurity, Issue of Future :

It is an essential phenomenon to minimize the

spread of the disease. However, FMD virus can spread on the wind therefore its stamping out is difficult but could help to reduce the risk. The important bisecurity measures are as under.

- People and Vehicles can be restricted due to their potential source of contamination.
- Essential visitors may be permitted with the provisions of boot and clothing at the entrance of farm.
- Visitor must be convinced to shower but ensure washed hand atleast.
- Possible limitation in the movement of people should be employed.
- Foot dips with disinfectant at the correct dilution in all the entrance should be needed.
- Pig movement should be minimized.
- Revision of disinfectant and cleaning procedures can be undertaken. Cleaned and disinfected vehicles can only be permitted in the farm.
- Loading ramps need special attention. Provision of designated boots for the use on loading ramps only is needed.
- Feeding and watering utensils must be cleaned thoroughly.
- Any residual organic materials can be removed after the surfactant spray of disinfectant or the emulsifying agents.
- Ensure the highest standard of hygiene maintenance.
- Livestock must keep away from household waste, bones or swill.
- Carcass disposal method should be by burial, burning and rendering.

Research in India :

Among the seven immunologically dissimilar strain types of FMD only four i.e. O, A, C, and Asia1 were ever recorded in India. ICAR had initiated a small project at Mukteswar (Campus of IVRI) with four regional centers, in the year 1971 to point out the involved strain and to know the prevalence and distribution of the disease in country.

Initiative of cross breeding of indigenous with exotics has also insisted the incidence of FMD and ultimately the project activity also. In these days the self-regulating Project Directorate on FMD, under the control of ICAR is working with 8 regional cooperating centers and 15 network units. These centers/units perform the laboratory diagnosis and sero-typing of suspected specimen by sandwich ELISA (LPBE). In the 85% of outbreaks, the causative serotype was 'O' was followed by 'A' with about 8-10% and the rest due to Asia 1, while serotype 'C' has not been recorded since 1995. A molecular epidemiological study has been shown that the Pan-Asian strain is the foremost cause of serotype 'O' involving FMD outbreak. Government of Denmark had provided the technical collaboration and started vaccine production in the year 1974 which is now working with three plants. They provide the facility for supply of diagnostic reagents, training and the research.

Virtually, there is no risk to human health from FMD. As the disease is highly infectious, the suffering country may loose its license for meat export. Control and eradication of any epizootic diseases would not be the only interests of developing countries but it would diminish the risk of the spread of such diseases across the globe.

Almost 95% animals recover within 2 weeks with little or without treatment. The virulence varies with the infecting strain and the variable virulence may exhibit due to the different virus species of the FMD. Vaccination may or may not prevent the infection of FMD or the establishment of the carriers. The costs associated with eradication or control can be high but in addition, imposition of trade restriction causes for surpassing indirect losses. FMD is a global threat. Early reporting of FMD results into the less severe economic losses. Success of their control and eradication needs more assistance to work on the problem in the endemic areas. Increasing awareness from the public and of governments would be the prime compulsion to empower the need to strengthen veterinary services to face this dilemma of FMD.

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Foot-and-Mouth Disease in Zoo and Other Animals

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"Those who give themselves up to the Lord do more for the world than all the so-called workers."

- Swami Vivekanadna

"In the beginning, to be sure, this world was water, nothing but a sea of water. The waters desired, "How can we be propagated" They kindled their own ardour, performming this very act with fervour. While summoning their creative energy they warmed up and a golden egg was produced."

- Satapatha Brahmana

Appropriate Samples for Diagnosis of Foot-and-Mouth Disease

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FMD-an overview :

Foot-and-Mouth Disease (FMD) is a viral disease of cloven-hoofed animals including cattle, buffalo, sheep, goat, pig, camel and deer. Humans rarely may become infected but only mild infection occurs. It is most contagious disease of animals causing severe epidemics and designated in OIE list A diseases. The disease is caused by an aphthovirus (family Picornaviridae), which has seven serotypes O, A, C, Asia-1 and Southern African Territories (SAT 1, SAT 2, SAT 3) and more than 80 immunologically and serologically distinct subtypes within these strains. Out of these seven serotypes, type O (most prevalent), A, C and Asia 1 are present in India. Virus is capable of infinite mutation, so new antigenically different subtypes are constantly appearing. There is no cross immunity between different serotypes; so immunity to one type does not confer immunity against any of the other six types.

The clinical signs particularly fluid-filled blisters/ vesicles on the tongue and in the mouth causing excessive salivation and lameness. The disease should be differentiated from vesicular stomatitis, vesicular exanthema, swine vesicular disease, blue tongue, pox infections, rinderpest & mucosal disease. The clinical symptoms in most of these diseases are quite similar so laboratory diagnosis of any suspected FMD case is therefore, a matter of urgency that will reduce the chances of further spread.

The laboratory diagnosis relies on collection and submission of appropriate samples so that disease outbreak can be successfully controlled and surveillance programmes on vaccination can be effectively monitored. The preferred tissue for FMD diagnosis is epithelium from unruptured or freshly ruptured vesicles.

Epithelium/ Vesicular Material :

The definitive diagnosis of FMD is based on the demonstration of virus or antigen in tissue samples. The preferred sample for diagnosis of FMD is epithelium from un-ruptured or freshly ruptured vesicles. The samples can be collected using sterilized blade from the tongue, dental pad, teats or feet lesions. It is advisable to take samples from many affected animals at a time as some samples may have low concentration of virus. The area should be washed with water to clear the food contents in the mouth or dung / soil from the feet but disinfectants should not be used for cleaning. Pigs and sheep can be sedated prior to sample collection. Collect as large a piece of epithelium as possible (minimum 5 gm epithelium). If un-ruptured vesicles are found, the virus-rich straw-colored vesicular fluid can be withdrawn with a syringe and put in transport medium (50% glycerol saline or 50% glycerol and phosphate buffer, preferably having antibiotics).

Somatic Tissues: The somatic tissues such as muscle and lymph may contain low quantity of virus antigen. But in young animals dying from myocardial form, the virus is present in

quite high concentration in the heart muscle. In such cases, necrotic myocardial tissue (present as 'tiger stripes' on the heart) from freshly dead animal should be collected in 50% glycerol saline or phosphate buffered glycerin (PBG).

Milk :

The virus excretion in milk is quite high for up to 4 days before the onset of clinical signs to several days after typical lesions appear. It is therefore useful to collect milk from lactating animals in which FMD is suspected. Milk should be collected into a plain glass tube and kept at refrigeration temperature until submitted to the laboratory.

For long period storage, milk samples should be frozen to prevent inactivation of the virus.

Esophagopharyngeal fluid:

In ruminants the pharynx, particularly the dorsal soft palate and the cranial esophagus are the sites of primary replication and virus persistence in the carrier animal. It is therefore possible to isolate FMD virus from esophagopharyngeal fluids under certain circumstances. There is no significance of collecting this fluid in pigs as these do not become carriers.

Blood:

Blood may be collected for virus isolation in tissue culture or antibody detection. For virus isolation whole un-clotted blood (one IU heparin per ml of blood; or 1mg of Ethylene diantine tetra acetic acid (EDTA) powder per ml of blood.) should be collected under sterile conditions. The whole blood should be collected from cattle which are in late phase of the incubation period and having fever and in sheep having less vesicular lesions. For antibody detection serum samples should be collected (minimum 5 ml serum; the vials containing blood are kept in a slanting position to allow the blood to clot. The serum is removed and transferred to clean tube/ vial).

Submission of Samples :

A strong glass container should be used with a metal screw cap fitted with a strong rubber washer. It should be taped around the cap to prevent leakage of fluid. The sufficient information regarding identification of the material should be written on a piece of adhesive tape attached to the bottle. The bottle should be wrapped in absorbent cotton and put in a snugly fitted container. Till transport, the sample should be kept refrigerated. It is important to avoid thawing and refreezing, so packing should be done quickly and stored at -70°C. Serum samples can be submitted without refrigeration, but refrigeration or freezing prevents the spoilage of samples.

Laboratory Diagnosis :

Virus/Antigen Detection :

The definitive diagnosis of disease is based on the demonstration of virus or antigen in tissue samples. Earlier, the complement fixation test (CFT) was test of choice for antigen detection but it was relatively insensitive.

Antigen Detection ELISA :

An indirect sandwich enzyme-linked immunosorbent assay (ELISA) approved by the World Reference Laboratory for FMD at Pirbright (UK) determines the presence of FMD virus antigen. The choice of subtypes to be detected depends on the virus strains prevalent in particular geographical area. Ideally, the ELISA can be used in combination with virus isolation to amplify low amounts of antigen that can not be detected by sandwich ELISA or the samples can be submitted from more number

of affected animals from the herd to increase the chance of detection.

Virus Isolation in Tissue Culture :
Tissue samples from infected animals may not contain sufficient antigen to give a positive identification of FMD virus in the antigen detection ELISA. Live virus in original tissue samples, however, may be amplified by culture on susceptible monolayer cell sheets to produce antigen-rich supernatant fluids suitable for this test. The presence of FMD virus in culture is determined by the observation of virus-specific cytopathic effect (CPE) after inoculation.

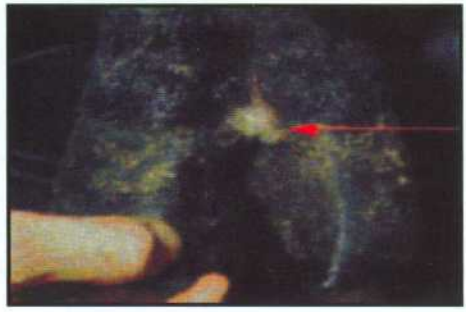
Polymerase Chain Reaction :
The Reverse Transcription Polymerase Chain Reaction (RT-PCR) is an extremely sensitive method of detecting FMD viral genome. The detection of viral genome has the advantage that viable virus or intact viral antigen is not required but there may be false-positive reactions.

Antibody Detection:
Virus neutralization test (VNT) and the liquid phase blocking ELISA are prescribed by OIE for FMD antibody detection. Liquid phase blocking ELISA is mostly used as the screening test due to its high sensitivity and the VNT is used as the confirmatory test (gold standard test) owing to its high specificity. The detection of specific antibody to FMD virus is evidence of previous exposure to FMD viral antigens but both these tests cannot differentiate between previously vaccinated and infected animals as these tests measure antibody to the structural capsid proteins of the virus which may be induced both by vaccination and infection. Now recently developed tests measure antibody to the non-structural (NS) proteins and are capable of differentiating vaccinated and previously infected animals.

Foot-and-Mouth Disease in Bovine

Bovine : Salivation and nasal discharge

Bovine : Extensive loss of epithelium on first day of clinical disease



Bovine : An unruptured vesicle at the anterior aspect of the interdigital cleft, one day after the onset of clinical signs.

Detection and Isolation of Foot-and-Mouth Disease Virus from Fresh and Frozen Meats

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India has a vast livestock population, which is the largest in the world. The livestock presently slaughtered in India includes 1.93 million cattle, 10.5 million buffaloes, 17.7 million sheep, 40.5 million goats and 23 million pigs per annum (Haleem & Rizwana, 1998). Mumbai has Asia's largest abattoir where about 10,000 animals are slaughtered daily for domestic consumption as well as for export. The total annual meat production in India is estimated to be about 1.2 million tonnes inclusive of private and government owned slaughterhouses. All this meat does not enter the domestic market and therefore, meat export forms one of the major sources of income for the country.

The far East and Middle East countries are the major markets for India, but following OIE guidelines wherein screening meats for pathogens before exports to any country is mandatory. As a risk-mitigation measure, screening of meats is carried out for several pathogens one of which i.e., Foot-and-Mouth Disease Virus (FMDV) has been described here. The identification of viruses from meats requires more specialized techniques than those for bacteria and fungi, as viruses are intra-cellular and are present in minute quantities within the tissues. Secondly, they do not alter the organoleptic qualities and neither they may cause any overt changes in the appearance of the meats. In the literature screened so far, there are no records on the isolation of viruses from frozen buffalo meat and meats of apparently healthy animals. Therefore the present study was conducted to compare the suitability of various serological tests and Polymeres Chain Recation (PCR) for the detection of FMDV from meats of buffalo, cattle, sheep, goat and pig.

Material and Methods :

A total of 253 samples comprising of 129 muscles, 54 hearts, 58 lymph nodes and 12 spleens were collected from buffaloes (67), cattle (74), sheep (25), goats (46) and pigs (41). These included 15 frozen buffalo meat samples collected from the retail outlets and 18 samples (6 meats, 8 hearts and 4 lymph nodes) from clinical cases of FMD in cattle. These samples were brought to the laboratory on ice in sterile containers and processed to make 10% suspensions in phosphate buffered saline (PBS). The suspensions were centrifuged at 3000 rpm and one part of the supernatant was stored in small aliquots at -20°C. The second part was subjected to chloroform treatment and the extracts were stored at -20°C till further testing.

Tests for Detection of FMD Virus :

(a) Micro Complement Fixation Test (MCFT): The test was carried out as described by Rai & Rao (1977) on chloroform extracts of the samples and also with the infected cell culture fluids.

(b) Sandwich ELISA: The sandwich ELISA was performed at the Disease Investigation Section, Pune which is one of the centres for the All India Co-ordinated Research project on FMD, as per the procedure of Venkataramanan (1988) on the PBS extracts as well as the infected cell culture fluids. The optical density was taken in an ELISA reader and the maximum OD obtained at 492 nm after taking into consideration of the controls.

(c) Suckling Mouse Inoculation Test (SMIT): The test was carried out on the PBS extracts of the samples as described by Rai & Rao (1977). Swiss albino suckling mice, 3-5 days old were used for inoculation. One litter (10-12 mice) was used for each sample among which one mouse was marked and kept as uninoculated control.

(d) Polymerase Chain Reaction (PCR): The test was carried out at the Indian Veterinary Research Institute, Bangalore as described by Suryanarayana *et al.*,(1996). Tissue samples collected from Deonar slaughter house were screened by mCFT and those found positive were subjected to PCR. The PCR products were analysed by gel electrophoresis along with marker DNA (lambda phage) using 1 % agarose. The PCR products were southern blotted onto nylon membranes and hybridized with a 35S radiolabelled (0.6kb) probe and autoradiographed.

(e) Isolation on Tissue Culture: The extracts / were found positive by mCFT and Sandwich ELISA in actively growing monolayer of BHK-21 cells obtained from National Center of Cell Sciences, Pune.

Results :

FMDV was detected and isolated from muscles and other organs of cattle, buffalo, sheep, goat and pig by mCFT, sandwich ELISA and growth in BHK 21 cells.

(a) Micro Complement Fixation Test: A total of 144 muscle, 69 heart, 58 lymph node and 12 spleen samples were subjected to mCFT for preliminary detection of FMDV from chloroform extracts of samples of buffalo, cattle, sheep, goat and pig. Out of these, 28 muscle, 10 heart, 9 lymph node and one spleen samples were found positive for FMDV. Virus was not detected in 8 muscle samples by direct mCFT but was recovered in cell culture. From cell culture fluids, virus was detected in 24 muscles, 7 hearts and 4 lymph nodes by MmCFT.

The virus serotype findings are summarized below species-wise:

Cattle: Mainly O serotype from infected meat, A and Asia 1 from meats of slaughter houses, were detected by mCFT.

Buffalo: From frozen meat, serotype O; from meat of slaughter houses serotypes O, A and Asia 1 were detected in chloroform extracts as well as in infected cell culture fluids by mCFT.

Sheep: The Serotypes O, A and Asia1 were detected in chloroform extracts and infected cell culture fluids by mCFT.

Goat: The serotypes A, O and Asia 1 were detected in chloroform extracts and in cell culture fluids by mCFT.

Pigs: The serotypes O and A were detected in chloroform extracts by mCFT and only serotype O was recovered in cell culture and confirmed by mCFT.

(b) Sandwich ELISA: FMDV was not detected by sandwich ELISA in the PBS extracts but was detected in cell culture fluids in which serotypes O and Asia 1 could be detected. A few samples showed mixed infection of the two serotypes.

The species-wise findings are summarised Below:

Cattle: The virus was detected in 5/10 muscle samples, 2 lymph nodes and one heart sample from slaughter houses. The serotypes O and Asia 1 were recovered in the samples.

Buffalo: The virus was recovered from one meat and one heart sample from slaughterhouse and 3/5 frozen meat samples. The serotypes O and Asia 1 were recovered from these samples.

Sheep: The virus was detected in 2/4 muscle samples and Asia 1 was recovered from these samples.

Goat: FMDV serotype Asia1 was detected in samples of one lymph node and one spleen.

Pig: The FMDV was detected in 2 muscle and one heart sample and both O and Asia1 serotypes were identified by ELISA.

(c) Suckling mouse inoculation test (SMIT): The FMDV was detected in meat from various sources using SMIT.

The findings are summarised below according to the animal species:

Cattle: A total of 7 muscle, one heart and 2 lymph node samples had viable virus by SMIT out of 18 positive by mCFT. The serotypes identified include 4 with O, 3 with Asia 1 and 3 with A from the various tissues.

Buffalo : Out of a total of 11 tissues positive for the presence of FMDV by mCFT, 9 samples contained live virus as detected by SMIT, which included 5 muscles, 3 heart and 2 lymph node samples. Altogether, 7 O, 1 A and 1 Asia 1 were identified.

Sheep: Out of 6 tissues positive by mCFT, only one tissue had viable virus detected by SMIT and that belonged to A serotype.

Goat: Out of 9 samples positive by mCFT, 4 samples had viable virus as detected by SMIT, which consisted of 1 muscle, 1 heart and 2 lymph nodes. In these tissues, 2 O and 2 Asia 1 serotypes were identified.

