COMPARATIVE SEROCONVERSION OF FOOT-AND-MOUTH DISEASE VACCINES IN GOATS

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Thesis submitted in partial fulfilment of the requirement for the degree of

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DECLARATION

I hereby declare that this thesis entitled **"COMPARATIVE SEROCONVERSION OF FOOT-AND-MOUTH DISEASE VACCINES IN GOATS"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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CERTIFICATE

Certified that this thesis entitled **"COMPARATIVE SEROCONVERSION OF FOOT-AND-MOUTH DISEASE VACCINES IN GOATS"** is a record of research work done independently by **Shri. M. Madhanmohan,** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to him.

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Introduction

1. INTRODUCTION

The goat is a versatile animal. It is known as the poor man's cow in India and as wet nurse of infants in Europe. India ranks first among the countries of world in goat population with around 114 million and in Kerala goat population is around 2 million. Goats provide a dependable source of income to 40 per cent of the rural population below the poverty line in India. The direct contribution of goats to the Indian economy is however estimated as Rs.59, 741.16 million annually. Any threat to the goat production, therefore will seriously affect the national economic scenario.

Among various conditions adversely affecting the small ruminants health and productivity, Foot-and-Mouth Disease (FMD) stands first which cripples livestock industry and adversely affect the export of livestock products. In small ruminants the infection is subclinical but the spread of infection to incontact susceptible animal is of great epidemiological significance.

India has a dense livestock population of cattle, sheep, goats and pigs, which are commonly grazed together under the system of extensive animal practices. Among those, regular vaccination is usually done in cattle against FMD while other susceptible small ruminants are vaccinated only at the face of an outbreak depending on the extent of infection in the animals. A clear idea about the disease in these animals will ultimately help in the prevention and dissemination of the disease across the susceptible species.

Being an FMD endemic country, India could not export many of our livestock products to FMD free countries. Because of all these reasons and repeated epidemic episodes have attracted the scientific community to develop satisfactory control measures.

The strategy adopted for control and eradication of foot and mouth disease varies from country to country depending on geographical location, technological and economical development and prevailing political attitude besides its endemic nature. Stamping out policy considered to be the most successful measure for

eradication of FMD and this has been practiced by countries like United Kingdom with satisfactory results. In India, stamping out policy is not socio-economically feasible. Strict control on cattle and other susceptible livestock movement across the state and national borders can be practiced, but having only limited results. The systematic large-scale vaccination is the most appropriate method to bring down the incidence of the disease.

The most commonly used FMD vaccine in India has been an aqueous vaccine prepared from inactivated antigen adsorbed on aluminium hydroxide gel and adjuvanted with Saponin. The vaccine however, still suffers certain disadvantages like repeated administrations to maintain protective levels of immunity in vaccinated animals. The immunity produced with aqueous FMD vaccine keeps the animal disease resistant only for a period of six months.

During the last few years, there is an increasing interest in the use of oil-adjuvanted FMD vaccine, and reported to be producing long lasting immunity for nine months. Hence the present study was conducted with the following objectives

1. To evaluate the level and duration of immunity by Foot-and-Mouth disease vaccination using oil-adjuvanted and aluminium hydroxide gel vaccines in goats.
2. To compare the immunopotency of oil-adjuvanted and gel FMD vaccines in producing effective seroconversion of neutralizing antibody in goats.
3. To assess the level and duration of maternally derived FMD antibodies in kids born to vaccinated does.

Review of Literature

2. REVIEW OF LITERATURE

2.1 HISTORY

Foot-and-Mouth disease first appeared in 1839 in dairies of Stratford London (Henderson, 1978).

The first systematic study of FMD in South-East Asia was initiated at the Indian Veterinary Research Institute, Mukteswar in 1943 for identification and typing of FMD virus using stock strains of type O, A and C from World Reference Laboratory, Pirbright UK (Natarajan *et al.*, 1993).

The earliest description of Foot-and-Mouth Disease was recorded by Fracastorius in 1546. FMD virus was the first animal virus reported by Loeffler and Frosch in 1897 responsible for causing Foot-and-Mouth Disease (Doel, 2003).

2.2 ETIOLOGY

Foot-and-mouth disease virus (FMDV) is a member of the genus Aphthovirus belonging to the family picorna viridae (Murphy *et al.*, 1999)

2.2.1 Virus Properties

Galloway and Elford (1931) reported the size of foot-and-mouth disease virus as eight to 12 μm using gradocol membranes.

Bachrach *et al.* (1964) observed that the Foot-and-Mouth disease virus had sedimentation co-efficient of 140 S.

Sellers (1968) found that chemical substances like phosphoric, sulphuric, citric and formic acids and sodium carbonate, sodium metasilicate and sodium hydroxide inactivated FMD virus in short time.

The antigenic property of the virus was discovered by Valle and Caree in 1922 in France (Brooksby, 1982).

Kumar *et al.* (1999) reported that a close antigenic relationship (r = 0.9 to 1) existed between type O FMD outbreak virus from Haryana during 1996-97 and vaccine strain thus, ruled out the possibility of a variant virus for the outbreak.

The virus occurred as seven major serotypes *viz.*, O, A, C, South African Territories (SAT) 1, SAT 2, SAT 3 and Asia 1. Within these major serotypes many antigenically and serologically distinct subtypes have been identified (Murphy *et al.*, 1999; Radostits *et al.*, 2000).

The FMD virus was icosahedral in shape with no envelope, core consisted of single stranded RNA and a small protein (3B vpg) co-valently linked to its 5^1-end. The capsid was composed of 60 protein subunits, each consisting of four proteins (Murphy *et al.*, 1999).

2.3 EPIDEMIOLOGY

2.3.1 Prevalence

2.3.1.1 Global

Nazlioglu (1972) reported that the number of sheep and goats infected with FMD was greater than the number of cattle and buffalo infected in Turkey.

Hafez *et al.* (1994) reported that nine per cent of goat serum samples in Saudi Arabia were found to be positive for FMD by serological survey.

During 1951-52, over 9, 00,000 outbreaks of foot-and-mouth disease were reported in Europe (Kumar, 1996).

Taylor and Tufan (1996) reported that 18.5 per cent of the total FMD cases in Turkey were associated with sheep and goats.

Kitching (1998) reported that during an out break of FMD in Bulgaria in 1993, approximately 7000 cattle, 13,500 sheep, 1500 goat and 450 buffaloes were vaccinated around the affected farm and 510 cattle, 1516 sheep and goats and 17 pigs were slaughtered and in Kosovo in 1996, where 2298 cattle, 734 sheep and goats and 496 pigs were slaughtered. Serotype O of FMDV was identified in 1996

from former Soviet Republics of Georgia, Azerbaijan and Armenia and the most recent episode in Greece during 1996 involved 39 outbreaks, in which 5000 sheep and goat were destroyed.

2.3.1.2 India

Datt *et al.* (1968) studied the incidence and distribution of different types of FMD virus in India, using complement fixation test on the vesicular materials collected from the field cases of FMD. An incidence of 55 per cent for type O, 23 per cent type A, nine per cent type C and 13 per cent Asia-1 were detected.

Natural outbreaks of FMD in sheep and goats in India were reported by Sharma and Dutt (1968); Sharma *et al.* (1981).

Sharma (1981) reported that only 17 per cent of the actually infected goats expressed the clinical signs of FMD.

Das *et al.* (1983) studied the outbreak and distribution of different types of FMD virus in Assam, using complement fixation test on the epithelium collected from the field cases of FMD. A total of 471 outbreaks were recorded in Assam during April 1973 to December 1981 and all the four serotypes of the virus, *i.e.* O, A, C and Asia–1 were isolated.

Negi (1986) studied the sero-epidemiology of FMD virus by serum neutralization test in Hill tract of UP and found 61.33 per cent of type O, 52.40 per cent of type A, 10.71 per cent of type C and 14.95 per cent of type Asia-1.

Sharma *et al.* (1991) reported that the seasonal contour of foot and mouth disease in India was associated with winter in the Western, Southern and the central region and with summer in the northern region of the country.

Natarajan *et al.* (1993) reported that number of FMD outbreaks occurred in India were 1,940 during 1988, 790 during 1989, 4,186 during1990, 524 during 1991 and 950 during1992. Serotypes O, A, C and Asia-1 were recorded every year.

Singh *et al.* (1994) observed a heavy mortality of small ruminants in Mathura region due to FMD virus type O.

Saxena (1995) reported that the average annual rate of FMD incidence in India was 23 per cent and recorded incidence rate of 29 per cent in indigenous cattle, 17 per cent in crossbred cattle, 20% in buffaloes and 16 per cent in sheep, goats and pigs.

Jana *et al.* (1996) described a severe form of type O FMD outbreak among vaccinated and unvaccinated cattle and pigs of different age groups and sexes at sampling area of Gorubathan in Darjeeling.

Mishra *et al.* (1997) described an outbreak of FMD virus type O in an organized goat farm in Rajasthan where 93.3 per cent of unvaccinated goats were affected.

Verma and Sarma (1997) reported a series of foot and mouth disease outbreaks due to virus type Asia-1 in mithun (*Bos gaurus*) of Arunachal Pradesh during the period between June 94 and February 95 and a total of 818 affected mithuns died in the course of the outbreaks.

Mann *et al.* (1998) studied the prevalence of foot and mouth disease virus types in Northwest India during 1994-96 and observed that type O was most predominant type (79.67 per cent) followed by A 22 (17.89 per cent) and Asia-I (2.44 per cent).

In a study of FMD outbreaks in wild and semi-domesticated animals in northern states of India Barman *et al.* (1999) Observed that the highest numbers of outbreaks were recorded in mithun followed by yak, elephant, sambar deer, spotted, barking deer and wild buffalo.

The pandemic serotype O virus was first isolated from an outbreak of FMD in northern India in1990 (Knowles *et al.*, 2001).

2.3.1.3 Kerala

Anon (1983) reported 9,122 cases of FMD among cattle, 636 cases among buffaloes and 1, 463 cases among goats of Kerala during 1983.

During 1990-91, out of 28 samples collected from FMD outbreaks in Kerala, 16 were found to be type O and two as type Asia-1 (Anon, 1991).

Anon (1994) reported an incidence of FMD in Kerala during 1994 where 800 cattle, 84 buffalo and 90 goats were affected.

Vijayakumar (1999) reported that the FMD outbreak that occurred in almost all districts of Kerala during 1998 was the most severe outbreak among those occurred in the state in last 12 years with a total of 14,905 cattle, 66 buffaloes, 910 goats and 22 pigs affected.

2.3.2 Host

Armstrong *et al.* (1967) reported cases of foot-and-mouth disease in man.

Hedger (1976) reported persistent infection of FMD in African buffalo (*Syncerus caffer*) without clinical lesions.

Chakrabarty and Manjumder (1990) reported a confirmed case of foot-and-mouth disease in elephants.

Farag *et al.* (1998) studied the susceptibility of camels to natural infection with FMD virus. None of the 645 camel sera samples tested were positive for virus infection associated antibodies against, type A Sau 41/91 and 01 Manisa/68.

Knowles *et al.* (2001) reported an outbreak of foot and mouth disease in cattle in UK caused by a pandemic strain.

2.3.3 Transmission

FMD spread by the wind under certain epidemiological and climatic conditions (Henderson, 1969).

Hyslop (1970) opined that the virus was likely to be present in all physiological fluids during viremic phase. Any voided secretion or excretion

must be regarded as a continuing source of infection for other animals .The greatest concentration of virus occurred in the fluid of the vesicles and in the overlying epithelium where leakage or rupture causes contamination of the saliva. By 24 hour after inoculation the majority of fully susceptible cattle excreted virus at titres of $10^{4.5} - 10^{6.0}$ in their saliva. Such animals would constitute a serious hazard to other stock.

Pigs were very potent emitters of airborne virus (Donaldson *et al.*, 1982).

Gibson and Donaldson (1986) reported that cattle were very sensitive to infection by the respiratory route.

Spread of FMD occured by variety of mechanisms including animal movement, contaminated animal product (meat, milk, semen), mechanically by people and fomites and by the wind (Thomson, 1994).

Infection spreaded by contact between animals. In densely populated areas the disease spreaded extremely rapid because of the high level of challenge from infected animals. Indirect transmission of infection by means of contact with contaminated persons (farmers, veterinarians, and transporters), equipment, the environment (saliva, fomites) and animal products such as milk was also important (Metcalf and McElvaine, 1995; Donaldson, 1997).

Donaldson and Alexandersen (2001) opined that pigs were relatively resistant to infection by airborne FMD virus.

2.4 ECONOMIC IMPORTANCE

Ellis and James (1976) estimated the economic losses due to FMD in bovine population of India as Rs.420 crores per year.

Saxena (1995) stated that FMD caused loss through death of animal, reduced milk yield in milch animal, abortion in pregnant animal, delayed maturity of animals and loss of workdays in drought animal.

Kumar (1996) reported that economic impact of FMD included export embargo on animal products and byproducts and repeat breeding.

The economic losses to Indian dairy industry caused by FMD came to more than Rs.5000 crores per annum (Manickam, 1998).

FMD caused significant financial losses (Perry *et al.*, 1999).

There were direct losses due to deaths in young animals, loss of milk, loss of meat and a decrease in productive performance. The costs due to eradication or control were high and there were major indirect losses due to the imposition of trade restrictions (Rweyemamu and Leforban, 1999).

Vijayakumar (1999) reported that an FMD outbreak occurred in Kerala during 1998 caused an economic loss of Rs.66.33 lakhs due to death of animals and Rs.121.50 lakhs due to reduced milk yield.

2.5 CONTROL

Control on cattle movement was important during FMD outbreak. Door to door vaccination was to be practised and should not allow congregation of animals (Azad, 1999).

Saseendranath (1999) suggested that livestock movement control, proper handling and use of vaccine, strict control over importation of livestock and animal products, increasing vaccine production, proper reporting of outbreak and proper disinfection of cattle premises were essential for control of FMD.

The most practised methods of control of FMD in countries where slaughter of affected animals were socially and economically not feasible was vaccination (Sulochana, 1999).

Procedures most widely employed for the control of FMD were eradication or vaccination or employing both methods together. For disinfection of barns, one to two per cent sodium hydroxide or formalin or four per cent sodium carbonate solution could be used (Radostits *et al.*, 2000).

2.6 VACCINES

Vallee *et al.* (1926) achieved inactivation of the virus by formaldehyde while retaining the immunogenicity.

Belin (1927) described attenuation of FMD virus.

Schmidt (1939) reported the immunopotentiating ability of alum hydroxide.

Frenkel (1951) achieved cultivation of the FMD virus on a practical scale in explantation of bovine tongue epithelium and suggested that FMD vaccines were either formulated, as aqueous vaccine or oil emulsion vaccine. Aqueous vaccines were adjuvanted with alum hydroxide and saponin.

The use of an aziridine, acetylethyleneimine, as a first order inactivant of the virus was first described by Brown and Crick (1959).

Martin and Chapman (1961) reported approximate correlation between neutralizing antibody titres and protection of cattle against virulent challenge.

Mowat and Chapman (1962) adopted the BHK monolayer cell line for the growth and titration of FMDV.

Acetylethyleneimine (AE1) and Ethyleneimine were more reliable as inactivant for FMDV (Brown *et al.*, 1963).

VanBekkum *et al.* (1967) concluded that the aqueous vaccines were less effective in pigs.

Hyslop and Morrow (1969) reported that the immunizing power of the vaccines could be increased in the presence of saponin.