Pig: Out of 4 tissues positive by mCFT, only one tissue had live virus as detected by SMIT i.e. one muscle sample having O serotype of FMDV.

(d) Polymerase Chain Reaction: PCR was performed on samples of cattle, buffalo, sheep and pig collected from slaughterhouse.

Cattle : The viral RNA was detected in 7 muscle and one-heart samples out of 13 samples subjected to PCR. Out of these, virus was recovered in tissue culture in 3/7 muscles and 1/1 heart sample as detected by ELISA, indicating the specificity of the test in detecting FMDV from meat samples.

Buffalo: The viral RNA was detected from one sample each of muscle and heart tissues, out of

which viable virus was recovered from muscle only and it was serotype "Asia 1".

Sheep: Out of the 6 samples tested by PCR, only 3 were found to contain viral RNA and viable virus was detected in one muscle sample only. Viral RNA was detected in one muscle in which virus was detected only by mCFT and not by ELISA. Only in one muscle sample viable virus was detected both by ELISA and mCFT but no viral RNA could be detected. This may be due to total degradation of viral RNA while processing. In the rest of the samples no viral RNA or viral antigens could be detected by PCR and ELISA respectively. In one of the sheep muscle sample neither viral RNA nor viable virus was detected by any of the tests, indicating a good correlation between the 3 tests.

Pig: In 2/4 samples viral RNA was detected by PCR and virus was isolated only from one heart sample. From the other sample (muscle) no virus was isolated, although viral RNA was detected by PCR and virus was detected in the chloroform extract by mCFT. In 2 samples, viable virus could be detected by mCFT and ELISA but no viral RNA was detected by PCR.

(e) Cytopathic effects of FMDV in BHK 21-cell culture : The CPE such as cell rounding, cell aggregation, pyknotic nuclei, syncytia and cell death and bar shaped eosinophilic inclusions were seen. A total of 56 samples were subjected to virus isolation on BHK 21 cells, out of which 35 samples showed viable virus as detected by characteristic CPE described earlier.

Discussion :

The meats are normally derived from ripened carcasses wherein the carcass has undergone maturing at 4°C for 12 hours. During this process the viruses are exposed to lactic acid, thereby inhibiting their multiplication (Astudillo *et al.,* 1998).

Nevertheless, FMDV was isolated from muscles and tissues of naturally or experimentally infected animals (Drieux 1975). This issue was discussed at national and international forums with special emphasis on export of buffalo meat. There was a controversy as to whether the viruses could be transmitted through meats, especially with reference to deboned, deglanded frozen buffalo meats subjected to plate freezing at -40°C for 8 hours followed by storage at -20°C before export or domestic consumption. Attempts were made to isolate viruses from processed beef wherein a series of concentration and filtration steps were used (Cliver *et al.,* 1983) and flexible pouch processing was also employed for the purpose (Blackwell *et.al.,* 1982). However, since viruses are intracellular and are released on disruption of cells, the warring blender was used in the present study to homogenize the meat. This method was found to be effective in releasing the virus from the meat, thereby simplifying the virus isolation process. Therefore, the present study was undertaken to develop a suitable protocol for virus isolation and identification in order to assess if viruses are transmitted through meat.

From the results of meat testing, it can be seen that FMDV survives in frozen meat, meat of infected animals and those of apparently healthy animals slaughtered at the abattoir. The

FMDV also survives in other organs such as lymph node and heart muscle. The presence of FMDV has been extensively recorded in meat of experimentally (Cox *et al.*, 1961) and naturally infected cattle (Drieux, 1975), but there is hardly any record of the presence of FMDV in deboned and deglanded frozen buffalo meat. Nonetheless, based on risk assessment studies the probabilities of transmission of FMDV through frozen buffalo meat have been established (Dazo & Benings 1999).

The FMDV serotypes were detected from PBS extracts by mCFT but not by ELISA. The reason for the lack of sensitivity of ELISA may be due to the predominance of 12S subunits rather than 146S units which may constitute only a minor fraction of FMDV antigens in the samples (Venkataramanan, 1988 and Suryanarayana *et al.*, 1996). This phenomenon may also be due to exclusive use of 146S guinea pig sera in the test (Paltnaik & Venkataramanan, 1984). In meat samples, the presence of serous exudates may have also resulted in a greater opportunity for antigen-antibody complex formation thereby blocking antigen detection both by mCFT and ELISA (Oliver *et al.*, 1988). Hence a combined use of mCFT and ELISA has been advocated, since the percentage of typed samples increased considerably when both tests were used in combination instead of singly (Hamblin *et al.*, 1984). It was also observed that maximum sensitivity and type specificity cannot be achieved within the same diagnostic test (Have *et al.*, 1984). Therefore, virus isolation was used as a definitive test of FMDV infectivity and was the most sensitive procedure for detection of live FMDV.

Another important observation was the presence of two serotypes of FMDV in some of the samples of cattle and pigs. Such multiple infected samples are common when samples are screened by indirect sandwich ELISA (Ferris *et al.*,1995). Apart from the World Reference Laboratory, Pirbright, most workers in India (Srinivasan, *et al.*,1992), Bangladesh (Rahman, *et al.*,1991), Saudi Arabia (Hafez *et al.*,1993) and Thailand (Blakshell *et al.*,1994) found sandwich ELISA more sensitive than mCFT in detection of virus from clinical samples and use of cell culture as the most efficient means of virus isolation, with further confirmation by sandwich ELISA.

SMIT is one of the reliable tests in detection and confirmation of FMDV (Rai & Rao 1977 and Skinner 1951). FMDV has also been isolated from tissues of experimentally infected pigs after storage for 55 days at -20°C in suckling mice (Wittman, 1957).

From most of the samples found positive for FMDV by mCFT, the virus could be recovered in BHK 21 cells by 24-48 hours post-infection (PI). The FMDV isolates so obtained were also confirmed by ELISA. Among the FMDV types encountered, O was the most common, followed by Asia 1 and A respectively. The CPE such as rounding of cells and syncytia were seen by 24 hours PI and there was detachment of the cell sheet by 48 hours PI. The stages of CPE such as margination of chromatin, bar shaped eosinophilic inclusions and pyknotic nuclei characteristic of FMDV were seen in the infected BHK 21 cells (Polatnick & Wool, 1983) by MayGruenwald Giemsa staining. CPE on fibroblastic cells of hamster kidney could

be detected within 16-24 hours PI and completed by 72 hours PI. Therefore, BHK 21 was found suitable for isolation of FMDV from meat and other organs of infected cattle and of other species obtained from different sources.

The BHK 21 was also used in earlier studies for isolation of FMDV from beef for studying the survival time of FMDV in uncooked and cooked beef products (Garcia Widal *et al.*, 1988). FMDV was detected in tissues of cattle, buffalo, sheep, goat and pig indicating the wide spectrum of hosts susceptible to the virus and its implications in the role of the virus in evolving a carrier status in the above mentioned species. India being endemic for FMDV, spread of the virus occurs irrespective of vaccination. Several factors such as the epidemiology and persistence of FMDV in the animals are responsible for the development of carriers, which play an important role in the spread of FMDV through meat (Kitching, 1992).

The serotype 'Asia1' was isolated from meat and other organs of cattle, buffalo, sheep and goat but not from pigs whereas type 'O' was isolated from all species. These animals were from the abattoir and were apparently healthy at the time of slaughter at antemortem inspection and hence isolation of FMDV from these animals indicated that they were preclinically infected (Sharma & Murthy, 1981) or they were carriers. Virus was also detected in the muscle of incontact pigs 20 hours before rise in temperature or the appearance of aphthae, suggesting a carrier status (Dhennin *et al.*, 1979).

All the types grew well in BHK 21 cells and the results correlated well with those of SMIT and mCFT. Highest percentage of tissues positive for O was from buffalo and that of Asia 1 from cattle. The overall percentage of tissues from various species with O serotype was highest, followed by Asia 1.

With the advent of newer specific techniques such as PCR minute quantities of the virus genome can be amplified to an amount detectable by radiolabelled probe or by restriction enzyme digests or by direct sequencing and therefore can be used instead of ELISA for the diagnosis of FMDV (Kitching, 1992). Since muscle is not the site of prediliction of FMDV, titers of virus may have been too low for detection by ELISA (Maan *et al.*, 1998) and hence virus was not detected in the tissue extracts by ELISA. Secondly, detection of FMDV by serological tests and virus isolation are time consuming and use of either test may not be reliable enough to confirm the diagnosis. Under such circumstances, PCR has shown to be of great value in detection of viral RNA from meat and other tissues (Rodriguez *et al.*, 1993). The results of PCR correlated well with isolation of FMDV on BHK 21 cells. Reverse transcriptase (RT) PCR has simplified the detection of FMDV-RNA from tissue samples making it more sensitive than ELISA (Rai & Rao, 1977).

Since pre-and-post PCR contamination of the product with other extraneous DNA is possible, PCR coupled with hybridisation with a specific radiolabelled probe, (0.6 kb) increased the specificity of the test as also confirmed in beef of experimentally infected cattle (Rodriguez *et*

al., 1993). In the present study, 15 out of 24 samples of meat and other organs from slaughterhouses contained FMDV-RNA, which correlated well with other tests. The Dot-blot hybridization signals also confirmed the presence of FMDV in the meat and other tissues. The probe used for hybridization was from the highly conserved region of the FMDV genome i.e. the 3D site for all 4 serotypes consisting of 620 bp and inserted in phagemed bluescript SK+. The probe was radiolabelled with 35S and used for hybridization. On dot-blot hybridization, the probe specifically hybridised with the DNA in 9 samples, probably in the rest of the samples, the quantity of cDNA was less and therefore, could not be observed. Using the primer pair of P1 and SV1 the presence of FMDV nucleic acid in meat and other organs could be detected.

The use of PCR for confirmation of FMD-RNA from meat of freshly slaughtered animals may be the first attempt since, from the literature reviewed so far, none of the workers have used PCR for the above purpose. The ability of PCR to reliably and rapidly obtain a positive or negative result in one or two days is a significant advantage particularly as the free exchange of biological and animal products is of increasing importance.

The presence of live virus in frozen meat indicated that FMD virus survived freezing at -20°C since it was recovered in cell culture. The use of susceptible cell culture proved to be easy and cost effective for isolation of FMD virus. Since the FMD virus strains isolated in the present study were virulent or infectious, their detection in meat focuses on the endemicity and the carrier status that this disease produces in a population, which may or may not have undergone vaccination prior to slaughter. Hence, ELISA can be adopted as a screening test for FMDV, at abattoirs to screen the carcasses and those testing positive may subsequently be further tested for viable virus in cell cultures. Only steps taken towards eradication of these diseases may help to alleviate this problem to some extent and reduce the restrictions imposed on the trade of meat to countries free of these diseases.

The elimination of FMD virus by suitable techniques such as irradiation treatment and use of chemical sanitizers in combination with irradiation opens up a new area of future research.

Acknowledgements :

The authors wish to acknowledge Agricultural Products Export Development Authority, New Delhi and Dr.A.T. Sherilar, Vice-Chancellor, Maharashtra Animal and Fishery Sciences University, Nagpur for financial support and facilities provided for the project.

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Foot-and-Mouth Disease Outbreaks in Vaccinated and Unvaccinated Cattle Herds

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The Food-and-Mouth Disease (FMD) is one of the most devastating disease of farm animals. Although the disease rarely leads to death, but high morbidity causes loss in productivity to the extent of 25 per cent. The disease is endemic in India and virus types 'O', 'A', 'C' and Asia 1 were identified in various outbreaks through out the country. To contain the disease, in our country, regular vaccination is only option. However, inspite of regular vaccination FMD outbreaks were record in immunized cattle herds (Goal & Rai, 1984 and Sarma & Hazarika, 1996). The present study was, therefore, designed to identify the virus associated with FMD outbreaks in vaccinated as well as in un-vaccinated cattle. Further, immune status of the susceptible animal was evaluated.

Materials and Methods :

FMD Outbreaks :

A total 34 of outbreaks occuring in the organized and un-organized cattle herds were attended. The outbreaks were categorised on the basis of vaccination history as vaccinated infected (10) and un-vaccinated infected (24). In vaccinated groups, the cattle were vaccinated at 4 months interval and the last vaccination was done 2-4 months earlier. The feet as well as tongue epithelia were collected from the affected animals in 50% phosphate glycerine buffer (pH 7.6) and preserved at 4^0 C until further processing The paired sera samples were collected at an interval of 21 days to estimate antibody titre.

Detection of Virus :

An approximately 10% tissue suspension was prepared in phosphate buffer Saline (PBS) and treated with chloroform. The sample was finally centrifuged at 3000 rpm for 30 minutes and supernatant was used to detect the antigen as well as to isolate the virus. Micro-complement fixation test (micro-CFT), following the method of Rai *et. al.*, (1980) and sandwich ELISA (Crowther & Abu Elzein, 1979) were used to detect viral antigens.

Isolation of Virus :

The FMD virus was isolated from the field sample in suckling swiss albino mice as well as in calf kidney cell monolayer. For each sample, a litter of mice was used and 0.1 ml of inoculum was given i.m. in the back muscle. The healthy cell monolayer grown in the bottle was inoculated with 1ml of inoculum. The presence of virus in infected mice and in cell culture fluid was confirmed by sandwich ELISA.

Assay of Antibody :

The virus specific antibody response was studied in the vaccinated infected, un-vaccinated infected and vaccinated un-affected cattle. Randomly selected 10 animals from each

group were taken for this study. The virus specific antibody titre in the paired sera samples was determined by the method of Abu Elzein & Crowther (1981). Viral antigen was added to the wells, coated with rabbit anti-FMD antibody. Test cattle sera diluted serially were added to the wells, followed by rabbit anti-bovine peroxidase conjugate. The colour reaction was read after adding substrate solution (OPD + H_2O_2). The corrected OD value of the highest dilution of the serum ≥ 0.1 was considered as titre.

Results and Discussion :

A total of 34 FMD outbreaks were attended and FMD virus types O was identified both in vaccinated and un-vaccinated cattle. Out of 53 tissue samples (23 of tongue epithelia and 30 of feet epithelia), viral antigen was detected by micro-CFT in 38 (71.7%) samples and by ELISA in 43 (81.1%) samples. The percent positive by ELISA in tongue epithelia and in-feet epithelia was 73.9% & 86.6%, respectively. The results of the typing of the samples, clearly indicate that the prevalent type 'O' virus was involved in all outbreaks. However, it is essential to characterize the antigenic make-up of type 'O' virus involved in vaccinated as well as in un-vaccinated herds. Comparing the tests for detection of viral antigens in clinical samples, ELISA was found more sensitive (Oliver *et al.*,1988). Further, the presents findings showed that viral antigen could be detected in feet epithelia, collected even after end of their disease course. Scott *et al.* (1966) mentioned in their findings that FMD virus persisted for long period in foot epithelia.

For isolation of the virus, a total of 15 clinical samples were processed. The rate of recovery of virus type 'O' was 40% (6/15) in mice and 60% (9/15) in primary calf -kidney cell culture. Primary cell culture provided uniform physiological condition in-vitro and thus, facilitated rapid propagation of the virus (Rai & Rao, 1977 and Anon, 1995).

The type 'O' virus specific antibody titres in affected animals are presented in the Table. The mean ELISA titre in affected cattle was quite lower (~80.0) than that of vaccinated un-vaccinated cattle (360.0). In the second serum samples, antibody titre increased ~4 fold in both the infected groups. Outbreaks of FMD in regularly vaccinated herds were reported by other workers (Goel & Rai, 1977and Sarma & Hazarika, 1996).

The breakdown of immunity was not only due to poor antigenic mass but also attributed to other factors associated with the hosts (Jana & Maity, 1997). However, to ascertain the actual cause of poor yield of antibody titre in vaccinated animals needed elaborative study.

Table: Mean (± SE) ELISA antibody titre against FMDV type 'O' in paired sera of vaccinated and un-vaccinated cattle.

Day post-infection	Mean ElLISA antibody titre		
	Vaccinated infected	Un- vaccinated infected	Vaccinated un-affected
1	80±10.95	70±10.95	360±35.78
21	280±43.82	320±4380	320±4380

Acknowledgements :

Authors are grateful to the Dean, Faculty of Veterinary Science, Assam Agricultural University, Khanapara, and Gawahati for providing necessary facilities, to Central FMD virus typing laboratory, IVRI, Mukteswar, Kumaon for providing anti-sera and reference vaccine virus.