Binary ethyleneimine (BEI) was the most preferred chemical inactivant for FMDV as reported by Bahnemann (1975) and Nair and Sen (1992).

Nair *et al.* (1985) compared the saponin extracted from the seeds of Acacia concinna for its adjuvant activity with the imported saponin as an adjuvant for foot and mouth disease vaccine and revealed that the indigenous saponin was as good as imported saponin for FMDV inactivated vaccine.

Radlett *et al.* (1985) reported that the Wellcome foundation Ltd. U.K. produced 350 million monovalent equivalent doses of vaccine in 1983 using BHK-21 suspension cells grown in a controlled cell culture environment in bioreactors.

Pay and Hingley (1987) reported that antibodies played an important role in the protection of animals against FMD and also made correlation between protection and serum titre of the virus neutralizing antibodies

Iyer *et al.* (2001) found that FMD vaccines formulated with virus concentrated using eight per cent polyethylene glycol were more immunogenic than the vaccine formulated with the untreated harvest virus.

2.6.1 Aluminium Hydroxide Gel Vaccine

The first inactivated vaccine was developed by Waldmann *et al.* (1941) using virus from the epithelium and vesicular fluid of tongues of deliberately infected cattle, which was inactivated with formaldehyde in presence of aluminium hydroxide gel.

The use of adjuvants with inactivated FMD antigen preparations was essential for satisfactory potency and aluminium hydroxide was eventually supplemented with a second adjuvant, saponin (Espinet, 1951).

Formaldehyde was reported to be stabilizing the immunizing potency of FMD vaccines (Mowat *et al.*, 1973).

Rivenson *et al.* (1977) reported that the FMD vaccines prepared by inactivating the virus suspensions with formaldehyde or acetyl ethyleneimine (AEI) adsorbing the antigen on aluminium hydroxide gel and adding saponin produced immunity of shorter duration and repeated vaccinations at four months interval was needed.

Dawe and Pinto (1978) assessed antibody responses to type specific virus infection associated antigens (VIAA) in cattle vaccinated with inactivated aluminium hydroxide gel polyvalent foot and mouth disease virus in North Malawi and found that the animals with the annual vaccination regime were

positive for antibody against virus infection associated antigen while sera from animals outside the annual regime were negative.

Nair and Sen (1993a) studied the antibody response in sheep to aluminium hydroxide gel saponified foot and mouth disease virus types Asia 1 and O vaccines and found that antibody titres were detected at seven days post vaccination which increased gradually and reached maximum between 21 and 28 days post vaccination and then declined. A booster vaccination further increased the antibody level after two to four weeks.

Nair and Sen (1993b) observed that immunogenicity of aluminium hydroxide gel and oil-adjuvanted FMD vaccines in sheep did not differ significantly over a period of eight weeks.

Roy *et al.* (1999) assessed the immunity in Jersey cross, Holstein Friesian cross and Haryana cattle vaccinated with aluminium hydroxide gel foot and mouth disease vaccine and revealed that gradual acceleration reached the maximum at 28 days of post inoculation (Dpi) and then declined slowly during 90 Dpi and 150 Dpi.

2.6.2 Oil-adjuvanted Vaccine

Cunliffe and Graves (1963) compared the response of formalin inactivated vaccine combined with either aluminium hydroxide or oil adjuvants and found that the antibody response was higher and of longer duration with the oil adjuvant.

Arias *et al.* (1977) studied antibody response of tropical range cattle to foot and mouth disease virus and revealed that antibody levels to all three subtypes *viz.* O1, A27and A18 were sustained over the 15th sampling period and mean antibody titres observed for O1 slightly exceeded those of A27 for both VIA+ and VIA- groups.

Mc Kercher and Graves (1977) reviewed the current status of oil adjuvants in foot and mouth disease vaccine and concluded that oil adjuvants gave superior stimulation of antibody production and increase in the duration of immunity of FMD vaccines.

Solyom *et al.* (1977) studied the efficiency of foot and mouth disease vaccines prepared from strain C with different adjuvants and suggested that the aluminium hydroxide adsorbed preparation was the most immunogenic for calves, oil adjuvant for adult cattle and oil and Dextran containing adjuvant for pigs.

Oil adjuvant vaccines were also satisfactory for use in swine as reported by Gomes (1980).

The 9th meeting of South American Commission of the control of FMD (COSALFA) recommended use of oil adjuvant vaccine in endemic areas (Auge-de-Mello, 1982).

Sharma and Murthy (1985) conducted vaccination trials in sheep with FMD polyvalent vaccine and reported that the neutralizing antibody appeared between eight and 10 days and peak titre was noticed between 21 and 35 days post vaccination. The satisfactory neutralizing titres could be observed in animals with booster dose given four months after a primary vaccination.

In a multifactorial study of the influence of antigen dose and potentially competitive immunogens of FMD vaccine on the response of cattle of different ages it was found that doubling the antigen dose increased the serum antibody titre against both A2 cruzeiro, and O1 campos by approximately 0.15 log10 and there was no evidence of competitive inhibition or enhancement between the virus strains included in the vaccines (Black *et al.*, 1986).

Olascoaga *et al.* (1986) suggested that the oil-adjuvant vaccine were superior to those prepared with aluminium hydroxide.

Misra *et al.* (1991a) studied the antibody response in dairy cattle to inactivated polyvalent foot and mouth disease vaccine adjuvanted with Bentonite and saponin and found that the mean antibody titre (log_{10} SN_{50}) ranging between 1.37 ± 0.1 to 1.91 ± 0.11 at 21st day post vaccination were maintained upto 180 days and thereafter declined gradually. Revaccination resulted increased antibody titre ranged between 1.44 ± 0.12 to 2.31 ± 0.1.

Misra *et al.* (1991b) suggested bentonite as an adjuvant in the inactivated foot and mouth disease vaccine and found that those vaccines were protective, as that of conventional aluminium hydroxide gel vaccine.

Nair and Sen (1992) tested the response of oil adjuvant FMD vaccine on cattle experimentally and found that the titre stimulated by the oil adjuvant vaccines persisted longer than that stimulated by aqueous vaccine.

Ananda Rao *et al.* (1993) compared three FMD vaccine formulations *viz.*, aluminium hydroxide-saponin, Marcol oil emulsion and paraffin oil emulsion, using concentrated viral antigen stored over liquid nitrogen which were administered to groups of calves and found that Marcol oil emulsion vaccine (OEV) induced better serological response than other two vaccines, the serum neutralizing antibody titer in calves administered Marcol OEV remained at satisfactory level on 270 days post vaccination (DPV), and the presence of maternal antibody did not affect the serological response of animals to OEV.

Rana and Nag (1994) studied the antibody response of foot and mouth disease vaccination in cattle and found that very poor seroconversion occurred against Asia-1 type and the maternal immunity interfered the seroconversion of subsequent first dosing.

Barnett *et al.* (1996) compared two novel oil adjuvants Montanide ISA 25 and 206 (Seppic, Paris) and the results indicated that the vaccines adjuvanted with these oils retained potency for longer periods following storage at +4°C and elicited good immune response in both pigs and cattle regardless of route of infection.

Hunter (1996) assessed the performance of oil adjuvanted SAT serotypes of FMD vaccine in cattle, sheep and goats and found that a commercial double oil emulsion vaccine elicited higher antibody titres and a more prolonged antibody response than conventional vaccines.

Chitravel *et al.* (1997) studied the antibody response in Danish Jersey heifers to foot and mouth disease vaccine and found that the onset of antibody

response was on day seven post vaccination, and the percentage of animal exhibiting protective titre (≥ 1.5) on day 21 pv as 84, 88, 92 and 92 against type O, A22, C and Asia 1 respectively.

Hammami *et al.* (1997) evaluated the immune response with foot and mouth disease vaccine in ovine population in Tunisia and found that around 59 per cent of sheep had protective titre for at least 180 days post vaccination.