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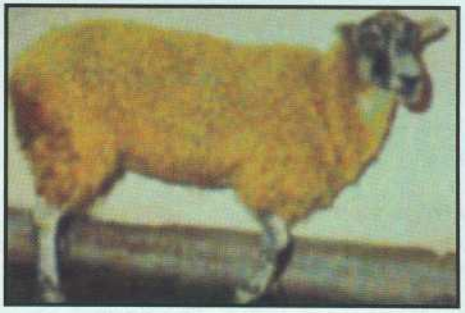
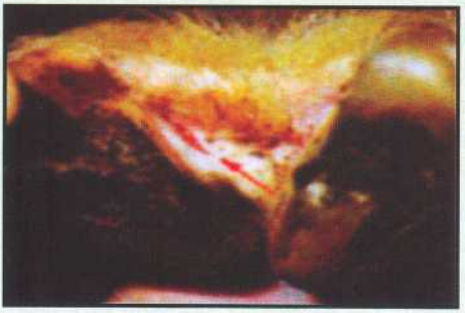
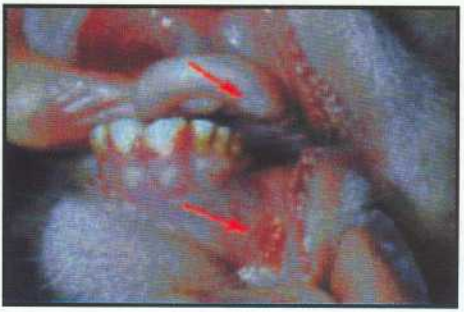
Foot-and-Mouth Disease in Sheep



Sheep : Lameness



Sheep Foot Lesions



Sheep Mouth Lesions

Evaluation of Protective Antibody Response in Mithun (*Bos frontalis domesticus*) after FMD Vaccination

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Introduction :

Mithun(*Bos frontalis domesticus*) is an important animal species in the hill regions of the North East India. The animal has an important role in the economic, social, cultural and religious life of the local tribal people. It is not only the source of meat and milk, but also the useful draught or pack animal on the steep hilly slopes. Though Mithuns are comparatively hardy, but foot-and-mouth disease(FMD) is considered as one of the most important infectious disease of the animal species. Severe outbreaks of FMD in Mithun has also been reported (Verma & Sarma,1997).Since vaccination can be the only way to prevent the disease in Mithun a trial was undertaken to evaluate the protective antibody response in mithun after FMD vaccination.

Materials and Methods :

Twenty healthy mithun of the National Research Centre(NRC), Mithun, Medziphema, Nagaland were selected for the trial. The animals were kept in stall fed and well management conditions. Commercially available tetravalent FMD vaccine (**Clovax®** from Intervet India Pvt. Ltd.) was used to vaccinate the animals as per the dose and route recommended by the manufacturer.

Serum samples from all the animals were collected on the day of vaccination and 28 days after vaccination. All the serum samples were tested for FMD virus types specific antibodies by liquid phase blocking ELISA as per the method of Hamblin *et al.* (1986) with slight modifications. Briefly the test procedure includes serial two fold dilution of each of the test serum samples from 1:16 to 1:128 in microtitre plastic plates and mixing in equal volume with each of the pretitrated FMD vaccine virus strains O, A, Asia 1 and C (IVRI) cell culture propagated antigen(OD value =1).After overnight incubation of the plates at refrigerator temperature the serum virus mixture was transferred to ELISA plates coated with FMD virus types specific antibody raised in rabbit and incubated for 1 hr. at 37°C.After washing of the plates FMD virus type specific tracing antibody raised in guineapig and diluted appropriately in blocking buffer(Phosphate buffer saline containing 0.01% Tween 20, 3% lactalbumin hydrolysate and 5% each of foetal calf and normal rabbit sera)was added and again incubated for 1 hr. at 37°C. Anti-guineapig IgG horse radish peroxidase conjugate (DAKOPAT) diluted 1:2000 in the blocking buffer was added after washing of the plates and further incubated for 1 hr. at 37°C. Finally the chromogen substrate (OPD +H_2O_2) mixture was added after washing of the plates and the reaction was observed after 10 minutes incubation at 37°C. Reading of the plates was taken in ELISA reader(DYNATECH) using 490 nm filter. The titre of each of the serum samples was calculated as reciprocal of the highest dilution showing 50% inhibition of OD value compared to the antigen control well. Any of the serum

samples showing a titre of 1:128($Log_{10}^{2.1}$) was considered as protective antibody titre against the FMD virus type.

Results and Discussion :

Results of the antibody titre in the serum samples of the vaccinated animals at 0 and 28 days post vaccination are given in the table. None of the serum samples on the day of vaccination showed antibody titre of 1:128. A few serum sample however showed antibody titre of 1.8 Log_{10} against the virus serotypes and this may be due to vaccination of the animals against the disease before 9 months of the trial. The serum samples at 28 day of the vaccination showed the presence of 2.1 Log_{10} antibody titre in 17(85%)samples against FMD virus serotype O and A and the same titre in 14(70%) samples against the virus serotype Asia 1. Only 5(25%) serum samples showed the protective antibody titre against the virus type C (Table).

Various workers(Hamblin *et al.*,1987 and Periolo *et al.*,1993) have reported that a titre of 2.1 Log_{10} or more in liquid phase blocking ELISA is 100% protective against FMD virus challenge infection . The study revealed that the vaccine elicited good protective antibody response against the serotypes O, A and Asia1 in Mithun as >70% of the 28 day post-vaccinated serum samples showed the protective antibody titre. The poor antibody titre against the virus type C is possibly due to less antigenic mass of the virus serotype in the vaccine. The study also suggested that the vaccine can be used safely without any adverse reaction and the animals can be protected against the disease by giving the vaccine in time. However further study on decline of the antibody level and the actual time for booster dose to the animal is required to undertake.

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Table : Antibody titre (Log 10) against different serotypes of FMD virus in 0 and 28 day post-vaccinated serum samples of Mithuns

Log 10	No. of serum samples(N=20) at 0 day showing the titre against the serotypes				No. of serum samples(N=20) at 28 day post-vaccination showing antibody titre against the serotypes			
	O	**A**	**Asia 1**	**C**	**O**	**A**	**Asia 1**	**C**
1.2	15(75)	13(65)	15(75)	17(85)	-	-	-	-
.1.5	4(20)	5(25)	4(20)	1(5)	-	-	-	10(50)
1.8	1(5)	2(10)	1(5)	2(10)	3(15)	3(15)	6(30)	5(25)
2.1	-	-	-	-	17(85)	17(85)	14(70)	5(25)

Figures in the brackets indicate percentage

Risk Management, Diagnosis and Response to Emergencies :

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Post-FMD Problems on Fertility and Productivity in Domestic Animals

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Foot-and-Mouth Disease is a major global animal health problem. As control and elimination programmes were undertaken regularly, its geographic distribution is shrinking in recent years. The disease is prevalent in all parts of the country. The Foot-and-mouth disease is one of the most contagious diseases of cloven-footed animals such as cattle, buffaloes, sheep, goat and pigs.

The full potential of fertility and productivity is expected only from young and healthy stock. However, the viral attack on these profitable animals leads to losses. The young animals and healthy stock are more commonly affected and hence, economic losses are more due to FMD. The acute phase of the disease in dairy herds results in loss of milk yield, abortion and even death of young stock. The exotic breeds of cattle are highly susceptible to the disease, so the introduction of crossbreeding programme in India further magnified the loss.

The complications post-FMD are more severe and chronic. The affected animals fail to recover rapidly. Infertility, reduced milk yield and weight loss due to prolonged inappetence and heat intolerance are most common complications of FMD. The virus primarily affects the epithelia of body systems and reduces immunity. Hence, there is equal risk of emergence of pneumonia, theileriosis in affected animals and secondary bacterial infections leading to further losses. Pneumonia is commonly encountered in cattle and buffaloes during post-FMD period.

Severe stress and reduced immune status for prolonged time reduces the lifetime productivity of animals in FMD affected cases. In vaccinated cases, the immunity produced against the disease is basically of short period i.e., maximum upto one year and regular vaccination is therefore, necessary in case of FMD prevention campaigns.

Being an acute and highly contagious disease of domestic animals, Foot-and-Mouth Disease is studied well in India. Research work has been carried out to study the type of antigens, use of vaccines and effects of the disease on body mechanism. The disease is studied in naturally infected animals as well as in vaccinated animals as both the conditions affect the animal body systems. The present report deals with extent of post-FMD infertility problems due to loss of the condition, immunity and health, which in turn affect animal productivity.

On reviewing the available literature, it is evident that the observations on post-FMD fertility problems in domestic animals have not been well documented. This could be due to lack of such type of studies. The available

veterinary textbooks have not related the post-FMD complications at length with the extent of infertility problems. The fertility problems post-FMD in females have not been given due attention and therefore, the research on this aspect is scarce in the literature.

Pathogenesis of Infertility:

All secretions and excretions may be infective in FMD affected animals and are the potential sources of infections to the other healthy stock. The virus can be isolated from semen of the infected bull. The susceptible cow may contract the infection, if inseminated by semen from an infected bull.

Hyperthermia produced in response to the exposure to the field virus has long lasting effects on reproductive system. Failure of thermo regulatory mechanism and localized systemic infection caused by the virus elevates body temperature. Simultaneously, avitaminosis-A leads to inhibition of release of gonadotrophines. Hence, failure of reproductive activity and other fertility problems encountered in FMD affected cases are common in both male and female animals.

The entire tubular genitalia is lined by mucosa which is of epithelial origin and FMD virus has an affinity to attack the epithelial system. Vitamin A is required for maintenance of all body epithelial cells. In case of vitamin A deficiency, epithelium of the reproductive system is prone to other bacterial and viral infections. Hence, reproductive disorders and infertility problems are severe in vitamin A deficient male and female animals.

In FMD affected cases, due to the oral lesions animal could not take feed and due to foot lesions animals are reluctant to move. The anorexia and lameness leads to avitaminosis-A. Further, increased demands for maintenance of damaged epithelia aggravate the condition.. Probably endocrine damage leads to chronic syndrome of dyspnoea, anemia, over growth of hairs and lack of heat tolerance, which is cumulatively described colloquially as "panting"(Radostits *et al.*, 2000). The syndrome is commonly observed in crossbred cattle followed by indigenous cattle. Diabetes mellitus has also been observed by Bhikane (2003) in post FMD affection period in cattle and buffaloes and further reported the severe persistent hypoglycemia and decreased glucose tolerance in post FMD diabetes mellitus affected buffaloes.

Loss of body condition in FMD affected animals is responsible for reproductive failures. In absence of metabolic fuels, animal fails to get stimulus from hypothalamus for continuation of reproductive cycles. Hormonal imbalance or derangement of FSH and LH may

Lesion in the Teats of Cattle, effected with FMD

be the suspected cause for infertility in animals. Mohapatra *et al.* (2005) reported that serum concentrations of calcium, total protein, glucose, cholesterol and albumin were significantly reduced in the FMD affected cattle. They further reported mild anemia leucocytosis and lymphocytosis in FMD affected cattle.

The significant fall in blood glucose, total serum protein and albumin levels indicate liver damage in FMD affected cases. Liver is the principal endogenous source of blood glucose and its utilization as an energy source during FMD infection may be the possible cause of decrease in glucose level due to anorexic animal.

Effects on Male Reproduction :

Testicular epithelium is more sensitive than all other body tissues (Jubb *et al.*,1983). Febrile conditions of the body profoundly affect testicular epithelium (Hafez, 1968) by impairing the heat exchange mechanism of pre-cooling arterial blood in pampiniform plexus. Degeneration of seminiferous epithelium is the commonest type of bull infertility encountered after FMD vaccination or exposure.

The post-FMD vaccinal infertility problems in male animals have been given proper attention by the Indian scientists. Breeding bulls show reduced sperm production and also the abnormal production of sperms for a period extending up to two months of exposure. The sperm abnormalities and the failure of maturation of sperms affecting the spermatogenesis at the level of testes, epididymis or cauda and after ejaculation are well documented.

Both viral and bacterial vaccination cause deleterious effect. There is elevation of body temperature during the post-FMD vaccination period and the temperature of the testis causes derangement in spermatogenesis. The stress factors after vaccination may affect the semen quality. The FMD vaccination stress and thereby reduction in the quality of semen is basically of mild and transient nature. On inception of regenerative changes, the temporary period of altered semen quality is reduced and optimum semen quality like pre-exposure stage is observed regularly.

Singh *et al.* (2004) observed the significant decrease in the libido and service behavior with marked increase in reaction time in FMD vaccinated bulls. Venkata Reddy *et al.* (1991) reported increase in reaction time, volume of semen and total sperm abnormalities, whereas decrease initial sperm motility, mean sperm concentration, live sperm count and per cent of cold shock resistant sperms during post-FMD vaccination period.

Murugavel *et al.* (1997) reported the effect of FMD vaccination stress on the semen quality in Murrah bulls. No significant change was recorded in the semen volume. However, significant decrease in spermatozoal concentration, initial motility due to increase in resorption of spermatozoa in the epididymis and epididymal dysfunction were observed. Increased percentage of total morphologically abnormal spermatozoa was attributed to the disturbances in epididymal function and defective spermatogenesis by vaccination induced testicular degeneration.

Mangurkar *et al.* (2000) screened 2376 semen ejaculates of pure breed and various grades of Holstein Friesian and Jersey breeds before and after vaccination and opined that the vaccination did not affect the ejaculate volume, initial motility, pre-freezing and post-freezing motility of the semen. However, the most significant effect of the vaccination observed was increased variability in ejaculate volumes and reduction in number of ejaculates by six per cent during post vaccination period.

Intact male animals used for natural services on exposure to FMD may suffer the fertility problems. However, the percentage of such breeding bulls under field conditions is regularly getting decreased. Buffalo bulls are regularly used for natural breeding and on exposure to FMD infection; the buffalo bulls may carry poor quality semen for prolonged periods. The most serious point is that such affected breeding bulls may transmit virus through natural service and hence, regular vaccination of such breeding bulls is mandatory.

The andrological cases have been drastically reduced with wide spread use of artificial insemination. Impotentia problems in native bulls and FMD affected breeding bulls are rarely reported to the clinics. Crossbred and exotic bulls are more prone for FMD complications than that of the native bulls. Post-FMD infertility problems in breeding bulls are related with testicular degeneration. Fertility problems are not of much significance in case of male animals of castrated category exposed to FMD.

There is dire need of maintaining crossbred and exotic bulls for crossbreeding. All breeding bulls from artificial insemination stations need regular vaccination against bacterial and viral diseases. Irrespective of extent of vaccination stress and post vaccination transient changes in semen quality, the semen samples collected are being used for AI programmes only after getting optimum quality. The programme needs to be emphasized for improved breeding activities.

Effects on Female Reproduction :

Post-FMD fertility problems in females have not been given due attention and therefore research on this aspect is scarce in the literature. Hyperthermia, endocrine dysfunction and stress is commonly observed in FMD affected females. Failure of exhibition of oestrus cyclicity is most common fertility problem in FMD affected animals and unless the body condition along with basal metabolic rate is optimized, no treatment is successful for the condition.

Follicular structures are basically of epithelial type and FMD affections at the level of follicular cells may inhibit the further growth and development of follicles from cortical

FMD effected buffalo

ovarian pool. High stress produced by post-FMD complications leads to high level of ACTH or cortisol and hence release of gonadotrophines is drastically reduced which leads to failure of cyclicity.

Ali *et al.* (1991) reported that low calcium and inorganic phosphorus levels with resultant improper serum calcium phosphorus ratio might be responsible for anoestrous status in crossbred heifers. Similarly Pal *et al.* (1991) stated that the higher cholesterol level in the cycling heifers and cows vis-à-vis the non cycling once is indicative of more secretion of steroids during oestrus due to increased ovarian activity. Also they found that blood protein level was decreased in non-cycling animals. These observations indicate that the FMD affected animals show non-cyclicity and becomes anoestrus.

Infertility in post-FMD affected female is basically affecting the neuro endocrine system thereby affecting the reproductive cyclicity. On exposure to the viral infection, many of the animals regain and resume oestrus cyclicity. However, these animals show cyclic non-breeding tendency for months together. These affected animals have excessively large amounts of secretary oestrual activity and the ovulations are delayed invariably. Thus delayed ovulations coupled with increased susceptibility of uterus to infections leads to repeat breeding problem, which is very commonly observed in post-FMD period in case of cows and particularly in crossbred cows. The affected animal become non productive or poor productive for long periods.

Early pregnant animals exposed to the FMD infection may lead to early embryonic deaths and subsequent resorption of foetuses in crossbred cattle. Loss of immunity and stress are the important factors in pregnant animals, which are responsible for abortions at any stage of gestation.

Abortion is the one of the clinical signs shown by FMD infected pregnants. Garg *et. al.*(2001) reported abortion at the stage of fourth month of pregnancy in cow and the FMD complications in both cows and buffaloes. The FMD virus does not cross placenta but cattle abort presumably as a consequence of fever. The abortificient action of FMD is related with hyperthermia, followed by maternal hypoxia leading to fetal hypoxia and fetal death.

Pregnant animals, which succeed in completion of term, may give birth to young ones that are very weak, immature and also having low birth weights. On exposure to FMD infection, young growing heifers show delayed growth rate and there is subsequent delay in maturity. Vesicular lesions have not been recorded in reproductive system in FMD affected females.

Embryo Transfer and FMD Infection:

Morrow (1986) reported that based on infertility assays and animal inoculations, FMD virus does not penetrate or attach to the zona pellucida. The zona pellucida intact embryos were found to be non infected with FMD and there development was normal. Similarly, FMD virus was also not found to be associated with the embryos collected from FMD affected cattle during acute stages of the disease. However, hatched bovine embryos were carrying infectious virus even after washing. It is

proposed that transfer of zona pellucida intact embryos from FMD viraemic donors to uninfected recipients must be studied. Conclusively, the embryo transfer is the best technique to boost fertility in animals with simultaneous prevention of transmission of FMD virus.

Effect on Productivity:

The virus infects the parenchyma of the mammary gland resulting in complete loss of alveolar function. The FMD virus replicates in the secretary epithelium of bovine mammary gland. Acinar lesions are the direct result of virus replication. The acini and ducts in the necrotic areas contain mainly sloughed epithelial cells, cellular debris and small number of leucocytes, which leads to reduced milk yield in the affected animals (Jubb *et al.*, 1983).

Animals regain and resume oestrus cyclicity. However, these animals show cyclic non-breeding tendency for months together. These affected animals have excessively large amounts of secretary oestrual activity and the ovulations are delayed invariably. Thus delayed ovulations coupled with increased susceptibility of uterus to infections leads to repeat breeding problem, which is very commonly observed in post-FMD period in case of cows and particularly in crossbred cows. The affected animal become non productive or poor productive for long periods.

Early pregnant animals exposed to the FMD infection may lead to early embryonic deaths and subsequent resorption of foetuses in crossbred cattle. Loss of immunity and stress are the important factors in pregnant animals. Saxena *et al.* (1994) reported that milk production is affected upto 30 per cent and the milk losses as well as non milk losses accounts for rupees 180 million each year due to FMD attacks in domestic animals. In dairy herds, the FMD results in the loss of milk production and shortens the rest of the lactation period, which often results in permanent loss of more than 25 per cent of milk production.

Therapeutic consideration in FMD affected cases includes prevention of secondary bacterial complications with antibiotics. Supportive therapy is continued with tonics, minerals, vitamins, etc. Herbal preparations and anti stress agents are commonly employed. Vitamin E, vitamin A and selenium preparations may have encouraging results.

The rapid loss of body condition is common and hence immuno potentiater or immuno modulator drugs are essential for early recovery. Herbal immune-potentiator can be use to prevent the post-FMD mastitis challenge. The herbal drugs increase the functional capabilities of macrophages, neutrophills and plasma cells (Singh & Pachauri, 2001). They also enhance the phagocytic and bactericidal activity of neutrophils at mammary glands and shorten the duration and severity of post-FMD mastitis challenge. Herbal immune-potentiator coupled with antibiotics can be used for the effective treatment of post-FMD mastitis and control.

Bottom Line :

The post-FMD complications are more common in crossbred and exotic animals as compared to resistant indigenous breeds of cattle and buffaloes. Further, the problem is

less severe in buffaloes as compare to the cows. Since, milk productivity has to be increased through crossbreeding as an accepted policy, and the crossbred animals are more prone for the disease, it is necessary to stress the research on post-FMD fertility problems in crossbred animals.

Some research aspects needs to be stressed in domestic animals, which includes studies on reproductive and endocrinal pattern, studies on ovulatory pattern, cyclicity and conception rates and studies on management of post-FMD stress for improvement of fertility and productivity in farm animals in FMD affected cows and buffaloes.

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"Human beings were invented by water as a device for transporting itself from one place to another."

- Tom Robbins

Strategies of Vaccination in National Mass Immunization Programs and Dealing with Special Circumstances

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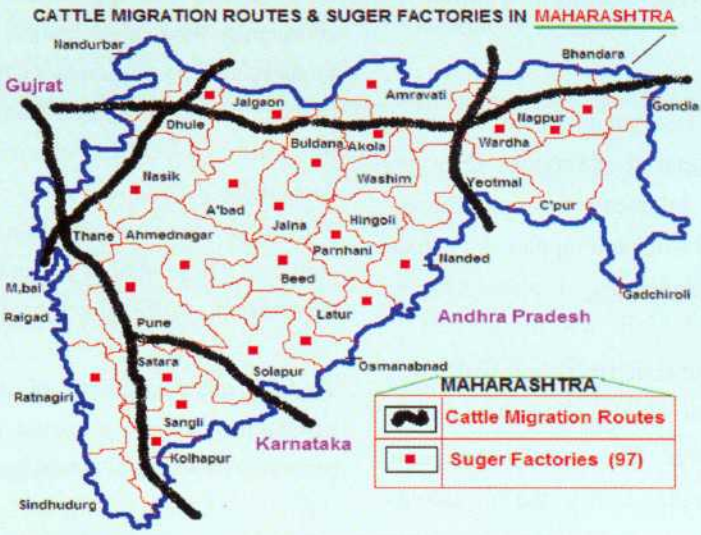
The Planning of National programs of creating disease free zones or creation of immune belts or to the matter of fact the disease eradication programs in state or National disease control technique needs different strategies for different situations.

The presence of an epidemic disease or carrying out mass vaccinations in highly mobile cattle herds and sheep and goat flocks in Asian countries and especially, in countries like India, complicates the eradication / immunization process greatly.

Migratory Flocks - The migratory herds are among the most knowledgeable of livestock farmers and they are amenable to cooperation with veterinary authorities if their confidence has been gained and they are given the opportunity to participate actively in decision-making. Many are amenable to quarantine procedures as a part of their traditional disease management practices, provided they are carried out sympathetically with full consultation. This is important because changes in climate and weather, which have profound implications for the seasonal availability of feed and water, may affect their willingness to conform to quarantine regulations. Virtually all the migratory farmers are now familiar with the value of vaccines in controlling major epidemic diseases and also the significance of National mass immunization programs.

The mapping of migration routes and an understanding of the factors that drive migrations



are the keys to anticipating future livestock movements and managing the risk of disease introduction and success of National mass immunization programs .

The knowledge and mapping of traditional livestock movements are the keys to anticipating the spread of emergency animal diseases and the factors risk of the disease

The confidence building in these critical area of migratory farmers are achieved largely through communication and improvements in the veterinary-farmer interface must start well in advance of any disease emergency / National mass immunization programs this is a most important and fundamental activity of animal health services. The dairy development authorities or milk cooperatives and participatory animal health programmes involving community animal health workers have an important role in building confidence and cooperation as well as in undertaking many of the actions of National mass immunization programs

In cases of disease emergency, involvement of the migratory communities, is essential to have confidence of the community elders in decision-making and implementation of control activities from the outset. Further, it is also advisable to cover the area on either side of the migratory routes, especially in the case of planning of ring vaccinations and immune belt development strategies.

Insecure or Otherwise Inaccessible Areas : The relative inaccessibility of areas as a result of natural causes (climate or topography) or insecurity resulting from the civil unrest present a major challenge to the successful control and elimination of epidemic diseases. The same also applies for the national immunization programs.

These areas often shared a number of characteristics :

- These are remote, often inaccessible by road, distant from the centralized services. They may be inhabited by migratory farmers who see other agricultural work as a supplement to their livestock-raising activities.

- These may be inhabited by people with a well established traditional way of life who are not inclined to change. Their decision-making processes are complex as they take into account climate, economic considerations (both monetary and non-monetary), social concerns, political factors, legal constraints or incentives and other ecosystem variables, etc.

- These have been marginalized in that the inhabitants have relatively little development contact in terms of education, outside trade and government services, including veterinary services.

These characteristics have precluded the successful implementation of conventional vaccination programmes which have a "top-down" approach with pre-determined targets for vaccine coverage and sero-surveillance results, a tight time schedule for pre-defined activities and contact with communities is primarily only through local officials. Such a model fails to accommodate the dynamics of special action areas and lacks the inherent flexibility required to work in such areas. It is now realized that approaches that use local community-based participation are more likely to succeed. The participatory-based approach to the elimination of disease and the provision of animal health services promotes decentralized, community-based and privatized delivery of vaccination and other animal health services. These should be under the general supervision of official veterinary services.

To carry out a successful disease eradication programme or the national immunization programs in a special action area.

- A thorough understanding of the complexities of the area and positive interaction and dialogue with a substantial cross-section of the local community are required.

- An extensive publicity through print and electronic media in local language will be really helpful.

- The use of thermostable (it available) vaccines, which are less reliant on refrigeration is preferred.

- Alternatively strengthening the cold chain facility is to be installed right at the taluka and village level.

Wildlife or Feral Animal Involvement in Epidemic Livestock Disease Outbreaks or in National Mass Immunization Programs :

The actual role of wild or feral animals in the epidemiology of the disease should first be considered. In some diseases they may act as a

reservoir for the disease and be a genuine threat for transmission of infection to domestic animals, but in others they may simply be acting as an indicator of infection that is already occurring in livestock in the area.

Vaccination Schedule of wildlife has been extremely successful in eliminating fox rabies from some regions, but as yet has very limited application for other diseases.

It may be possible to limit contact between susceptible wild and domestic animals and thereby reduce the chances of transfer of infection from one to the other. This could be done by fencing, livestock-free buffer zones or removing livestock from epidemiologically important wildlife. In the case of epidemic poultry diseases such as highly pathogenic avian influenza (HPAI) and virulent Newcastle disease, poultry sheds can be wire-netted or otherwise sealed to prevent direct access to wild birds. Steps should also be taken to prevent faecal contamination of poultry feedstuffs. In the case of HPAI, faecal contamination of water supplies by wild water-birds is an important source of infection for chickens and other domestic poultry. This may be prevented by using water from town-water or underground water supplies. Alternatively, water drawn for poultry farms from dams, lakes or rivers where water-birds congregate may be treated by chlorination to remove any HPAI virus contamination.

If none of these measures is likely to be practicable and/or successful, it will probably be necessary to mount ring or blanket vaccination programmes for the livestock in those areas where infection in wildlife constitutes a continuing threat.

Further more, the surveillance activities should be extended to wild and feral animal populations, in collaboration with wildlife authorities.

Final Concluding Stratagies :

When the clinical disease appears to have disappeared from either a region of a country or the whole country, it is time to take stock of the situation and to carry out a thorough epidemiological and economic assessment of the future options. It may prove desirable to maintain strategic vaccination if there is still a high risk of a new incursion of the disease from a neighbouring country. On the other hand, it is often advantageous to change direction completely by stopping vaccination programmes altogether and moving to a disease search-and-destroy policy. The efforts should be directed away from routine vaccination to increased activities directed to early warning and early response. There must be a willingness to enhance active disease surveillance activities and to maintain preparedness against the disease at a high level. In this way any disease breakdowns can be detected and eliminated quickly before they have done much harm by either a short, sharp targeted vaccination campaign or by eradication procedures.

Let us all come together in planning the state and the country to be a disease free nation.

Cost Benefit Analysis of Foot-and-Mouth Disease Control Program in Maharashtra State

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FMD is a highly contagious disease of all the cloven hoofed animals, the more susceptible species being cattle, buffalo, sheep, goat and pig. It is a nationally important notifiable disease, because of its economic impact on the farmer. FMD has immediate and delayed losses. Immediate being financial losses incurred by farmers through treatment of affected animals, reduction of milk production, hire charges and death of animals. Delayed costs are defined as losses due to the after-effect of disease, further treatment for infertility or chronic mastitis, weight loss and reduced value of animals and products. The major economic losses are in terms of reduced milk production, loss in draught power and transportation, cost of treatment and disinfectant, chronic mastitis, abortion and infertility. These losses directly affect the economy of farmers to whom the dairy animals and working animals are the major source of income. The AICRP FMD project estimated the economic losses of FMD at approximately 15,000 million rupees per year. Ellis & James (1976) estimated Rs. 4200 million per year loss due to FMD in India due to loss in milk production and loss in draft power, without estimating losses for small ruminant and pigs. Efforts are made by Mahajan & Deshpande (1997), AICRP FMD project, regional centre, Pune to calculate the actual economic losses to the farmers. The economic analysis of cost and benefit of FMD control program in Maharashtra could help to justify investment in prevention, control and eradication program.

Costs and Benefits of FMD Control Program in Maharashtra :

Harrison & Tisdell (1997) stated that an examination of the economic impact of FMD should be the starting point for formulating the disease control program as well as assessing the economic desirability of the control program. Costs of disease and benefits of control could be estimated using the cost benefit analysis methodology.

FMD hampers the economy of farmers directly, by causing a setback to the valuable animals as well as to farmers. Government is reluctant to give any necessary funds for such control programs unless convinced that the benefits for the nation comfortably exceed the cost of government support and intervention. For this complex reason, economic analysis has to be made from two different points of view, that of farmers and that of the nation or state concerned. The economic analysis of FMD control and eradication made by Harrison & Tisdell, (1997) from the Department of Economics, University of Queensland for the ACIAR Thai- Australia animal health program could be a suitable example for economic analysis of planned control of FMD in Maharashtra state.

Cost of FMD in Maharashtra :

The cost of FMD is high for milking animals, draught animals and crossbred cattle, and most of which is borne by farmers. The cost of disease can be divided into immediate cost and delayed cost. The direct and indirect costs of disease differ between producers, traders and government.

Immediate Cost :

Production Losses :

A reduction occurs in production of milk, meat, wool and live weight loss. Most of the farmers and landless labourers depend on milk production because they sell milk daily either to consumers or to a milk society. It was observed that in the area surrounding the Baramati Milk Union FMD reduced the volume of milk collected by 30,000 liters per day over a period of two months. The total loss to the milk union was Rs.12.6 mio due to milk loss that could be the cost of disease control for that area.

The cost of treatment of affected animals :

In FMD to avoid the secondary complications and to reduce after-effects, treatment with antibiotics, vitamins and sodium carbonate is essential. Providing treatment to dairy and crossbred animals reduces the chance of bacterial infection, e.g. with Haemorrhagic Septicemia, which may lead to death of the animals. The cost of treatment depends on the severity of the outbreak and varies from area to area. The cost of disease could be high in areas where the crossbred cattle population is high as the owners of valuable, high-producing dairy cattle do not hesitate to pay for treatment if the disease is prevalent. In contrast, owners of low-grade animals or small ruminants in extensive grazing system, or pigs in smallholdings, may be unwilling or unable to treat their livestock.

Loss of Draught Power and Transportation :

In affected cases, because of lesions in inter-digital spaces animals can't walk properly due to lameness and wound formation. Irrespective of good treatment and hygienic condition average recovery period is 15 days. The infection of FMD to transport animals disturbs the livelihood of bullock cart owners because their source of income is totally depending on the bullocks. If a working animal is infected with FMD during the period of agricultural operation then replacement of the animal is essential for the farmer. Replacement charges depend on the type of work and whether the owner has to hire a tractor.

Death of Animals :

The morbidity percentage is very high in FMD, whereas the mortality percentage is usually low. However, in young calves 40 to 50% mortality is seen. In adult animals, mortality occurs from susceptibility to secondary disease such as HS. Death of animals means direct loss equal to the value of the animals, which varies from Rs.1000 to 25000. The loss would be greater than the market value of an animal, due to transport, time and of the costs in obtaining a suitable replacement.

The Delayed Costs Due to FMD :

Delayed losses are generally noticed mainly because of after-effects of the disease, and include the following

- Abortion, infertility, decrease in carving rate, increase intercalving period
- Weight loss or reduced weight gain
- Increase frequency of other diseases due to immunosuppression e.g. chronic mastitis.
- Permanent disability such as lameness and loss of lactation
- Secondary complications, eg. Maggot-wound, panting, rough coat appearance.

- Limitation on genetic improvement.
- Reduced value of livestock and by products.

These do not constitute an immediate financial loss to Government, consumers and traders even when FMD is endemic in the region or country with no strict control measures in place. This is may be one of the reasons for neglecting FMD control and eradication programs in Asian countries.

Delayed costs of disease can impact on animal health programs of government such as cross breeding and genetic improvement. Indirect economic cost from an FMD outbreak arises from long-term production losses and the declines in price received for livestock products, from the FMD- free areas compared with those from FMD endemic areas. A loss of an opportunity to export livestock products in international market has great impact on the economy of farmers and country.

Cost of Disease to Government :

In countries where FMD is endemic with no control and eradication program, the government does not have any direct losses from FMD. The indirect cost of disease is mainly in cost of vaccine, loss of an opportunity to earn foreign exchange through export, developing and maintaining diagnostic laboratories, vaccine distribution and cost of staffing and maintaining checkpoints. The government has to look after the control of outbreaks, collection and analysis of samples, epidemiological studies and extension costs. These are the indirect costs of disease to state governments. Other costs include those of establishing and maintaining a livestock health information system.

Benefits of FMD Control Program in the State

The benefits of a FMD control program could be estimated on the basis of experience and work done by Harrison (1996) and Harrison & Tisdell (1997) in the Thai-Australia Animal Health Project. The state of Maharashtra has so far not worked on benefits of the FMD control program The costs and benefits observed by Harrison & Tisdell (1997) could be more or less applicable to Maharashtra state. However, it is required to work on costs and benefits to producers, consumers, traders and government separately. The benefits of the FMD control in Maharashtra state could include benefits to farmer, government, community health and environment. The farmers benefits are in terms of reduced loss in production, reduced treatment cost for morbid animals and reduced losses in draught and transportation. A government benefit includes reduced cost of vaccine purchase, reduction in expenditure on control of FMD, foreign exchange through international trade.

Farmer Benefits :

1. Reduced Loss in Milk Value :

In FMD affected animals, milk production is reduced to 25% during clinical envisage. The loss of milk production depends on the stage of lactation when the disease occurs and when milk production recovers in that lactation. There is evidence that FMD sometimes also leads to mastitis, which further affects production and incurs additional cost for treatment. The AICRP FMD project estimated the losses in milk production, which averaged approximately Rs. 472 per animal.

2. Reduced FMD Treatment Costs :

The treatment and palliative care for of infected animal is expensive for livestock owners. It involves use of antibiotics, vitamins and

disinfectant, and the service of veterinary officers. The approximate cost of treatment is Rs. 320, again a major loss to farmers. The saving in treatment cost is the annual cost in the absence of the control program. Saving in treatment cost could be a significant benefit to farmer from FMD control program.

3. Draught and Transport Benefits :

The working bullocks are widely used in the state for pulling carts and agricultural implements. The affected animals cannot be used for work until recovery and owners may have to replace affected animals. The cost of treatment and cost of replacement, if important agricultural work is to be done, are severe losses to owners. The AICRP FMD project at Pune estimated the average losses for working animals at Rs. 895 per animal, which includes cost of treatment and loss of working hours value. This loss can be avoided through a FMD control program and it is an important benefit to the owners. The replacement of animals for transportation and draught purpose may not required to the farmers.