Cox *et al.* (1999) studied about the emergency vaccination of sheep against foot and mouth disease and suggested that both oil and aqueous emergency vaccines provided a rapid and protective immunity in sheep as early as three days following vaccination. These vaccines reduced virus replication in the oropharynx, consequently decreasing virus excretion and thereby limiting the transmission of the disease to susceptible nonvaccinated sheep.

Doel (1999) stated that the potential variables in vaccination against FMD like use of oil adjuvant for cattle were less critical when compared to elements like selection of appropriate strains and proper and timely administration.

Blanco *et al.* (2002) reported the serological evidence of FMD subclinical infection in sheep population during the 1999 epidemic in Moroco and demonstrated the presence of FMDV specific antibodies in 77 clinically normal sheep by using liquid phase blocking ELISA.

Deghaidy *et al.* (2002) studied the immune response of sheep to FMD vaccine containing different adjuvants and revealed that the double oil emulsion (DOE) FMD vaccine using the new emulsifier spane showed much higher antibody titres and longer duration of immunity than other two vaccine preparations.

Patil *et al.* (2002a) studied the early antibody response of cattle to FMD quadrivalent double oil emulsion vaccine and found that the early antibody response against all the four serotypes was detected as early as the fourth day following vaccination. The duration of immunity maintained for a long period.

The neutralizing antibody was maintained well above 2 $\log_{10}$ even after six months of vaccination irrespective of serotypes.

In a comparison study of double oil emulsion and aluminium hydroxide gel vaccines in eliciting immunity in goats it was found that the oil adjuvant elicited superior immune response at any given period than aluminium hydroxide gel vaccine and the rapidity of development of response was quicker. The duration of immunity also appeared to be maintained for long period. The difference in immune response between two adjuvant groups was statistically significant ($P<0.05$) (Patil *et al.*, 2002b).

2.6.3 Synthetic Vaccine

Kleid *et al.* (1981) reported that the recombinant DNA technology could be used to produce vp-1 of FMD virus in *Escherichia coli.*

Dimarchi *et al.* (1986) suggested that the experimental vaccine prepared with biosynthetic VP-a or synthetic peptide elicited neutralizing antibody in cattle but challenge experiments in cattle were often disappointing.

2.6.4 Maternal Immunity

Uppal *et al.* (1975) found that calves below one month of age, which received single dose of 40 ml polyvalent vaccine, did not withstand virulent challenge after six weeks where as by use of split dose of vaccine at an interval of 21 days they withstood the virulent challenge.

Roncha *et al.* (1983) conducted vaccination trial in young cattle with oil adjuvant FMD vaccines and recommended that vaccination of young cattle should be performed at least three times at intervals of six months followed by annual vaccination.

In a study to detect the antibody response of buffalo and cow calves to aphthovirus vaccine Sharma *et al.*, (1984) found that the neutralizing antibodies were appeared at seven days following primary vaccination, the titre peaked by 21 and 22 days and the titre remained at relatively higher level until revaccination, which resulted in a sharp rise of antibody response.

Francis and Black (1986) reported that young pigs, devoid of maternally derived antibodies (MDA) were capable of responding to FMD vaccination at one week of age whereas piglets born to FMD vaccinated sows, the MDA had a suppressive effect on the early vaccination response.

Shankar and Uppal (1986) found that the prevaccination sera of most of the calves born to FMD vaccinated cows showed varying levels of maternal antibodies with the SN indices ranging from zero to three while the calves born to unvaccinated cows showed negligible levels and calves of both the groups showed significant rise in SN antibody titres at 21 day post vaccination.

Calves born to vaccinated dams did not respond to the aqueous FMD vaccine 30 or 90 days post vaccination where as calves which were 30 or more days old responded to oil-adjuvant FMD vaccine like adult cattle (Sadir *et al.*, 1988).

Dorairajan *et al.* (1989) studied the antibody response against FMDV type O in pregnant ewes vaccinated at different periods of gestation and concluded that vaccination during the later stage of pregnancy would result in better antibody response at the time of lambing so that the lambs have protection through maternal antibodies.

Nair (1995) studied the immune response to type O FMD vaccine in pregnant ewes and lambs born out of them and concluded that pregnant ewes can be vaccinated at 12 weeks of pregnancy without any apparent untoward reaction. The colostrum fed lambs from vaccinated mothers showed high level of neutralizing antibody from 12 to 48 hours of birth and satisfactory level of maternal antibody persisted upto four weeks of age.

Calves aged three to four months with non-protective level of colostrum derived antibodies responded with high antibody titres to oil adjuvanted FMD vaccination (Spath *et al.*, 1995).

Gajendragad *et al.* (1999) reported that calves born to protected dams and fed with colostrum possessed antibodies against FMDV, though below the

presumed protective levels could still prevent the calves from getting clinical disease.

2.7 ASSESSMENT OF IMMUNE RESPONSE

The tests generally used for the detection of antibodies to foot and mouth disease virus was Virus Neutralization Test (VNT) and Enzyme Linked Immunosorbent Assay (ELISA) (OIE Manual, 1992).

2.7.1 Virus Neutralization Test

Golding *et al.* (1976) described a standard procedure for the detection of antibodies against FMD virus using Virus Neutralization test.

Sutmoller and Vieira (1980) reported that virus neutralization titres of FMDV obtained had a direct correlation with protection against FMDV challenge in cattle.

Results of serum neutralization test for quantitative estimation of serum neutralizing antibodies in dairy cows indicated that there was good level of neutralizing antibodies against all the four types of FMD virus even after 11 month of vaccination (Das, 1983).

The virus neutralization test was specific, sensitive and quantitative and takes two to three days to provide result. Low titre false positive reactions expected in a small portion of sera (OIE Manual, 1992).

Kalanidhi *et al.* (1993) carried out Micro Neutralization Test (MNT) for serum antibody assay to compare the efficacy of FMD vaccines prepared from concentrated antigens stored at low temperature and results obtained showed that vaccines formulated using antigen stored at +4°C and in liquid nitrogen for 18 to 30 months induced satisfactory titres for all the four virus types.

Dekker and Terpstra (1996) employed virus neutralization test to detect foot and mouth disease antibodies in dairy herds of Netherlands four years after vaccination. Virus neutralization titres equal to or higher than the titre at which 95 per cent of the cattle would be expected to be protected against challenge were

found in 57 to 73 per cent of the younger age groups and in 100 per cent of the older animal.

Bayri *et al.* (1999) assessed the protective immunity to a recombinant protein encoding C-terminal of the VP1 protein of type Asia-1 in guinea pigs using virus neutralization test. The sera collected at intervals of 21, 42, and 63 days after booster showed high titres, which could be protective.

2.7.2 Enzyme Linked Immunosorbent Assay

Abuelzein and Crowther (1978) employed indirect ELISA technique for quantifying antibodies to FMD virus from cattle sera. On comparison of the results from ELISA and neutralization test, a low degree of correlation was obtained ($r = 0.693$) between the two tests.

A rapid double sandwich enzyme-linked immunosorbent assay for the identification and type differentiation of foot and mouth disease viruses in epithelial tissue was found to be more sensitive and specific than CF test (Hamblin *et al.*, 1984).

Have *et al.* (1984) described an enzyme-linked immunosorbent assay for the primary diagnosis of foot and mouth disease using epithelial samples of type O and found that it was approximately 500 times more sensitive than complement fixation test.

Mc Cullough *et al.* (1985) developed a liquid phase ELISA to detect antigen/antibody reactions in liquid phase, which was to be six to eight times more sensitive than the indirect ELISA.

Hamblin *et al.* (1986a) described liquid phase blocking sandwich ELISA for the quantification of antibodies against foot and mouth disease, which can replace the virus neutralization test. The assay was rapid, relatively simple to perform, economic and results may be recorded within 24 hours.

Hamblin *et al.* (1986b) found that a titre of one in 16 in virus neutralization was equivalent to one in 40 by ELISA as indicated by the overall regression between the ELISA and the VN data.

Hamblin *et al.* (1987) evaluated the antibody titre against foot and mouth disease after infection and vaccination using ELISA. The antibody titres recorded by ELISA were compared with virus neutralization test results and concluded that results were similar following primary vaccinations and until five days after secondary vaccination.

A highly sensitive indirect sandwich enzyme linked immunosorbent assay, a suitable diagnostic and typing test for FMD virus of all seven serotypes was described by Roeder *et al.* (1987) and found that the sensitivity was approximately 125 times that of complement fixation test.

Ferris and Dawson (1988) compared indirect sandwich ELISA in parallel with CFT for the diagnosis of FMD and suggested ELISA as the test of choice for its superior sensitivity, reproductivity and economical use of reagents.

Westbury *et al.* (1988a) compared ELISA, CFT and virus isolation for foot and mouth disease diagnosis and found that the ELISA was at least three times more efficient than the CFT.

A single dilution blocking ELISA was developed and evaluated for measuring serum antibody to foot and mouth disease virus by Westbury *et al.* (1988b) and found that a positive correlation between ELISA and VN titres with the overall correlation coefficient being $r = 0.8990$.

A sandwich ELISA was described for subtype analysis of FMD by Pattnaik and Venkataramanan (1989) and the test was very much specific and time rating.

Villinger *et al.* (1989) developed an ELISA to detect antibodies to FMD virus infection associated antigen (VIA) in cattle sera using a bioengineered VIA (Bio VIA) protein antigen.

Ferris *et al.* (1990) compared the titres obtained with live or inactivated antigens and found that either live or inactivated antigens could be used in the liquid phase blocking ELISA.

Smitsaart *et al.* (1990) reported that an FMD diagnostic monoclonal antibody based inhibition-ELISA was found to be more sensitive than CFT and approached that of virus isolation.

VanMaanen (1990) developed a complex-trapping blocking ELISA for the detection of antibodies against FMDV, which was sensitive, type specific and more reproducible ($P<0.05$) than serum neutralization test.

Mc Cullough *et al.* (1992a) assessed the antibody response of cattle after vaccination against FMD using SNT, the sandwich ELISA, liquid phase ELISA, sandwich competition ELISA, Liquid phase competition ELISA and the liquid phase sandwich blocking ELISA and found that competition ELISA (Blocking ELISA) was the most collective at detecting reactivity in these cattle sera.

Saha and Sen (1995) compared different types of ELISA for detection of antigen and antibody of FMDV (SW ELISA, SWCOM-ELISA and BLK-ELISA) and found that the sandwich ELISA was superior because of its sensitivity than routine complement fixation test and BLK-ELISA was found to be more sensitive for estimation of antibody.

Araujo jr *et al.* (1996) described a BLK-ELISA for detection of antibodies against foot and mouth disease virus in water buffalo sera and found that a positive correlation existed between VNT and BLK-ELISA. Correlation coefficients for FMDV type O, A, and C were 0.83, 0.88 and 0.89 respectively.

Armstrong (1997) reported a LPB-ELISA and a specific isotype assay (SIA) for detection of antibodies against FMDV type O -Manisa in cattle milk.

Smitsaart *et al.* (1998) studied the herd immunity level induced in cattle by foot and mouth disease oil adjuvant vaccines using ELISA. Ninety nine per cent of the native cattle serum samples had titres below $\log_{10} = 1.2$ and none had a titre above $\log_{10} = 1.5$.

2.8 VACCINATION FAILURE/VACCINE FAILURE

2.8.1 The Virus/Antigen Factor

The use of purified or concentrated virus had made it possible to reduce the volume of the dose administered to as little as 0.1 ml and thus problem of adverse reaction in cattle caused by oil adjuvant was eliminated (McKercher and Graves, 1977).

Srinivasan *et al.* (1983) established the serological relationship between six O type FMD virus isolates from different parts of India. The vaccine strain O IND 53/79 exhibited broadest serological spectrum.

Goel and Rai (1984) detected antigenic drift taking place in FMD virus type O strains in India during 1967-82. This could be the reason for the incidence of general outbreaks of FMD in vaccinated herds.

Sarma *et al.* (1985) reported cross-reacting strains of FMDV isolated from field outbreaks in the north eastern region of India.

Formaldehyde inactivated Frenkel vaccine stored for as long as five years while aqueous vaccines formulated with azridine inactivated virus did not last that long because formaldehyde chemically cross linked the viral coat and this improved antigen stability (Bartelling and Anemaet, 1987).

Belwal *et al.* (1989) carried out a two dimensional micro neutralization test revealing considerable antigenic variation among 24 Asia-1 strains of FMD virus of Indian origin.

Nair and Sen (1992) studied the effect of inactivant in the immunogenicity of FMD vaccines in sheep and found out that no significant difference existed in antibody response to vaccines inactivated with formaldehyde or binary ethyleneimine.

Serological study of type A Indian FMD isolates by Azad *et al.* (1995) indicated that type A IND 17/82 had a broad immunogenic spectrum and could be

considered as a candidate vaccine strain for incorporation in FMD vaccines in India.

Gleeson *et al.* (1995) observed a close antigenic relationship between vaccine virus and outbreak virus (r = 0.61). The investigation suggested the requirement of close contact between animals for FMD to spread in tropical environment.

Sikdar *et al.* (1996) studied on subtype variation of FMDV strains of O and C type group, isolated from regularly vaccinated cattle in West Bengal and got the R value as 35 for O type and 63 for C type which indicated that the former was falling under variant group and the latter at the margin of homologous strain character, respectively.

Wani *et al.* (1996) characterized the foot and mouth disease virus type O isolated from immunity breakdown cases and revealed that the entire field isolates showed close relationship with reference strain.

Kumar *et al.* (1999) compared the FMD type O virus isolated from outbreaks in Haryana during 1996-97 with vaccine strain. For all field strains tested, r-value obtained was 0.9 to one indicating close relatedness of vaccine strain with outbreak strain.

2.8.2 The Host Factor

According to Pay and Parker (1977) immunity of foot and mouth disease in cattle appeared to be mainly depending on serum neutralizing antibody levels present at the time of exposure to infection. A linear correlation has been described between the log SNT produced in cattle following a primary vaccination and the log antigen dose (140S) and suggested that the variation in the serum neutralizing antibody response produced in cattle, even of the same age and breed following primary vaccination with a fixed antigen dose was quite large with standard deviation of the mean log SNT of 0.4.

A number of protozoan diseases were known to cause immuno suppression and trypanosomosis showed to suppress the response of cattle to FMD vaccination (Sharpe *et al.*, 1982).

Ahmad *et al.* (1991) studied the immune response to FMD vaccines (monovalent type A22) in *Trypanosoma evansi* infected guinea pigs. The infected animal showed a significant suppression of both humoral and CMI response.

Pay (1991) reported that the presence of maternal antibody depressed the response of young animals to FMD vaccination in varying degrees depending on the level of antibody present and the antigen mass present in the vaccine.

2.8.3 The Human Factor (Handling And Storage)

Pay (1991) reported that FMD antigens were relatively labile, and their decay rate would be proportional to temperature and time.

Kumar (1996) reported that aqueous as well as oil adjuvanted type of vaccines containing inactivated FMD virus needed to be kept at +4°C. Shelf life of conventional commercial FMD vaccine was 12 months even at refrigeration temperature and stated that the maintenance of cold chain was most difficult in India

A study on the effect of storage temperature on the shelf life of FMD vaccine revealed that the vaccines stored at 6 to 8°C and 35 to 37°C maintained the protective immunity upto 30 days, but those samples kept at 41 to 43°C were not efficacious even on 10 days of storage (Anon, 1998).

The use of chemicals to sterilize the syringe, excessive use of alcohol while swabbing skin, administration through unconventional routes or inadequate dose resulted in failure of an effective vaccine to stimulate protective immunity (Tizard, 2000).