4. Avoidance of Weight Loss :

In disease affected animals generally, loss of weight is common due to inability to eat, anorexia and high temperature. Weight loss reduces the market value of animals, relative to normal healthy animals. Further study is required for to estimate this loss more precisely, and hence the benefits of avoidance of weight loss in future FMD control program.

Producer and Traders Benefits:

Increased Revenue from live Animal, Meat and other Exports :

The control of FMD and acceptance of free status by meat importing countries, could lead to substantial new export markets for livestock products. This applies particularly to buffalo meat and will be of benefit to meat producers and traders. FMD control will help to improve animal health, and higher prices of live animals will also provide benefits. Once the state achieves the status of FMD-free zone, the export of buffalo meat will increase with probable high prices, and the owners, traders and exporters will benefit. Apart from meat, export of milk products will also be possible. The scope for export of leather and leather product will be seen. The export of live animals, meat and other products could have a major impact on FMD control and its economic impact, with government gaining more foreign exchange.

Benefits to Government :

The long-term benefits of a FMD control program will be important from a government point of view. The government could support producers for export to increase the various livestock production and their products. The quality of product will be improved. It could be easy for government to decide on a suitable breeding program for genetic improvement of valuable livestock in a disease free environment. The chances of improvement of the existing crossbreeding program in the state could be higher with improved health and reproduction of dairy animals. The disease-free status will enhance industry and national development programs. International recognition of disease free status in relation to a particular disease and consequent increased export opportunities potentially has very large economic benefits. These benefits would be more than the expenditure incurred on control. Similarly, the government will ultimately benefited in saving of petroleum because farmers could use the healthy working animals

for transportation and draught purpose. Improved animal health has the potential to improve human health, both through improved nutrition and by prevention of zoonotic diseases such as Salmonellosis and Brucellosis.

Table 1: Immediate FMD costs for affected milk and draught animals

Cost Category	Assumed parameter values	Amount (Rs)
		Rs
Immediate loss per milking cow	6 l/day of milk by 75% for 15 days @ Rs 7/l	473
Loss in milk production	2 visits by veterinarian @ Rs 150	300
Cost of treatment and medicines	Local treatment and Disinfectant	20
Cost of mortalities	2% of animals @ Rs 10,000	200
Total expected loss per milking cow		**993**
Immediate loss per young calf		
Cost of treatment and medicines		200
	2 visits by veterinarian @ Rs 100	20
Cost of mortalities	Local treatment and Disinfectant	200
Total expected loss per calf	20% of animals @ Rs 1000	**420**
Immediate loss per draught animal		
Loss in working capacity		375
Cost of treatment and medicines	15 days @ Rs 25/day	320
Hiring replacement	As per milking cattle	100
Cost of mortalities	approximately	100
Total expected loss per draught animal	2% of animals @ Rs 10,000	895
Immediate loss per buffalo		
Loss in milk production		472
Cost of treatment and medicines	3 l/day of milk by 75% for 15 days @ Rs 14/l	320
Cost of mortalities	As per milking cattle	
Total expected loss per buffalo	Nil	**792**

Approximate immediate losses for Sheep, Goats and Pigs including production losses, cost of treatment and deaths of animal are Rs. 300 per head.

Cost Benefit Ratio :

Understanding cost-benefit analysis is required to identify the constraints, to identify the alternatives, to determine the scope and objective of the program and to identify the cost of disease and control benefits to farmers, consumers and Government (Ramsay *et al.*, 1999). An annual ratio of benefits to expenditure of Maharashtra state is tentatively calculated here on the basis of report of Harrison & Tisdell, (1997). All the costs considered for ratios are average values. Ellis (1994) stated that minimum 15 per cent of animals are affected every year due to FMD in India.

Table : Livestock populations, vaccine dose and approximate FMD incidence (Provisional figures 1997 livestock census)

Species	State population (million)	Vaccination million doses per year (twice)	FMD incidence (15%) million head per year	Economic loss / animal @ Rs.	Economic losses in million (Rs.)
Female cattle (in milk & bredable)	9.30	18.6	1.39	993	1380.27
Working male cattle	9.23	18.46	1.38	895	1235.10
Female buffaloes (in milk and breedable)	4.91	9.82	0.74	792	586.06
Sheep	3.10	3.10	0.47	300	141.00
Goats	11.0	11.0	1 .65	300	495.00
Pigs	0.52	0.52	0.08	200	24.00
Total	38.06	61.5	5.71		3861.43

For the vaccination of all susceptible animals in the state a total 61.5 million doses of vaccine will be required per year. The benefit of control program would be saving in the morbidity and mortality of animals due to FMD. If only 15% of animals currently are affected out of total population of state. i.e. 5.71 million. So the cost of benefit will be around 3861.43 million rupees.

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Administrative and Operational Aspect of Ring Vaccination

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The most of the manufacturers, engaged in production of veterinary vaccines and biologicals, follow utmost care and precaution in day-to-day functioning to achieve the basic norms of disease containment standards. Many of the functions require the usage of various established primary and secondary cell lines, and different media used for the growth, production and testing of the vaccines in experimental animals. These processes involve the use of highly potent bacteria (like *Brucella, Leptospira, Anthrax,* etc.) and viruses (like (Foot-and-Mouth disease, Rabies, etc.) at different stages of production and testing. The GMP standards and norms of controlling at every production and QC step needs various experiments utilizing live animals that are administered with the vaccines and challenge organisms, both bacteria and viruses, to arrive at the best safety, efficacy and potency for the commercial release. The best standards are always adhered to Good Laboratory Practices, for example; in rabies, the persons engaged in handling of live virus materials are always subjected to prophylactic vaccinations, periodic testing of their antibody profiles which assure that no untoward or adverse effects, happened to them. The adequate bio-security and disease containment measures are taken to prevent any escape of such dangerous organisms in the surrounding environment.

This needs a very close vigil and supervision over the human and animal population residing in the surrounding villages of the Vaccine production plant. As in the case of the Foot and Mouth Disease (FMD), such animals are the best indicator animals to suffer from any infection in the event of virus escape.

Apart from the bio-security and disease containment facilities adapted, frequently reviewed and improved upon by the vaccine production units, ring vaccination measures is such an important aspect of disease control around the vaccine units. Its importance in disease control especially against FMD is well emphasized (Toma *et al.,* 2002 and Barnet & Carabin, 2002).

Considering the importence of ring vaccination in FMDV, an attempt has been made in this article to highlight some of the vital aspects of ring vaccination (RV) in two broad categories: Administrative and Operational (Technical) requirements.

FMDV is a highly contagious virus agent responsible for deadly and costly outbreaks in various ruminants (especially cattle, buffaloes, sheep, goats and pigs). The consequences are always devastating as entire livestock industry suffers impacting the dairy farmer the most in the process. To create an immune belt around

the vaccine production units. Ring vaccination measures are taken up to offer the benefits of vaccinations to such animals in the surrounding villages (say: a radius of 5 km zone, around vaccine production units) and keep the immune status of such animals at maximum levels so withstand and counter infections. Achieving disease control status in ring vaccination zone warrants the role and precise participation of various agencies and Personnel. The vaccine producer, animal husbandry departments, public health authorities, and civil population should plan and execute their functions and responsibilities.

Administrative Requirements :

To begin with, a map/shape of the proposed area of the ring vaccination showing all the villages surrounding the vaccine production unit should be obtained. The help of the municipal/civic authorities is required here. The Animal Husbandry department provides the livestock census (Cattle, Buffaloes, Sheep, Goats and Pigs) that hare to be dewormed and vaccinated regularly.

The program may require creation of animal check posts, if any animal trade routes fall under the RV zone. In this situation, one should either relocate the existing check post /animal trade route or evolve a mechanism for safe passage of such animals without hampering the immunity of the vaccinated animals.
Before starting of the program, it is necessary to discuss with AH department and gather their confidence. One has to ensure and avoid any duplication of vaccinations in such animals. The responsibility for carrying out the vaccinations lies with the vaccine producer. The Staff, specifically identified to plan and execute ring vaccinations, should be recruited, and trained.

Such employees are required to execute declaration stating that they will not enter any animal zoos or farms and animas other those falling under the RV zone. Besides, such personnel should not have any access to enter the vaccine production units that there is a possibility of contaminating the premises as they might be coming from infected areas or villages.

The person may however operate from a nearby office having facilities for data storage (computer with a printer) and proper storage of vaccines that include refrigerators, deep freezers, ice packs, instrument sterilizer, etc) and efficient transportation of vaccines, vaccination equipment and related materials (like syringes, needles, isothermic containers, disinfectants, anti-histaminics, and dewormers, etc). The vaccine production unit should ensure the supply of vaccines and logistics required for this function be accurate and precise.

The program requires persuasion / commitment on the part of all concerned as there may be some resistance from the field which can result in declining interest by the executors. The program involves huge financial commitment. Nonetheless, it is a long-term investment and can help to create FMD free zone around the vaccine plant.

Operational / Technical Requirements :
The important documentation required for the

successful execution should be available with the person, in-charge of RV and the vaccine production unit, as well. The detials are as under -

1) A map of the RV area

2) The census report on livestock population census of each village falling in the RV area with owner's name and addresses details.

3) The deworming and Vaccination card showing age of the animal, sex, breed, date of primary vaccination, date of booster, date of repeat vaccination, vaccinations done other than FMD, any treatment, date of blood collections (pre-and post-vaccinations)

4) The report of the periodic inspection and confidential surveys by the responsible officers.

5) An authenticated recording of outbreaks, if any, in the RV area.

6) A Standard Operating Procedure (SOP) has to be in place, detailing the vaccination procedure, the hygienic measures required to be adapted before moving from one area to another or a farm with emphasis on the sterilization of the equipment, and personal hygiene required in the mass vaccination campaigns.

7) The veterinary extension activities are required to promote the program, explain the benefits, dos and do nots of vaccination, and basic animal management activities. These indirectly contribute towards the social responsibility by the vaccine producer.

8) A mechanism to identify both the vaccinated and unvaccinated (either by ear tagging or colour painting of the horns) needs to be worked out. This is essential as every year, there would be at least one vaccination dose per animal.

9) All young animals are required to have regular dose of deworming, and specially deworming is advised before each vaccination is carried out. It is always ideal to give a gap of 7 to 10 days after deworming and then to proceed for vaccinations to achieve better efficacy.

10) The preferred timings for vaccinations are in the morning (before 8 am) to avoid stress and physical exertion of the animals. After field work in the morning, daily reports are prepared and to plan for the next day/week engagements in advance.

11) It is necessary to maintain vaccination coverage of more than 85 percent of the target population to achieve good and intended herd immunity. Some animals which are below the vaccination age, or pregnant, or diseased would not receive the vaccination, but they should be vaccinated once they become normal/healthy.

12) It may happen that owners may show reluctance to allow blood collection from their animals after 2 - 3 visits, hence incentives such as antibiotics, mineral supplements, etc. should be provided.

13) There is a possibility of some outbreaks due to contact with infected animals spreading to the RV villages & appropriate support

(including field visits by experts, counseling, sample collection and rapid diagnosis) should be provided to ease the situation and regain the confidence of the animal owners in the program.

14) There should be a fortnightly/monthly audit, inspection and confidential surveys by reporting officers are an integral part of the program to identify further improvements.

Benefits of the Ring Vaccination :

The area under the RV becomes a comfortable zone as far as FMD is concerned after few years of systematic application of vaccine. This leads to creation of a buffer zone thus becoming a good source for trade of animals and animal products.

One can conduct sero-conversion studies in RV villages by collecting pre-and post-vaccination serum samples from various species (cattle, buffaloes, sheep, goats and pigs), age groups, and sexes, etc. The results of the studies will also build a substantial data regarding the performance of the vaccine in the field conditions. Purchase and procurement of animals for experiments can be sourced from the ring vaccination zone.

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"One should be extremely careful about making His service perfectly flawless. But the truth is, God knows our foolishness, and therefore, He forgives us."

- The Holy Mother - Saradamani

"The earth is a garden, The Lord its gardener, Cherishing all, none neglected."

- Adi Granth

"This life is short, the vanities of the world are transient, but they alone live who live for others, the rest are more dead than alive.

- Swami Vivekananda

Foot-and-Mouth Disease: Review in Relevance to Disease-free India

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Foot-and-Mouth Disease (FMD) is a highly contagious viral disease of cloven-hoofed animals. It causes severe losses, which adversely affects milk production, reproductive capacity in breeding bulls and also is therefore, a major constrains in international livestock trade. In a global scenario, livestock is an important economic tool for human welfare not only in India but also in Asian, Southeast Asian and SAARC countries.

This article reviews the successful eradication of FMD in Europe and in the South East Asian countries. It also highlights those successful implementations of programmes which could be of relevance to establish the disease free zones and the eradication of of FMD from India.

Control and Eradication :

Looking into the global economic situation and current development in e-business much attention has been focused on the prevention and control of FMD. In the 1920'and 1930's the universally severe epidemic of FMD had towards the declination after the second world war but it again surged into the early of 1950's (Donaldson, 1993). In India, the disease ebbed and flowed according to the rise and fall of the naturally acquired immunity of the livestock populations for certain time still in dramatic situation India suffered with several outbreaks per year.

Control Measures in Europe :

The first major endeavor of the control measures occurred in mid of 1960's when the Netherlands introduced mass and annual vaccination for its cattle herd (Donaldson 1993). This annual mass vaccination was followed by countries would not restrict themselves to follow them. Methods for large-scale production of the vaccines had been developed but its use was not in a systematic way. Only during mid-60's, the first vaccine was applied in a organized manner (Frenkel 1951).

The success of the policy quickly became apparent as the prevalence of outbreaks was begun to decline within a decade and the number of outbreaks in Western Europe fell down from more than 20,000 to less than 4,000 per year. The declination was in progress, which was reached upto the less than of 400 by the 1980's (Mowat, 1989).

Success did not result from the vaccination alone, but the other control measures brought into operation had been also shared into the success. It was booster vaccination around the foci, prevention of movement of animals and products in the farm premises and burning of swills from the farms. Harbors and airports must be protected to reduce the risk of virus entry from other countries through livestock and animal products.

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Influence of Experiences of Other Regions :
Southeast Asia had a lesson from the experiences gained in the control and eradication of FMD from other regions of the world. Indonesia mounted a very successful programme during the 1974-1981. It helped to eradicate the disease from Bali and Madura in 1978, and from South Sulawesi and East Java in 1981.

The last case of FMD was reported in Kebumen, Central Java in December 1983, while the last vaccination in Java against FMD was carried out at the end of 1985. Indonesia was declared free from FMD in 1986 (Soehadji & Setyaninagsih, 1994).

The Southeast Asian countries, Cambodia, Indonesia, Lao PDR, Malaysiya, Myanmar, Philippines, Thailand and Vietnam started the South East Asia Foot and Mouth Disease Campaign (SEAFMD) Phase II in the year 2002. It is a regional strategy for the progressive control of foot and mouth disease in Southeast Asia. In Phase II, the programme will concentrate on :

- Regional cooperation.
- The development of progressive regionalisation and zoning approaches.
- Public awareness and communications.
- Disease surveillance, diagnosis, reporting and control.
- Programme management, resources and funding.
- Policy and legislation.
- Improving animal health services within the livestock sector.
- Monitoring and evaluation.

Importance :

I. Healthy livestock contribute for food and poverty alleviation:

- Provide draught power for agricultural production.
- Generate and maintain cash reserves.
- Convert agricultural by-products to protein.
- Produce organic fertilizers to maintain soil quality.

II. The FMD causes direct and indirect economic losses

- The FMD affects cattle, buffaloes, pigs, sheep and goats. It causes mouth sores, lameness
- Poor appetite and loss of condition in cattle and buffalo, severe lameness in pigs and
- Sometimes death in young livestock.
- It severely impairs the ability of animals to work, which affects farm production.
- Milking animals reduce production.
- It spreads rapidly, once introduced to an area.
- FMD is barrier, against the development of export trade.

The successful campaigns in Europe, including the former USSR, South America, Southern Africa and Indonesia were due to certain common factors. It must be looked into account when a plan will be projected on following points for control and eradication of FMD in Indian regions.

1. India should formulate a national plan under the National Advisory Board for the control and eradication of FMD. The legal and financial support from the government at the appropriate level would be the prime need in this context.

2. The technical requirement of the campaign i.e. surveillance, diagnosis, implementation of control measures, vaccine availability and delivery system etc. must be enough resources for effective output. It should be sufficient and free to protect the possibility of regional economic crises.

3. Representatives of the livestock industry from each state should be the part of control campaigns from the earliest possible stage of spread up the disease.

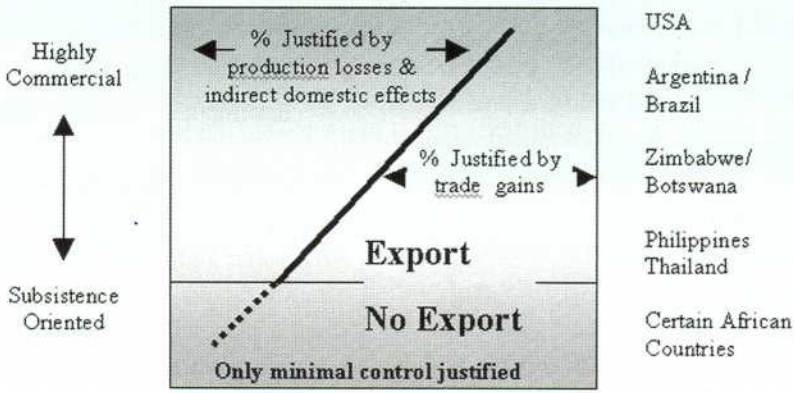
4. The borders based neighbor states must be provided benefits for implementing common strategies at boarders.