Materials and Methods

3. MATERIALS AND METHODS

The study was carried out at the Department of Veterinary Epidemiology and Preventive Medicine, College of Veterinary and Animal Sciences, Mannuthy during June 2002 to May 2003.

3.1 MATERIALS

3.1.1 Glassware and Reagents

All glassware used was of either Borosil or Vensil brand and chemicals were of analytic or guaranteed reagent grade. All materials were processed by standard procedures and sterilized by either keeping in hot air oven at 160°C for 60 minutes or autoclaving at 121°C for 15 minutes at 15 lbs pressure, depending on the material sterilized.

3.1.2 Experimental Animals

Thirty healthy goats above four months of age, which were not vaccinated against FMD, were selected at random, from Kerala Agricultural University Goat and Sheep farm. They were grouped into two groups each consisting of 15 animals.

3.1.3 Vaccines

Two different commercial Foot-and-Mouth disease vaccines were used for the study. They were

Vaccine I : Raksha*

Vaccine II : Raksha-O Vac**

* Raksha- Inactivated quadrivalent aluminium hydroxide gel vaccine against O, A, C and Asia-1 strains of Foot-and-Mouth disease, manufactured by Indian Immunologicals Ltd.

** Raksha-O Vac Inactivated oil-adjuvanted vaccine against O, A, C and Asia-1 strains of Foot-and-Mouth disease, manufactured by Indian Immunologicals Ltd.

3.1.4 Liquid Phase Blocking Sandwich Enzyme Linked Immunosorbent Assay (LPB-ELISA)

3.1.4.1 ELISA Plates

Flat bottom 96 well ELISA plates (MAXISORP) were used as the test plates and 'U' bottom 96 well plates (NUNC) were used as the carrier plates for the LPB-ELISA.

3.1.4.2 Reagents

a. *Coating buffer* (0.5 M corbonate – bicarbonate buffer) pH 9.6

Sodium carbonate	1.59 g
Sodium bicarbonate	2.93 g
Distilled water to make	1000 ml

(First dissolved the reagents in 500 ml distilled water and made upto 1000 ml)

b. Dulbecco's phosphate Buffered saline (DPBS) pH 7.2

(i) Stock solution (5x)

Sodium chloride	40.0g
Potassium chloride	1.0 g
Magnesium chloride ($MgCl_2.6\ H_2O$)	0 .5g
Potassium dihydrogen orthophosphate	1.0 g
Disodium hydrogen orthophosphate	5.7 g
Calcium chloride ($Cacl_2.\ 2H_2O$)	0.5 g
Distilled water to make	1000 ml

(Dissolved $Cacl_2.\ 2\ H_2O$ separately in distilled water and added).

(ii) Working solution (1x)

DPBS stock solution (5x)	1000 ml

	Distilled water	1000 ml
c.	Dulbecco's phosphate buffered saline – Tween-20 (DPBS-T)	
	Tween-20	0.5 ml
	DPBS (1x)	1000 ml
d.	Citrate buffer (substrate buffer) pH 5.0	
	Citric acid	5.11 g
	Disodium hydrogen orthophosphate	7.3 g
	Distilled water to make	1000 ml
e. (i)	Substrate solution	
	Orthophenylene diamine dihydrochloride (Sigma)	30 g
	Citrate buffer	56.25 ml
(ii)	Activated substrate solution	
	30% hydrogenperoxide	0.001 ml
	Substrate solution	2 ml
f.	Reaction stopper solution (1 M H_2SO_4)	
	Con. sulphuric acid	63 ml
	Distilled water to make	1000ml
g.	Blocking buffer	
	Normal bovine serum	10 ml
	Normal rabbit serum	5 ml
	DPBS-T	85 ml

3.1.4.3 Biologicals

a. Antigen

Inactivated O, A, C and Asia 1 Foot and Mouth disease virus antigens were used.

b. Anti –146S immune rabbit serum (IRS)

Type specific rabbit antisera against O, A, C and Asia-1 FMDV antigens were prepared by two subcutaneous inoculation of inactivated 146S FMDV in Freund's Complete Adjuvant (FCA) (Have and Jensen, 1983). Animals were bled 28 days after the inoculation. Sera were dispensed into aliquots and stored at –20°C.

c. Anti –146S immune guinea pig serum (IGPS)

Type specific guinea pig antisera against O, A, C and Asia-1 FMDV antigens were prepared by inoculation of inactivated 146S FMDV in Freund's Complete Adjuvant (FCA) as described by Ferris and Donaldson (1984). Guinea pigs were bled after 28 days. Collected sera were pooled, dispensed into aliquots and stored at –20°C.

d. Anti-guinea pig – Horse radish peroxidase conjugated IgG

Anti guinea pig horse radish peroxidase conjugated IgG (Sigma) was used at a working dilution of 1 in 2000 in blocking buffer.

3.2 METHODS

3.2.1 Vaccination of Animals

Group I:

All the 15 goats of this group were vaccinated with vaccine I as follows.

Primary vaccination	:	at 4 months of age
First booster dose	:	at 5 months of age
Second booster dose	:	at 11 months of age
Dose	:	1 ml
Route of vaccination	:	Subcutaneous

Group II

All the 15 goats of this group were vaccinated with vaccine II as follows:

Primary vaccination	:	at 4 months of age
First booster dose	:	at 13 months of age
Dose	:	1 ml
Route of vaccination	:	Deep intramuscular

3.2.2 Collection of Serum Samples

All the goats were bled before vaccination separated the serum and inactivated at 56°C for 30 minutes in water bath. The samples were stored at –20°C which formed the prevaccinated, 0th day samples.

All the goats were bled at 7 day post vaccination (dpv), 14^{th} dpv, 21^{st} dpv and 30^{th} dpv and there after at monthly intervals for a period of one year from the date of primary vaccination.

3.2.3 Collection of Serum Samples from Kids

Blood samples were collected from seven kids born out of the vaccinated dams of each group immediately after birth (before taking colostrum), then 24 hours, 72 hours 1^{st}, 2^{nd} , 3^{rd} , 4^{th} , 8^{th} and at 12^{th} week of age. Serum samples were separated and inactivated at 56°C for 30 minutes in water bath. All the serum samples were stored at –20°C.

3.2.4 *Liquid Phase Blocking Sandwich Enzyme Linked Immunosorbent* Assay (LPB-ELISA)

LPB-ELISA was employed for the assessment of serum neutralizing antibody titre against O, A, C and Asia-1 foot and mouth disease virus antigens. The procedure was carried out as per Hamblin *et al.* (1986a).

3.2.4.1 Antigen

BHK-21 cell adapted, azridine inactivated O, A, C and Asia-1 FMD virus antigens were used for the LPB-ELISA.

3.2.4.2 Standardization of Reagents

The working dilution of antigens, immune rabbit serum, immune guinea pig serum and anti-guinea pig HRPO conjugated IgG were assessed by checker board titration procedure.

The working dilution of different reagents are as follows:

A. Antigen

O	:	1 in 8
A	:	1 in 4
C	:	1 in 4
Asia-1	:	1 in 4

Antigen dilutions were made in DPBS-T.

B.Immune rabbit serum (IRS)

O	:	1 in 1000
A	:	1 in 1000
C	:	1 in 1000
Asia-1	:	1 in 1000

IRS dilutions were made in coating buffer.

C.Immune guinea pig serum (IGPS)

O	:	1 in 1000
A	:	1 in 1000
C	:	1 in 1000
Asia-1	:	1 in 1000

IGPS dilutions were made in blocking buffer.

D. Anti-guinea pig HRPO conjugated IgG (Sigma) working dilution 1 in 2000 (in blocking buffer)

3.2.4.3 Test Procedure

3.2.4.3a Coating of Test Plates

All the four types of IRS were made into corresponding working dilutions with coating buffer (0.5M carbonate bicarbonate buffer). Flat bottom 96 well ELISA plates (MAXISORP) were used for coating with IRS. Added 50 µl of IRS at working dilution to all the 96 wells. Sealed the plates and kept at room temperature in a moist chamber overnight for coating.