5. High healthy status of particular area should be protected by the control of movements of livestock within and between the states. It will need checkpoints and barriers to reinforce the control measures. Colour coded ear-tags have been found useful for identifying the origin of animals and to deter the illegal movements.

6. Adequate supplies of safe, potent vaccines of appropriate antigenic specificity are essential to reduce the prevalence of disease to stamping out and move towards the final goal of virus eradication.

7. Campaigns should have the publicity group to ensure the awareness of campaign and its potential benefits for farming community and the livestock industry.

Justification of Control*



* Source : B.D. Perry (2002)

Diagnostic Requirements to Accelerate FMD Control in India :

When national laboratories are routinely using their diagnostic and surveillance tests they should consider the expansion of their capacities to acquire a tissue culture capability. A tissue culture capability enables a laboratory or the WRL to do antigenic and genome analysis. Laboratories with the capability can also confirm ELISA results by using virus neutralization tests. The liquid phase blocking-ELISA is highly sensitive and ideal for screening large number of sera samples. However, a small number of samples will ultimately give obscure results and so further testing by virus neutralization, the definitive confirmatory test, is necessary to obtain a final result. Clearly, this requires a tissue culture capability.

Several different types of tests have been developed to differentiate infected animals among the vaccinated animals. Most depend on the fact that cattle that have been infected with FMD virus can be differentiated from those, which have been vaccinated with purified vaccine without the non-structural (NS) proteins of the virus. Presumably, this will be on the agenda, which will be included during the next FAO/IAEA Coordinated Research Programme.

Differential Diagnosis :

The differential diagnosis have to be carried for the Vesicular Diseases (Swine vesicular disease, vesicular exanthemas, vesicular stomatits)

- Infectious Bovine Rhinotracheitis (IBR)
- Rinderpest
- Bluetongue
- Bovine popular stomatitis
- Mucosal disease
- Peste des petites ruminants
- Mycotic stomatitis
- Phototoxic dermatitis
- Foot rot
- Chemical irritants and scalding
- Traumatic lesions of mouth and feet

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The use of Cell Culture in the Commercial Production of Foot-and-Mouth Disease Virus Vaccines

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The introduction of cell culture techniques in the production of vaccines against Foot-and-Mouth disease in the late 1940's greatly increased the production capacity. Until that time vaccines were prepared according to Waldmann's protocol of harvesting virus from the tongue epithelium and vesicular fluid of experimentally infected cattle. This virus was subsequently inactivated with formaldehyde and formulated in aluminium hydroxide gel (Waldmann, 1937).

In 1947, Frenkel and co-workers described the use of epithelium from tongues of freshly slaughtered animals. The tongues of healthy animals were obtained from slaughterhouses and epithelial tissue fragments were prepared from it. These tissues could be maintained in vitro and infected using virus seed material. This method no longer required deliberate infection of animals for the preparation of vaccine antigen. The method was relatively simple and resulted in high and constant virus yields. Furthermore, because the cells in use are primary bovine cells, no adaptation of the foot and mouth disease virus to the cell substrate was required and hence there was no risk of decreased antigenicity of the produced virus (Barteling, 1987).

This method still had some drawbacks. First and foremost, the risk of bacterial contamination was quite big and it was difficult to maintain sterility throughout the production of virus antigen. Also, the collection of sufficient tongue material from the slaughterhouses proved to be difficult. Despite the obvious disadvantages, this method was used in various European laboratories until the vaccination of animals against FMD was stopped in 1991 (Barteling, 2002) and the vaccine performed well.

A new method for the production of foot-and-mouth disease virus was described by Mowat & Chapman in 1962. Baby hamster kidney (BHK) cell line BHK21 clone 13, developed by MacPherson & Stoker, was found to be a suitable host cell for FMD virus infection. This resulted in the development of a production process in roller bottles as the cells needed to grow attached to a surface. This meant that vaccine production on an industrial scale was very labour intensive and involved many 'open handlings' and thus was prone to contaminations (Doel, 2003).

The breakthrough of the cell culture technique for industrial application of BHK cells for the production of foot-and-mouth disease virus came with the adaptation of BHK cells to growth in suspension (Capstick *et al.*, 1962) and the subsequent production of BHK cells in large-scale bioreactors (Telling & Ellsworth,

1965). Nowadays the BHK cell line is the cell line of choice for most FMD vaccine producers in the world. The cell line is also widely used for the production of other veterinary vaccine viruses such as Rift Valley Fever virus, pseudorabies and rabies virus. A genetically engineered BHK21 cell line is used for recombinant factor VIII production for human therapeutic purposes (Boedecker *et al.*, 1994).

The use of a cell line for vaccine antigen production greatly improves the quality of the product as the cells can be frozen down in liquid nitrogen as a Master Cell Seed (MCS) which can be fully characterized for growth performance, virus propagating properties etc. The cell seed can also be tested for absence of extraneous agents such as viruses, mycoplasma etc. Once this MCS is tested and released it can be used to make a Working Cell Stock (WCS). This is the cell stock from which at regular intervals vials are thawed and cells revived to be used for the production of virus antigen. The establishment of a cell bank system as described above ensures that the cells that are being used for the production of antigen will show a uniform and repeatable growth pattern in the production process and thus not give rise to variations in the process or the product. As cells can be stored in liquid nitrogen more or less indefinitely without any loss of viability, the production of sufficiently large cell banks assures the possibility of long-term antigen production without having to replace the cell source.

Once cells are revived from liquid nitrogen, they can be used for antigen production for a limited number of passages only. In the case of BHK cells for FMDV production the use has been validated for more than 30 passages from the MCS. Once this passage level has been reached, the cells need to be replaced with fresh cells, revived from another vial of the frozen cell stock.

In practice, a vial of cells is taken from liquid nitrogen and in about 6 passages scaled up to a 1000L volume. From this point onwards, the cell bioreactors are subcultured daily until the maximum allowable number of cell passages has been reached. A large portion of the contents of the cell bioreactors is transferred to a sedimentation tank and the remaining cell suspension is diluted back to the original volume with fresh culture medium and allowed to grow for 24 hours. A close eye is kept on important parameters such as oxygen consumption, pH, cell morphology etc. Regularly samples are taken for cell count and packed cell volume (PCV) tests. The latter test is carried out in a graded tube in which a fixed volume of the cell suspension is added. The tube is then spun down at 100-200g for 10 minutes. The cells will have pelleted in the bottom of the tube and the percentage of the total volume that they occupy can be read from the graded tube. This is another method of quantifying the cell density than the more traditional microscopic trypan-blue exclusion test using a counting chamber. An advantage of the PCV analysis is that the error from one analyst to the next is generally smaller, but in this test no distinction is made between dead and live cells. Therefore, microscopic observation using a dye that does not stain live intact cells but does stain dead cells, which no longer have an intact cell membrane, remains an important test. Generally speaking, healthy cultures will have a dead cell count that is less than 5% of the

total cell count (**Fig. 1.**)

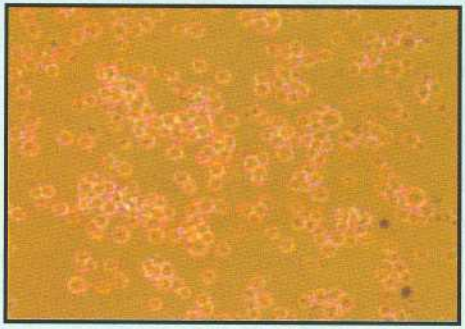

Fig. 1. Microscopic observation (Magnification 100x) of BHK cells in suspension

Bioreactors :

Bioreactors can provide the ideal environment for optimal growth of mammalian cells as important parameters such as temperature, pH, dissolved oxygen concentration (DO_2) can be closely monitored and controlled. (Fig. 2.) This is not the case in monolayer cultures in roller bottles. In roller bottles, the temperature is controlled by the incubator, pH can only be checked off-line or by the color of the medium and dissolved oxygen concentration cannot be checked at all.

Fig. 2. Wave bioreactor for growth and scale-up of BHK cells in suspension

Revival :

When a vial is taken from the liquid nitrogen environment it should be thawed as quickly as possible in 37°C water. As soon as the last bit of ice has thawed, the vial should be kept on ice. The medium in the vial contains DMSO, which is added to the medium is a cryopreservant, preventing the formation of ice crystals while freezing the cells. This DMSO is toxic to the cells above temperatures of 4°C at the concentrations present in the freezing medium. Once it is further diluted by addition of icecold medium it is no longer harmful to the cells. At that point the cell culture can be transferred to the 37°C incubator. Regularly the cell density is checked by means of counting with a hemocytometer or PCV analysis and once the cells reach the end of the logarithmic growth phase, the cell culture is subcultured. This means that the volume of the cell suspension is increased in such a way that the cell density remains high enough to keep the cells in the logarithmic growth phase. For BHK this generally means that split ratios of 5-10 can be obtained. In 48-72 hours the cells will again reach the end of the logarithmic growth phase and the subculturing has to be repeated. At regular intervals, samples are taken for sterility testing. (Fig. 3.) A schematic representation of the cell production process up to the point of virus infection is shown.

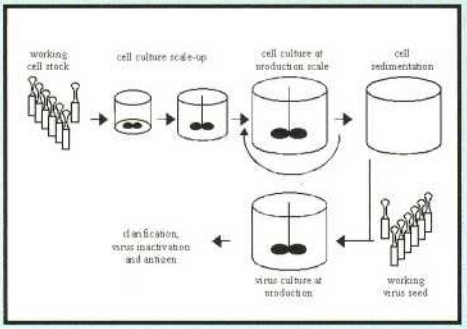

Fig. 3. Schematic representation of the revival, scale-up and production of cells for FMD antigen production.

Quality Control :

Also for quality control purpose the use of cell culture has resulted in big improvements in quality of the vaccines available. For various tests the use of cell lines has become indispensable.

Until the use of the BHK cell line for production of antigen, the only way in which virus titres could be determined was *in-vivo* by titration either in suckling mice or cattale tongue (Barteling, 2002). The establishment of the cell culture techniques meant a significant reduction in the number of animals required for QC purposes, as well as an increased product quality due to the higher sensitivity of the *in-vitro* tests. The tests which BHK cells are used are given below.

Titration and Inactivation Kinetics :

For the determination of the virus titer and the inactivation rate of the virus by chemical incactivation, BHK cells are used. For the virus seed used to infect production batches the titer needs to be known, but it is also of paramount importance that at the end of the inactivation process the virus is completely inactivated before it is used in the formulation of vaccine. To assess the complete inactivation of the virus in the produced antigen batch, two tests are used. First of all, the inactivation kinetics is established, i.e. the rate at which the infectivity of the virus is neutralized. Samples taken during the first hours of inactivation are serially diluted after neutralization of the inactivating agent and the dilutions are added to the wells of a 96 wells microtiter plate, containing BHK monolayer cells. After incubation of the plates at 37°C for 1-3 days, the presence of virus in the wells can be detected microscopically by the cytopathic effect, i.e. the effect the FMD virus has on the BHK cells. The virus titer of the samples can be calculated using method of Spearman & Karber. The individual titers of the various timepoints are plotted in a logarithmic graph of residual titer versus inactivation time. The slope of the linear plot given the inactivation rate. The extrapolation of the inactivation curve will give a theoretical titer at the end of the inactivation. Guidelines state that the titer at the end of the inactivation should be less than I virus in 10,000L of antigen.

Innocuity :

In a second test, a volume of inactivated and concentrated antigen equivalent to 200 doses is added to a BHK monolayer culture. The cell culture is incubated for 1 or more days to allow any live virus that might be present in the sample to multiply. Subsequently, a sample of this first passage is addred to to a fresh BHK monolayer and again incubated. This step is repeated once more. Absence of development of cytopathic effect implies the complete

inactivation of the 200 dose sample, as required by the European authorities (Council of Europe, 2002). This repeated susculturing results in a very sensitive test, much more sensitive than the in vivo test in which only 2ml per animal can be tested (Barteling, 2002). To achieve the same sensitivity a large number of animals would have to be used for every single batch of virus antigen produced.

Serum Neutralization Test :

Seroconversion of animals after vaccination is a good indicator for protection of the animals against foot-and-mouth disease virus (Baterling, 2002). Seroconversion is tested by measuring the virus neautralizing antibody levels in the blood of vaccinated animals in a so-called serum neutralization test (SNT).

A fixed volume of serum is mixed with a quantity of serially diluted virus and this sample is transferred to a 96 wells plate with BHK monolayer cells. If the antobody level in the serum is high all infectious virus particles will be neatralized and no CPE will develop in the BHK cells. If the antibody level is not high enough to enable completed virus neutralization, CPE will develop. The antibody levels can be calculated using the method of Spearman & Karber.

Conclusion :

To conclude, this can be said that the development of cell culture techniques for producton and QC testing of Foot-and-Mouth disease vaccines has enabled the yearly production of hundreds of millions of doses of safe vaccine required to reduce the number of outbreaks of FMD and to establish successful eradication programmes in various parts of the world.

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Selection of Foot-and-Mouth Disease Virus Vaccine Strain

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Foot-and-Mouth Disease (FMD) has still remained world wide the most important and for economic reasons, the most meaningful animal disease. This highly diffusible infection affecting thirty or more cloven-footed animals include some of the man's most valuable livestock: cattle, buffalo, swine, sheep and goat. The disease is generally characterized by the vesicular lesions on the feet and mouth of the infected animals besides the involvement of skin, teats, snout, rumen and myocardium. In cattle, there is profuse drooling of ropy saliva and rupturing of tongue vesicles, exposing large raw areas on the tongue.

The disease affecting hundreds of thousands of animals and leaves in their wake a multitude of infertility, sterility, impaired work ability and a very large number of dead young calves. It has the potential for causing enormous economic impact party because of the effect on trade. The Government should make a considerable effort to generate a greater awareness of the disease and its economic importance among the people by implementing proper and successful control programme. Successful disease control involves a number of measures used in combination. The availability of an appropriate high quality vaccine is an essential element in any successful campaign for the control of FMD, besides a range of zoosanitary measures, including restrictions on the movement of animals and effective cleaning and disinfection procedures. The vaccines consist of FMD virus grown in suspensions of Baby Hamster Kidney (BHK21) clone 13 cells has been inactivated with acetylethylenimine (AEI) or binaryethylenimine (BEI) and adsorbed into aluminium hydroxide gel particles.

Selection of Vaccine Strains :

Vaccination is likely to be the corner stone around which any future policy that will be built. Potent, safe and cheap vaccines will be required for both prophylactic and emergency purposes. To manufacture a polyvalent vaccine as in the case of FMD, the first and foremost duty is to select vaccine strains of all the types prevalent in a particular country.

There are three important points which should be kept in mind while selecting vaccine strains of FMD virus to be incorporated in polyvalent vaccine.

Serological Specificity of Vaccine Viruses :

FMD virus, giving its high mutation rate, is constantly changing to produce a range of antigenic variants. So, it is necessary to renew the vaccine strains time to time with the strains having wide coverage to get the desirable result. The basis for the evaluation of serological specificity is best expressed as the r-value relationship either in complement fixation test (CFT) or micro-neutralization test (MNT).

$$R = \frac{\text{Titre of reference serum against field isolate}}{\text{Titre of reference serum against homologous reference strain}}$$

This criterion recognizes that the serological relationship between vaccine and field strain is not always symmetrical. If the r-values of the field strains are closer to 1.00, it is certain that the current strain is able to give protection in animals against all the strains available in the field. If the r-value are very far from the 1.00, it is desired to replace the current vaccine strain with a suitable strain. The ideal FMD vaccine strain, therefore, would be one whose antiserum is highly reactive with many virus strains of the same type derived from the different parts of the world. It should be noted that factors influencing serological results such as test method employed, the intrinsic error of the test method, variation in the avidity of antiserum and the quality of the reagents used should be taken into account while interpreting the data.

Physio-chemical Characteristics of Vaccine Viruses :

The immunogenicity of FMD virus resides in the intact virion particles (75 S) lacking in RNA genome is equally immunogenic and stable over a period of at least 2 years at 4^0C. In selecting a vaccine strain for vaccine manufacture, it is important, therefore to identify the immunogenic component for that strain. It is equally important that the capsid should be stable to the inactivant used to render the virus non-infectious. FMD virus strain of almost similar serological specificity may be different in their capsid stability to inactivants used in vaccine viruses.

Growth Characteristics of Vaccine Viruses :

It is not sufficient to identity a FMD virus strain having wider antigenic spectrum and higher capsid stability to be used in manufacturing vaccine. Its growth characteristics in BHK_{21} suspension cells should be such that even at low multiplicity of infection, extra-cellular infectious virus yield would reach peak titre of 6.5 $\log_{10}$ pfu/ml within 24 hours of culture inoculation. Virus isolated from the same epizootic may differ markedly in their growth characteristics in BHK21 suspension cells. It has also been seen that even plaque mutants derived from the same virus isolate may differ greatly in their growth characteristics in BHK21 suspension cells and small plague variants generally grow readily in BHK21 to a higher titre than large plaque variants.

Conclusion :

The higher antigenic variation and low vaccine potency resulting from the inherent instability of the FMD virus results in a vaccine failure and disease outbreak. Due to its high mutability, emergence of a large number of strains quite different from vaccine strain are no longer taken care of by vaccine strain. It is of prime importance to select the vaccine strains from time to time to renew the existing strains to obtain a better and successful result. It is suggested, therefore, that an ideal FMD virus vaccine strain should fulfil the following criteria:

- Wide antigenic spectrum or coverage.
- Grow rapidly and regularly in the cell culture to a higher titre.
- Give rise to a high content of immunizing antigen which is stable after inactivation of infectivity.
- In the formulated state, the antigen must be capable of provoking immunity in vaccinated animals. The antigen must be stable for at least one year at refrigerated temperature.
- Lastly, formulated vaccine containing FMD virus strains of all serotypes having desirable characteristics should be reached to the users in ideal condition to vaccinate the entire susceptible animal, otherwise the whole idea of controlling and eradicating the notorious disease will be jeopardized.