3.2.4.3b Preparation of Carrier Plates

All the four types of antigens were made to corresponding working dilution with DPBS-T.

'U' bottom 96 well microtitre plates (NUNC) were used. Added 50 µl of DPBS-T to all the 96 wells. Added 50 µl of test serum samples in 1 to 10 wells of first row (ten samples on a single plate). Two fold dilutions were made column-wise (A to H wells of 1 to 10 columns).

Added 50 µl of corresponding antigen at working dilution to all the wells except in 12^{th} column of the carrier plate. Sealed the plates and kept at +4°C overnight for neutralization.

3.2.4.3c Transfer of Serum-Antigen Mixture to Test Plates

Washed the IRS coated plates five times with DPBS-T and tapped to dry. Transferred the contents to the corresponding wells of test plates. Only 50 µl of serum antigen mixture was transferred from each carrier plate well. Sealed the plates and incubated at 37°C for one hour with intermittent shaking.

3.2.4.3d Addition of Detecting Antibodies

All the four types of IGPS were made into corresponding working dilution with blocking buffer.

Test plates were washed five times with DPBS-T and tapped to dry. Added 50 µl of corresponding IGPS at working dilution to all the wells. Sealed the plates and incubated at 37°C for one hour with intermittent shaking.

3.2.4.3e Addition of Conjugate

Anti guinea pig HRPO conjugated IgG (Sigma) was made into a working dilution of 1 in 2000 with blocking buffer.

Test plates were washed five times with DPBS-T and tapped to dry. Added 50 µl of conjugate at working dilution to all the wells. Sealed the plates and incubated at 37°C for one hour with intermittent shaking.

3.2.4.3f Addition of Substrate

Washed the test plates five times with DPBS-T and tapped to dry. Added 50 µl of activated substrate solution to all the wells. The plates were kept in darkness for 10 minutes.

3.2.4.3g Addition of Stopper Solution

After 10 minutes, plates were taken out and added 50 µl of 1 M H_2SO_4 to all the wells.

3.2.4.3h Reading of the Plates

The optical density (OD) values were observed using a multi-scan spectrophotometer at a wavelength of 492 nm after setting the 12^{th} column as the column blank.

3.2.4.4 Control

The 12^{th} column of each plate was taken as the blank where antigen was not added. The 11^{th} column of each plate was taken as the antigen control for corresponding antigen where test serum is not added.

3.2.4.5 Interpretation of Readings

The serum neutralizing antibody titre against the corresponding foot and mouth disease antigen was estimated as the 50 per cent optical density and point of each serum dilution obtaining from the mean OD value of antigen control and expressed as $\log_{10}$ of the serum dilution.

3.2.4.6 Statistical Analysis

Statistical analysis of the results was done as per Snedecor and Cochran (1985).

Results

4. RESULTS

All the serum samples collected from the test animals were subjected to liquid-phase blocking ELISA for estimation of serum neutralizing antibody titres against FMDV types O, A, C and Asia-1. From the optical density values obtained, SN_{50} log_{10} values were calculated and were taken as antibody titres. Comparison of results between groups for all the four serotypes were done by analysis of variance, comparison of results between adjacent months were done by paired student t-test and the tables of t-value obtained are presented in Tables.

4.1 SEROCONVERSION FOLLOWING VACCINATION IN DIFFERENT GROUPS

4.1.1 Seroconversion of Type O Antibodies

4.1.1.1 Group I

The type O antibody titres of all the animals belong to Group 1 from zero to 12th month is given in Table 1. Following the primary vaccination the mean type O antibody titre was increased to 2.022 ± 0.097 by 21 days post vaccination. The highest mean titre of 2.150 ± 0.097 was obtained during the second month and the lowest mean titre of 1.343 ± 0.052 was obtained in the 11th month of study. The mean type O antibody titre of Group I before vaccination was 0.611 ± 0.0 and became 1.344 ± 0.043 in 12th month of study (Fig.1).

There was a significant rise in antibody titres ($P<0.01$) from 0.611±0.0 to 2.022±0.097 during the zero day of vaccination to 21st day post vaccination and significant fall in antibody titres ($P<0.01$) from 2.150 ± 0.097 to 1.446 ± 0.06 during second to sixth month. The mean type O antibody titre of 1.887 ± 0.082 during the eighth month was reduced to 1.343 ± 0.052 at 11th month, which was significant $P<0.01$(Table 4).

4.1.1.2 Group II

The mean titre of type O antibody of all the animals in Group II is given in the Table 2. The mean type O antibody titre of Group II animals before vaccination was 0.611 ± 0.0 and became 1.560 ± 0.113 at 12th month of study. After the primary vaccination the highest mean type O antibody titre of 2.117 ± 0.116 was observed on 21st day post vaccination and the lowest mean titre of 1.370 ± 0.1 was obtained during the ninth month of study (Fig.1).

There was a highly significant increase in mean type O antibody titre ($P<0.01$) from 0.611 ± 0.0 to 2.117 ± 0.016 following primary vaccination during zero to 21st day post vaccination. After the booster vaccination at ninth month the mean antibody titre increased from 1.370 ± 0.1 to 2.110 ± 0.136 which was significant $P<0.01$ (Table 4).

4.1.1.3 Comparison of Type O FMD Antibody Titres Between Groups

The comparison of type O antibody titres between two groups in each month is shown in Table 3. A significant difference between Group I and Group II was observed during second, third and 11th month. A highly significant difference was obtained during seventh day, seventh month, eighth, ninth and 10th month (Fig.1a).

4.1.2 Seroconversion of Type A antibodies

4.1.2.1 Group I

The mean type A antibody titre of all the animals belonging to Group1 from zero day to 12th month is given in Table 5. The mean type A antibody titre of Group 1 before vaccination was 0.611 ± 0.001 and became 1.607 ± 0.05 in 12th month of study. After the primary vaccination the highest mean type A antibody titre of 1.981 ± 0.092 was observed during the first month of vaccination. The highest mean type A antibody titre of 2.292 ± 0.08 was observed during the

second month of study and the lowest mean titre of 1.607 ± 0.05 was obtained during the 12th month of study (Fig.2).

There was a significant rise in mean type A antibody titre (P<0.01) from 0.611 ± 0.001 to 1.970 ± 0.092 from zero to 21st day post vaccination. After booster vaccination at first month the mean antibody titer increased from 1.981 ± 0.092 to 2.292 ± 0.08 which was significant P<0.01 (Table 8).

4.1.2.2 Group II

The mean type A antibody titre of all the animals in Group II is given in the Table 6. The mean type A antibody titre of Group II was 0.615 ± 0.05 before vaccination and became 1.730 ± 0.15 at 12th month of study. The highest mean type A antibody titre of 2.009 ± 0.12 was obtained during the 21st day post vaccination and the lowest mean antibody titre of 1.320 ± 0.115 was observed during the ninth month of study (Fig.2).

There was a highly significant increase in mean type A antibody titre (P<0.01) from 0.615 ± 0.005 to 2.009 ± 0.12 from zero to 21st day post vaccination. After booster vaccination at ninth month there was significant (P<0.01) increase in the mean antibody titre from 1.320 ± 0.115 to 2.005 ± 0.13 (Table 8).

4.1.2.3 Comparison of Type A FMD Antibody Titres Between Groups

The comparison of type A FMD antibody titres of two groups is given in the Table 7. Highly significant difference (P<0.01) was noted at second, seventh, eighth, ninth and 10th month (Fig. 2a).