History of Foot-and-Mouth Disease Vaccine Development: An Overview

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The development and use of vaccines against Foot-and-Mouth disease virus (FMDV) has been complicated by the presence of seven different immunologically distinct virus types, antigenic variation within virus types, the continuous emergence of new virus subtypes, the relatively short time for which FMD vaccines provide effective protection. Killed vaccines against FMDV are produced by growing virus in cell culture, inactivating the virus and combining it with an adjuvant, a substance which enhances the immune response. In recent times, further processing may be carried out to concentrate the antigen to reduce the volume required for vaccination of each animal, and allow storage of antigen for prolonged periods without loss of efficacy. Testing during vaccine production ensures safety and efficacy of vaccines produced under standards of Good Manufacturing Practice. Considerable progress has been made in areas such as virus growth, inactivation, purification and the development of effective adjuvants to boost the immune response to the vaccine. In recent years "vaccine banks" have been developed containing purified concentrated antigen in certain parts of the world which may be used to produce high potency emergency vaccines at short notice. This concept may take many more years to implement in developing countries. The effectiveness of vaccination in practice depends not only on the standard production of the vaccine but also on other logistics aspect of its strorage, transportation and proper administration under the expert supervision. This is much more relevant in the present scenario of an ongoing control program against FMD in India. In spite of many new developments around the world, killed vaccines continue to play a major role for at least another decade or so.

History of Inactivated Vaccine Development

In preliminary vaccine production initially, inactivated FMDV vaccines were developed by treating suspensions of virus taken from naturally-infected animals with a dilute solution of formaldehyde and/or by heat. Vaccines with greater potency were developed by extracting virus from infected tongue epithelium, adsorbing onto aluminium hydroxide gel followed by controlled-temperature treatment with a formaldehyde solution. Further progress in virus growth and harvesting has been made in the following ways :

- The virus from lesion material or viraemic blood of naturally infected cattle was used as a source of virus. Virus suspensions were produced by treatment of this natural material. Dose volumes of 30-100 ml were required. Potency of vaccine of preparations made from blood varied due to variations in time of maximal viraemia in donor cattle.

- The virus was taken from tongue epithelium and vesicular fluid of deliberately-infected cattle. In such cases, the problems

were related to the requirement to infect cattle to produce virus, limited production depending on handling of the infected cattle, variation in time to maximal virus titer in different individuals, risk of infected cattle to the surrounding livestock and hence, isolation / containment facilities were needed, required disposal of infected carcasses. It was ethically undesirable to use live animals for such vaccine production.

- The virus was grown from deliberately infected laboratory animals like rabbits, hamsters, guinea-pigs, day-old chicks or chicken eggs and then inactivated. But the limitations of this type of vaccine production were variation in time to obtain maximal virus titer in different individuals, risk to nearby livestock and hence also isolation / containment facilities were required. It further needed disposal of infected carcasses and it was of course ethically undesirable to use live animals for any vaccine production. Hence, further developments, mainly alternatives to live animal usage, took center stage for the improvements in FMD vaccine production.

Cultivation of Cell Culture and Virus :

The idea of tissue culture usage for virus growth has become major technological breakthrough in FMD vaccine production particularly on commercial scale. First of its kind is the Frenkel culture in which epithelium and superficial tissues collected from the tongues of freshly-slaughtered cattle were incubated and then virus was grown in this surviving tissue culture. This development allowed greater quantities of virus to be grown and processed. However, further increasing the production to meet the increased demand during phase of an outbreak became difficult. It required the constant supply of fresh tongue epithelium and this could be obtained relatively easily with a good organisation of collection at slaughter houses. But it required co-operation of the slaughter house and good hygienic conditions. A high and constant virus output could be achieved. No adaptation of virus to the culture system was needed. The method reduced the risk of allergic reactions to foreign proteins. Simple, cheap medium for culture, not requiring any serum. However, it was difficult to prevent low-level contamination with bacteria and yeast, and required substantial use of antibiotics (although product could be sterile filtrated). Then comes the use of primary cell culture for growing the virus in calf kidney or pig kidney cells in monolayers. However, this required the constant supply of donor organs for cells seeding. Also the prevention of extraneous pathogens is a constant concern. At last growth of virus in continuous cell line culture has been developed, which is at the moment being used widely all over the world. Specifically BHK (baby hamster kidney) cells are being used. With these cells, growth in suspension culture is possible, by which greater production capacities could be achieved. These cells lines can be maintained at different passages and stored in Liquid Nitrogen for the years. Also only once, at the Master seed level, a screening for the absence of extraneous agents is needed. Whenever required, the cells can be revived and scaled-up to desired production level under in-vitro conditions.

Inactivation of Virus :

The inactivation is one of the most important steps in producing a safe efficacious FMD vaccine. Vaccine should be inactivated beyond any doubt. With heat alone, inactivation is

incomplete. Hence, one started to use formaldehyde solutions of 0.02-0.10 %, buffered to pH 7.6-9.2 while heating to 23-26°C for 24-48 hours. This method of inactivation does not follow first order kinetics and hence problems have occurred with incompletely inactivated vaccines. Even prolonged treatment times with 0.05% formalin and incubation at 37°C could not always completely inactivate the virus. With beta-propiolactone (BPL) the inactivation is rapid, but care is required to avoid a sudden pH fall which results in complete denaturating of viral antigen. Today the most important inactivation agents are Aziridine compounds such as acetylethylenimine (AEI) and similar chemicals such as binary ethylenimine (BEI) and propylenimine. In practice Acetylethylenimine (AEI) at 0.05% and 3-5 mM Bromoethylenamine hydrobromide (BEA) are used, the latter at a pH of 8 (at which it is transformed to the active substance, ethylenimine). The inactivation is most reliable and produces first-order kinetics. "Tailing off" of reaction may occasionally occur under certain conditions and thus testing to ensure complete inactivation is important. Unlike formaldehyde EI's act directly on the viral genome.

Concentration and Purification of viral antigen

The inactivated antigen may be concentrated either by physical precipitation (for instance using polyethylene glycol, PEG) or by Ultrafiltration. Purification of viral antigen is important as impurities present in a vaccine may cause both local reactions at the site of vaccine inoculation as well as systemic reactions. Virus may be precipitated using polyethylene glycol (PEG) or polyethylene oxide (PEO) at particular concentrations. Continuous centrifugation may be used for large scale collection of precipitated antigen; alternatively filtration may also be used.

Addition of Adjuvants :

Adjuvants are essential to increase the immunogenicity of the antigen used in FMDV vaccines. Aluminum hydroxide is readily available, easily sterilized and simple to standardise. Saponin adjuvant can be used as such but aluminum-hydroxide plus saponin (Alsa) may be superior. These are commonly used for vaccinating ruminants since it is effective and cheaper than oil emulsion vaccines, but these Alsa vaccines are not effective in pigs. Oil adjuvants like Freund's incomplete adjuvant and others are recently more commonly used for vaccination of cattle, especially in South America. In pigs, the single oil emulsions may produce unacceptable tissue reactions and remain as a residue in the meat. But, tissue reactions are reduced by the use of double oil in water emulsions. However, in cattle, local reactions can also be reduced if the dose volume is decreased and the vaccine given subcutaneously. Dose volume may be reduced to 1.0 ml if concentrated and purified antigen is used; this then does not produce adverse reactions. Light mineral oil with a suitable emulsion can be used as an adjuvant. Purity of the ingredients for oil emulsions is extremely important for stability of the vaccine and to avoid adverse tissue reactions. Double-emulsion (water in oil in water) are obtained when water in oil emulsion is re-suspended in an aqueous phase containing a polysorbate such as 2% Tween 80. Vaccine emulsions may separate upon storage but homogeneity of the

vaccine can be restored by shaking. An advantage of a double oil emulsion is the lower viscosity than a normal single oil emulsion and a long-lasting immunity can be induced in both cattle and pigs. Often emergency vaccines are prepared with ready-to-use oil-in-water emulsion such as Montanide ISA 25/50 or water-in-oil-in-water emulsion Montanide ISA 206, using high antigen payload of BEI-inactivated, PEG-concentrated filtrate of antigen. For use of intra-muscular injection in pigs, double emulsions have low viscosity, low tissue reactivity and high potency. However, individual manufacturers may have their own experience and expertise to manufacture their own suitable adjuvants.

Quality Control of the Vaccine :

The vaccines must be quantified for their antigen content. The biological test systems may be used; originally cattle tongue and suckling mice tests and later cell culture tests. The complement fixation tests, which was used to give a quantitative estimate of total antigenic mass of the major 146 S immunogen. ELISA's have been developed as well to estimate 146 S particles. The sucrose density gradient analysis is most often used as the quantitative test, estimating the 146 S antigen in µg/ml. This test is now accepted internationally as the standard antigen test. Prior to vaccines being released for use, stringent tests must be passed for safety and efficacy. Testing using intra-dermolingual inoculation in cattle is prescribed by the Indian Pharmacopoeia as a final proof of safety. The *in-vitro* tests are more reliable than the intradermolingual test. More antigen can be screened in one test and for detection of virus that was not released from the cells, blind passages can be made. In vitro testing of inactivated vaccines by infection of tissue culture cells may be an effective alternative to testing using intradermolingual inoculation of cattle, for all stages of testing before adjuvants are added. However, as per local country requirements the monograph has to be followed. Inactivation kinetics may be used to indicate when total inactivation has occurred, at least if the inactivation process follows first-order kinetics. During production repeating inactivation may be applied following transfer of suspension to a second vessel may be used to avoid the risk of particles avoiding contact with inactivant, e.g. on container lids or in tubes or valves.

Vaccines prepared from virus grown in tissue culture cells and suitably inactivated with an imine have proved effective when (a) their quality is assured by adequate potency testing, (b) they are stored and transported at refrigerator temperatures and (c) they are administered under the supervision of an efficient veterinary service.

Vaccine efficacy depends on a number of factors including the potency of vaccine itself. To be approved for use, FMD vaccines have to pass stringent potency tests to prove their efficacy. Originally, vaccines were tested by the ability to protect cattle against the development of secondary lesions on the feet following inoculation of FMD challenge virus into the tongue (intradermolingual challenge). Vaccine dose extinction point has been adopted by the European Pharmacopoeia (EP) as their definitive reference method. Based on its correlation to protection the batch release could be done by serological methods, e.g. virus neutralising test or Elisa.

Vaccine Storage and Transportation :

The concentrated inactivated FMDV antigens can be stored at ultra-low temperatures which allow stable storage for many years and can be formulated into vaccine when required.

The European Union Vaccine Bank contains inactivated antigen of six different FMD virus strains (O1 Manisa, O1 BFS, C Noville, Asia 1 Shamir, A22 Iraq, A24 Cruzeiro) sufficient for the production of five million cattle doses of vaccine for each strain. In India, the vaccine bank concept is not in use, since the FMD control program in progress, is consuming, huge amounts, that is all of the vaccines produced. If ready-to-use vaccines are used, after storage for too long or storage or transport in incorrect conditions (e.g. allowed to get too warm), their efficacy decreases significantly. Optimum storage temperature is between +2 and + 8° C. FMD vaccines must not be frozen. These are most likely the problems occurring in areas where rapid transport and cold storage facilities are not reliably available.

Proper Use of the Vaccine :

Efficacy is decreased if handling (e.g. gentle shaking of contents) and dosing (site, quantity) are not carried out correctly. Administration under the supervision of an effective veterinary service provides the best chances for the desired efficacy.

Live FMD Vaccines :

Historically, live vaccines have been used but has been ceased due to the observed reversion to virulence under field conditions. FMD vaccine virus strains were attenuated by passage in mice (varying ages), rabbits, guinea pigs, chick embryos and later tissue culture to balance between loss of ability to cause disease and maintenance of capability to induce protection against severe challenge. The problems arose because strains which appeared to have been successfully modified under laboratory conditions turned out to be pathogenic under field conditions. Vaccine reactions and incomplete protection of livestock has been observed. Moreover, attenuation for one species does not guarantee the attenuation for other species. For example, a safe live vaccine for cattle proved to be very virulent in pigs especially after some passage in that species. Time taken to develop correct degree of attenuation is disadvantageous when faced with an outbreak involving a new virus subtype requiring the development of a new vaccine. Many countries prohibited the import of meat and other animal products from areas where live modified vaccines were used. Hence, the usage of live vaccines is almost none on the globe.

Modern Developments in FMD Vaccines :

There has been considerable interest in the development of peptide, protein or expression vector vaccines which would, for example, no need to be handled in high-containment facilities or to be kept refrigerated during storage. There are as yet no fully-developed alternative (not based on inactivated virus) FMD vaccines. There has been considerable interest in the development of vaccines which would overcome one or more of the limitations on the FMDV vaccines presently available. Disadvantages of the presently available FMDV vaccines are the requirement of including different vaccine strains for protection against different virus types and subtypes. There is a potential reversion to virulence (for live attenuated vaccines) apart from potential escape of live virus from vaccine production units.

Sometimes, improper inactivation of virus leads to many consequences. There is a requirement for cold storage and for refrigeration during transportation. Relatively short endurance of immunity and therefore requirements for frequent booster vaccinations. Any new vaccine would, for full usefulness, have to match the current inactivated vaccines in their ability to elicit a protective immune response, after a single vaccination, against severe experimental challenge (10000 LD_{50} inoculated into the tongue of cattle) and against close contact with infected animals. The following attempts have been made worldwide.

Peptide Based Vaccines :

Based on VP1, multimeric forms of the peptides involving the neutralizing epitopes (linear dimer or tetramer) have been e.g. attached to the N-terminus of B-galactosidase or presented as part of the core protein of hepatitis B virus, or polymerized with glutaraldehyde.

These candidate vaccines elicited good responses in guinea pigs but not in cattle and pigs. Hence, if at all feasible further work is required for the development of efficient peptide-based FMD vaccines.

Plasmid Based Vaccines :

A plasmid vaccine encoding two FMDV VP1 epitopes (amino acid residues 141-160 and 200-213) has been designed. Better response was obtained by a vaccine administered by gene gun at the back of the ear rather than at the inner side of the thigh. Pigs vaccinated twice with this vaccine were protected against the development of clinical signs, such as increased body temperature, foot lesions or mouth lesions when inoculated with FMDV. This is in the homologous situation, however the heterologous protection even within a serotype was not considered sufficient.

Recombinant proteins (Capsid proteins expressed in vector systems) :

The VP1 peptide from A12 strain of FMDV, was expressed in *Escherichia coli* as a fusion protein with 190 amino acids of the LE' protein of the tryptophan operon of *E. coli.* 58 μg of viral peptide, emulsified with oil adjuvant has been tried. Vaccine reactions of up to 2.5-3cm was seen in about 90% of cattle or pigs for about four weeks after vaccination, but not visible by six weeks (still detectable by palpation and on dissection at post-mortem examination). Good serological response has been obtained in cattle and they were resistant to challenge exposure at 28 days post-vaccination. An adequate serological response in pigs and was observed and they were also protected against challenge with homologous virus strain (A12), but only one of four was protected against a heterologous strain of the same type (A24).

Extracts from *Escherichia coli* expressing FMDV proteins from a construct containing P1-2A gene, portion of P2 gene and the 3C protease gene were also tested. High neutralizing antibody titres developed (mouse protection assay) and three of four animals were protected against the development of clinical signs whereas two were protected against virus replication.

FMDV P1-2A structural protein precursor gene and a portion of the P2 gene, were expressed in a recombinant baculovirus. With this recombinant, low neutralizing antibody response has been induced (mouse protection assay). Only two of four animals inoculated were found to be protected against clinical disease but not against virus replication.

Vaccines Development

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Vaccination Against Foot-and-Mouth Disease: Current Status and Future Outlook

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Foot-and-Mouth disease (FMD) is endemic in many parts of the world: South America, Africa, Middle East, Several Asian countries, and in several countries of the former Soviet Union. Countries in these areas are, only with intervals, successful in eradication of the disease for longer or shorter periods.

The viruses causing FMD can be distinguished in seven so-called serotypes: O, A, C, Asia1, SAT1, 2 and 3. More recently, the viruses are even considered to be completely different viruses within the aphtovirus group. Within each group again a great deal of variation occurs, so that in practice over 60 subtypes are recognized with variable cross reactivity within each of the serotypes. Still new subtypes are emerging, especially when a certain subtype is going around for some time in a given area. The typing of field isolates is done by the World Reference Laboratory Center in Pirbright, UK. They also do the sequencing of field isolates, which allows the matching with existing strains, most importantly those strains, which are adapted for vaccine use. The matching is done by cross-neutralization tests and expressed in R-values. The pairs of strains with R-values between 0.3 and 1.0 are considered sufficiently similar to consider vaccination with the respective vaccine strain.

The virus replication involves the production of one single polypeptide from the viral genome. This polyprotein is processed by viral and cellular proteases, firstly into a number of large fragments named P1 – 3 and then successively in to smaller fragments given the suffixes 1ABCD, 2ABC, 3ABCD. De proteins comprising the capsid are derived from fragment 1ABCD, also named VP1 –VP4. To some of the non-coat or non-structural protein functions could be attributed. For example, the protein with polymerase function comes with fragment 3D, whereas fragment 3B represents the small protein VPg that is covalently coupled to the 5'-terminal of viral genomic RNA (Fig. 1).

Deletions in the genome in fragment 3A are found in attenuated strains, therefore 3A is assumed to correlate with virulence in cattle. Probably, 3A plays a role in host range specificity as well, as virus strains with deletions in 3A that were attenuated in cattle were fully virulent in swine.

Vaccination has been shown to be very effective in preventing clinical disease as well as effectively decreasing spreading of the virus. However, it has been shown that after challenge infection of vaccinated animals, virulent virus can be recovered from the tonsils for an extended period of time; more than year for cattle and sheep. This persistence of the virus in animals that have at the same time antibodies against Foot and Mouth Disease Virus (FMDV) is considered a main threat for trading of animals from vaccinated herds. Despite the fact that in numerous trials under controlled conditions, quite surprisingly so far, it has not

been possible to obtain any evidence of transfer of virus from persistently infected animals to susceptible sentinel animals. The role of persistently infected animals in maintaining FMD in endemic areas is still not clear.

Since, early 90's, a virus strain is going around the world, named the Pan-Asia strain. Roughly from India, it passed through Afghanistan and Iraq to Turkey, somewhat later, it was found in China, Korea and Japan, in 1999, it appeared in South Africa. It was, however, the appearance in 2001 of this strain in Europe, which affected heavily the UK, that greatly increased the interest in strategies for control of FMD.

FMDV Vaccines :

Live vaccines have been used for a while with disastrous results. In the sixties in Israel and in the eighties in Indonesia reversion of the vaccine strain to virulence gave rise to outbreaks following vaccination. Also the FMD outbreak in swine in Taiwan in 1997, was most probably caused by the smuggling of swine from the mainland of China where live vaccine is used. A deletion characteristic for a virus attenuated for cattle was found in the viral genome. However, for swine such vaccines are fully virulent. In this particular case, the virus was also very well adapted to swine and wiped out almost the complete pig population in Taiwan.

Since 1953 and for many years thereafter, the classical inactivated FMD vaccines based on the ideas of Valée-Schmidt-Waldman-Frenkel were used. Basically, these vaccine viruses were grown on bovine tongue epithelium cells and after absorbing to aluminium hydroride gel inactivated with formaldehyde. Later on also a purified natural product, namely Quillaia saponin, was included in the vaccines as an additional adjuvant. The stability and relative potency of these vaccines was quite good. However, the major drawbacks were the availability of bovine tongues and to maintain sterile conditions in the tissue culture; high amounts of antibiotics in the medium were required. Furthermore, the use of formalin as activation agents was a source of constant concern. Complete inactivation was very difficult to obtain under all circumstances and as a result a number of outbreaks of FMD occurred following the vaccination route of the veterinarian. Here the breakthrough in inactivation procedures was the use of aziridines in 1963, especially the use of the much safer aziridine, bromo-ethyleneamine (BEA) which *in-situ* is converted into the active form binary ethyleneimine (BEI), was introduced by Bahnemann in 1975. From this time on the proper and complete inactivation of FMDV could be ascertained.

Another major improvement with respect to consistency and quality was the introduction of BHK cell culture for virus growth by the Pirbright Animal Virus Research Institute. Today, almost worldwide, BHK cells are used for propagation of any FMDV strain. Although most of the vaccine strains in use are quite well adapted to BHK, the adaptation of newly emerging strains from the field, however, is not always an easy task.

Historically, but even at present, there is quite some variation in dosage use. For cattle a dose has been as much as 10 ml, but that has decreased to volumes of 2 - 5 ml depending on the composition or formulation, the number of strains incorporated in a dose and even the country were it is applied. For sheep and goat half a cattle dose is often advised.

In addition, the adjuvant incorporated plays a role in the application. Aluminum hydroxide - saponin (Alsa) vaccines are mostly used for cattle and sheep, and mainly in the Middle East countries. For emergency vaccines the more potent oil-based adjuvants are taken. For immunization of swine Alsa-vaccines proved less efficacious, therefore in this species always oil-based vaccines are applied to insure a good immunity. Especially double oil emulsions (w/o/w) are preferred in pigs, as while maintaining efficacy it induces less adverse effects in this species.

In case of emergency vaccination, high-grade vaccines with oil adjuvant surprisingly provide an earlier protective immunity than the aqueous Alsa vaccines. Protection has been shown as early as 2-4 days after vaccination.

The larger volumes of vaccines and the use of serum in the virus growth medium gave rise to appreciable local reactions, both i.m. and s.c. The s.c. injection site in cattle was most often at the cossum (dewlap). To reduce the local reactions, the protein burden, consisting among others of the added serum and proteins released by the lysis of cells, was strongly reduced by introducing clarification and purification steps in the down-stream processing procedures.

In addition to the former also the wish to have a stock of virus, so-called "antigen banks" for emergency use added to the interest to develop procedures to concentrate as much as 100-times or more the original antigen content. Several ways can be followed to arrive at this point: Precipitation of viral antigen by polyethyleneglycol (PEG) is a well known example, whereas ultrafiltration can also be used as an alternative method for concentration. The difference is, however, that using ultrafiltration, part of the protein present in the harvest is concentrated as well together with virus particles. In contrast using PEG precipitation, the virus particles are concentrated and the protein burden is removed to a large extent. The concentration process is a prerequisite to allow storage of large amounts of antigen in the antigen banks as kept these days by governments for emergency use.

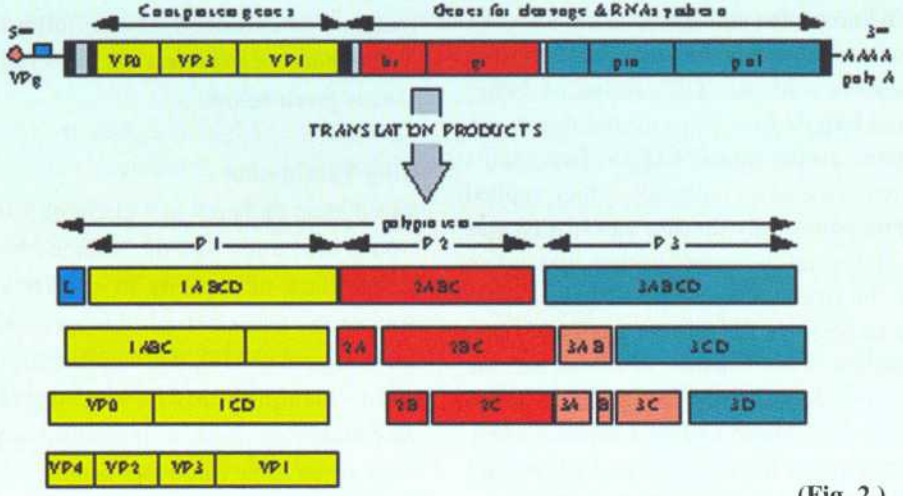
In the purification steps also the non-structural proteins are removed. During replication of the virus in the host cells "non-structural" (NSP) or better "non-capsid" (NCP) proteins are produced. The purification procedure now provides the possibility to develop test systems that can differentiate infected from vaccinated animal. Vaccinated animals only produce antibodies against the viral coat proteins, whereas infected animals in addition to antibodies against the viral particles also have an immune response against the non-capsid proteins. The NSP/NCP proteins have very conserved functions and as a result the immune response they elicit is the same for all serotypes, this in contrast to serotype specificity of various FMD strains.

Test for Differentiation of Vaccinated from Infected Animals :

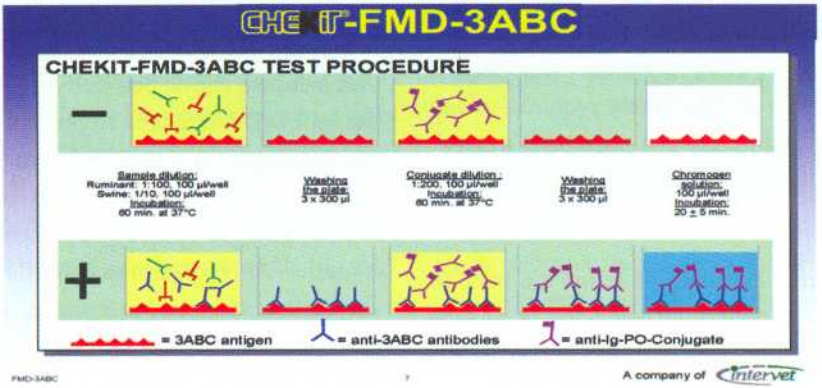
Of all the non-capsid proteins made during replication of the FMD viruses the choice has been made for the so-called 3ABC protein. Antibodies induced by these particular proteins can be readily detected in Elisa or Western-blot. Already in 1985 Bergmann et al. published on the use of antibodies against non-core proteins for this purpose. Later a consortium of institutes from a number of countries including e.g. Germany, The Netherlands, UK and Denmark in a concerted action declared that" Any

testprogram to differentiate between vaccinated and infected animals should start with the 3ABC test as a basis". To date the 3ABC test is validated for use in cattle, sheep and swine. Regarding sensitivity (simply said; the correct identification of true positives) and specificity (simply said; correct identification of true negatives) the total antibody Elisa and the 3ABC Elisa perform similarly. However, the antibodies against the nonstructural proteins tend to have a lower titer and tend to appear somewhat later. The 3ABC test is not suitable (yet) for monitoring of individual animals and it is advised to use the test on herd basis only. Therefore sampling rates have to be statistically sound. An outline of the ready-to-use Elisa, which is commercially available, is given (Fig. 2) Bergmann *et al.*, 1993 showed the usefulness of the 3ABC-test system in demonstrating the absence of circulating virus in vaccinated areas in Latin America. In Europe suspected outbreaks were monitored by the Pirbright 3ABC Elisa in the Balkan area, whereas a comparable Elisa test from Brescia was successfully used for instance in Albania and Macedonia. A strong point of the 3ABC-based

(Fig. 1.)



(Fig. 2.)



test is that it detects infection with any FMDV serotype and is thus very suitable for monitoring negative areas close to areas with endemic FMDV of various serotypes like Turkey and Iran. If positive serum samples are detected, a second test (so-called LPBE, Liquid Phase Blocking Elisa) can be performed to identify the serotype. The latter test also is the test of choice to perform sero-surveillance in vaccination programs.

Vaccination Programs :

After years of FMD-outbreaks, preventive vaccination became common use for several decades in many countries in Europe. From 1991 in Europe, the preventive vaccination was no longer allowed because it was not compatible with the OEI status of being declared FMDV-free. Once having this status, countries could retain FMDV free status quickly in case of an outbreak, if they applied slaughter policy only. In case vaccination was practiced a much longer period was taken before the free status could be regained. One of the reasons for this policy was that it was not possible to tell whether an animal became seropositive because of vaccination or as the result of an infection. As outlined above today this is no longer the case as e.g., ELISA's are available that can make the differentiation.

It goes without saying that in addition to either vaccination or culling a number of additional measurements are absolutely required in any control program: enforced movement control, disinfection regimes, surveillance programs, etc.

In the media, a variety of terms are used to describe the possible and actual use of vaccine under the conditions of an outbreak like the one that has hit Europe.

Preventive Vaccination :

Blanket vaccination: the historical practice of yearly routine, preventive or prophylactic vaccination of all cattle. In this case the optimal vaccination regime is two vaccinations with three to four weeks interval and a booster vaccination after approximately another 6 months. In practise this becomes in effect a yearly booster. Preferably such a vaccine has a potency of at least 3 PD_{50}'s.

Emergency Vaccination :

In case of the emergency of an acute outbreak many variations on the theme are envisaged, without pretending to be complete a comprehensive overview of the terminology used is given below.

Ring Vaccination :

Vaccination of farms in a circle of 1 or 3 km around the farm where the outbreak started, in case of lack of capacity to quickly kill and remove the animals from this area (so-called stamping-out policy). With similar purposes the terms "damping down" or "suppressive" vaccination are used; to reduce the spread of virus in an area while performing a culling scheme.

Firewall Vaccination :

Vaccination a region between an endemic area and an FMDV-free area to prevent spreading. For example in some Eastern European countries vaccination is applied as barrier to FMDV-outbreak in Turkey and former Russian countries. Another term used for that is Barrier vaccination. If applied in a smaller area outside the "protected" zone, this approach is also called "preventive emergency vaccination".

Emergency Mass Vaccination :
This is used if an outbreak is not handled by a stamping out policy but by use of vaccination of all susceptible animals.

Vaccination-to-Live :
Any emergency vaccination program that allows instead of destruction the slaughter of vaccinated animals and (local) consumption of the meat.

Future Prospects :
Growth of large amounts of FMD virus antigen in FMDV-free regions is still considered as one of the great risks of conventional vaccine production.

The great advantage of all the alternatives (see below) to inactivated virus vaccines is that there is no longer a need for high containment facilities to grow infectious FMD virus. For these reasons over more than 25 years a lot of efforts have been put into peptide and protein based vaccines with, unfortunately, limited success.

The pioneering work of Strohmaier *et al*, 1982 on the identification of the peptides that represented the major immunodominant epitopes of the coat of FMD virus in the beginning of the 80's was the starting point of great efforts to develop new types of vaccines. The great advantages of peptide vaccines were anticipated as:

- Very safe in production, as handling of infectious virus is no longer needed.

- Large scale peptide. Isynthesis was perceived as cheap. Initially, a number of quite promising results were reported. However, despite the further input of many institutes all over the world peptide vaccines turned out to be rather problematic in terms of the protection obtained. Only in case of challenge with the field virus strain homologous to the one from which the peptide was obtained significant protection was observed. The rapid changes of the virus in its highly variable regions of the capsid proteins complicate this approach a lot. Moreover, against the background of the many subtypes the kind of protection observed was not even close to that of the existing inactivated whole virus vaccines.

Other new alternatives became available by the use of molecular biology technologies in FMDV research resulting in many trials with the major outer capsid proteins produced from a variety of expression vectors, like for instance E. coli and Baculovirus. The products were inducing antibodies, but neutralizing activity was lacking. It appeared that for induction of neutralizing antibodies the conformation of the proteins, as present in the intact virus particle, was necessary to preserve the correct 3-dimensional structure of the capsid proteins. Only under these conditions neutralizing antibodies and thus protective immunity could be induced. Again until now these leads and attempts were not successful in competing with the existing vaccines.

The successful use of the polio live vaccine in humans also was taken as an example that could be pursued for FMDV. In practice the modified live vaccines as tested in Israel for instance turned out to become a disaster as the vaccine reverted to virulence and outbreaks of FMD followed the vaccination. The modern

technologies to construct modified live mutants did not lead to a solution yet. Small deletions made in the FMDV genome gave rise to attenuation in cattle; however, the modified virus retained virulence in swine. Detailed studies and an almost base-by-base understanding of the (molecular) biology of the virus are needed to give sufficient insight whether future genetically engineered modified live virus vaccines can be made safely.

A more recent progress in vaccine technology is the use of DNA vaccines. Initially this approach appeared highly promising for many applications. At this moment the considerations for the FMDV field are: on one hand the expressing of individual peptides has the same drawbacks as discussed above for the other expression systems, i.e. how to obtain the correct conformation of the proteins and on the other hand, the use of infectious DNA clones implies again the use of infectious FMD virus with all its safety considerations.

The technique of having an infectious DNA clone available gives the possibility of further manipulation of the virus. Not all the serotypes are equally well and easily adapted to growth on BHK cells. To solve this problem and to have a quick answer to an outbreak with a new (sub) serotype the group of Peter Mason (Plum Island, USA) developed a DNA clone of an FMDV vector virus that grows well in BHK. By inserting the characteristic epitopes of the VP2-protein of a field isolate in to this vector a hybrid virus can be obtained. This hybrid virus should grow well in BHK and at the same time can induce neutralizing antibodies relevant for the field isolate. The final vaccine still would contain the hybrid virus in inactivated, adjuvant form.

Finally, one of the more successful recent approaches for alternative FMDV vaccines is again a development from the FMD group at Plum Island and involves the use of an Adenovirus vector that expresses the complete set of FMDV structural / capsid proteins (VP1 to VP4). After vaccination the onset of immunity is fast like with the conventional vaccines. However, large titers of "infectious" Adenovirus (up to $10^{10}TCID_{50}$) are required and in addition for each serotype another Adenovirus-construct is needed.

Even after a successful proof of principle it would take many years of development before any alternative to the conventional inactivated vaccine would be licensed and available for large scale use.

"In the beginning, to be sure, this world was water, nothing but a sea of water. The waters desired. "How can we be propagated?" They kindled their own ardour, performing this very act with fervour. While summoning their creative energy they warmed up and a golden egg was produced."

- Satapatha Brahmana

Future Outlook / Research :

Suggested Reading :

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GUIDELINES TO CONTRIBUTORS

"The Blue Cross Book" is published biannually. The contributions to the journal are accepted in the form of review articles, research articles (clinical / field studies), case reports, other information pertaining to animal health and production. The decision of the Editorial Board members will be final regarding acceptance of the article for publication. The manuscript should be typed on one side of the paper with double spacing except for footnotes and references for which single spacing be used. The style of reference citing should be strictly followed as shown below. The words to be printed in italics should be underlined. The manuscript should be arranged in the following order:

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	e.g. Chhabra, D., Moghe, M.N. and Tiwari, S.K. (1996). *Ind. Vet. J.*, **82,** pp : 1-3.
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	Radostits, O.M., D.C. Blood, and C.C. Gray (1994). Veterinary Medicine, **8th Edn.**, *English Language Book Society (ELBS)*, London, pp.:121-125.
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GIR-1/3-30128/12/04

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