4.1.3 Seroconversion of Type C antibodies

4.1.3.1 Group I

The type C mean antibody titre of all the animals belonging to Group I from zero days to 12th month is given in Table 9. The mean type C antibody titre of Group I was 0.610 ± 0.00 before vaccination and became 1.596 ± 0.048 at 12th month of study. After the primary vaccination the highest mean type C antibody titre of 2.102 ± 0.103 was observed during the first month of study. The highest mean type C antibody titre of 2.287 ± 0.081 was obtained during the second month of study and the lowest mean titre of 1.596 ± 0.048 was obtained during the 12th month of study (Fig.3).

On statistical analysis, a highly significant (P<0.01) increase was observed in mean titre from zero days to 21st day post vaccination (0.610 ± 0.00 to 2.096 ± 0.103). A highly significant increase in mean antibody titre was noticed after booster vaccination at first month from 2.102 ± 0.103 to 2.287 ± 0.081 (Table 12).

4.1.3.2 Group II

The type C antibody titres of Group II animals are presented in Table 10. The mean type C antibody titre of Group II at the beginning of the study was 0.610 ± 0.00 and increased to 1.750 ± 0.153 at the end of the study, during 12 month. Highest mean antibody titre following primary vaccination was noted during 21st day post vaccination (2.089 ± 0.130) and lowest mean antibody titre following vaccination during ninth month (1.337 ± 0.117) (Fig.3).

A highly significant rise in mean antibody titre (P<0.01) was recorded during zero to 21st day (0.610 ± 0.000 to 2.089 ± 0.130) and during ninth to tenth month (1.337 ± 0.117 to 2.050 ± 0.137).

A highly significant fall in mean antibody titre (P<0.01) was recorded during first and second month, second and third month, third and fourth and between tenth and eleventh month (Table 12).

4.1.3.3 Comparison of Type C Mean Antibody Titres Between Groups

The comparison of type C antibody titres of all the two groups in different months is shown in Table 11. A highly significant difference was observed during the eighth month and there was a significant difference observed during seventh, ninth and tenth month (Fig.3a).

4.1.4 Seroconversion of Type Asia-I antibodies

4.1.4.1 Group I

The type Asia-1 antibody titres of Group I animals are presented in Table 13. The Group I animals showed mean type Asia-1 antibody titre of 0.611 ± 0.001 before vaccination and it reached 1.523 ± 0.052 during 12th month. After the primary vaccination the highest mean type Asia-1 antibody titre of 1.978 ± 0.085 was obtained during the 21st day of vaccination. Highest mean antibody titre of 2.052 ± 0.084 was obtained during the second month following vaccination and lowest mean titre of 0.957 ± 0.043 was noted during seventh day post vaccination (Fig.4).

On statistical analysis, highly significant rise in antibody titres observed ($P<0.01$) from zero to 21st day post vaccination (0.611 ± 0.001 to 1.978 ± 0.085) and first to second month (1.957 ± 0.077 to 2.052 ± 0.084). Highly significant reduction in mean type Asia-1 antibody titre recorded ($P<0.01$) during third and fourth month (1.904 ± 0.072 to 1.745 ± 0.081) (Table 16).

4.1.4.2 Group II

The type Asia-1 antibody titres of Group II animals are presented in Table 14. The mean antibody titre of this group was 0.610 ± 0.000 before vaccination. The mean type Asia-1 antibody titre of 1.878 ± 0.152 was observed during 12th month. Following vaccination, highest mean antibody titre was observed during 21st day post vaccination (2.225 ± 0.136) and lowest titres during seventh day post vaccination (0.965 ± 0.043) (Fig.4).

Statistical analysis revealed highly significant rise in mean antibody titre ($P<0.01$) during zero to 21st day post vaccination (0.610 ± 0.000 to 2.225 ± 0.136) and during ninth to tenth month (1.478 ± 0.129 to 2.081 ± 0.136). The fall in mean titre during first to second (2.199 ± 0.133 to 2.047 ± 0.133), second to third (2.047 ± 0.133 to 1.906 ± 0.119) third to fourth (1.906 ± 0.119 to 1.727 ± 0.102) fifth to six and eleventh to twelfth month were significant $P<0.01$ (Table 16).

4.1.4.3 Comparison of Type Asia-1 Antibody Titres Between Groups

The comparisons of type Asia-1 antibody titres of two groups are presented in Table 15. A significant difference in antibody titres were observed during eighth and 12th month. A highly significant difference was obtained during tenth and 11th month of the study (Fig.4a).

4.2 MATERNAL ANTIBODY TITRES OF DIFFERENT GROUPS

4.2.1 Type O Maternal Antibody Titres

4.2.1.1 Group I

The mean titre of type O maternal antibody of all the kids in Group I are given in the Table 17. The mean type O maternal antibody titre of Group I immediately after birth (before colostrum feeding) was 0.610 ± 0.0. The highest mean type O maternal antibody titre of 2.180 ± 0.18 was observed at 24 hours after birth (after colostrum feeding) (Fig.5).

There was a significant rise in maternal antibody titres ($P<0.01$) from 0.610 ± 0.00 to 2.180 ± 0.18 during zero hours of birth to 24 hours after birth. There was a significant ($P<0.01$) fall in maternal antibody titres from 24 to 72 hours after birth, and from eighth week to 12 week after birth (Table 20).

4.2.1.2 Group II

The mean types O maternal antibody titre of Group II kids are shown in Table 18. The mean type O maternal antibody titre of Group II immediately after

birth was 0.610 ± 0.0. The highest mean type O maternal antibody titre of 1.908 ± 0.23 was observed during 24 hours after birth. There was a significant (P<0.01) rise in maternal antibody titres from 0.610 ± 0.0 to 1.908 ± 0.23 during zero hours of birth to 24 hour of birth. There was a significant (P<0.01) fall in maternal antibody titres from third to fourth, fourth to eighth and eighth to 12th week after birth (Fig.5).

4.2.1.3 Comparison of Type O Maternal Antibodies Between Groups.

The comparison of type O maternal antibodies between groups is presented in Table 19. There was no significant difference in the mean type O maternal antibody titres observed during the entire study period. (Fig.5a).

4.2.2 Type A Maternal Antibody Titres

4.2.2.1 Group I

The mean type A maternal antibody titre of all the kids in Group I are given in the Table 21. The mean type A maternal antibody titre of Group I was 0.610 ± 0.000 immediately after birth. The highest mean type A maternal antibody titre of 2.368 ± 0.25 was observed at 24 hours after birth (Fig.6).

There was a significant increase in maternal antibody titres (P<0.01) from 0.610 ± 0.000 to 2.368 ± 0.25 during zero hours of birth to 24 hours after birth. There was a significant fall in maternal antibody titres from 24 to 72 hours after birth. From 72 hours onwards the maternal antibody gradually declined and became 0.637 ± 0.027 at 12th week after birth (Table 24).

4.2.2.2 Group II

The maternal antibody titres of kids in Group II against type A antigen are presented in Table 22. Immediately after birth a titre of 0.610 ± 0.001 was observed, which was gradually increased after taking colostrum and the highest

mean type A maternal antibody titre of 2.012 ± 0.21 was obtained at 24 hours after birth (Fig.6).

A significant increase in maternal antibody titres (P<0.01) from 0.610 ± 0.00 to 2.012 ± 0.21 was observed from zero hours of birth to 24 hours after birth. Thereafter there was a significant decrease in maternal antibody titres, which reached at 0.610 ± 0.0 after 12 weeks of age (Table 24).

4.2.2.3 Comparison of Type A Maternal Antibody Titres Between Groups

The comparison of type A maternal antibody titres of two groups are given in the Table 23. There was no significant difference in the mean type A maternal antibody titre during entire study period (Fig.6a).

4.2.3 Type C Maternal Antibody Titres

4.2.3.1 Group I

The mean type C maternal antibody titre of Group I is given in the Table 25. The mean type C maternal antibody titre of Group I was 0.610 ± 0.000 immediately after birth. The highest mean type C maternal antibody titre of 2.357 ± 0.226 was noted at 24 hours after birth, thereafter declined to 0.637 ± 0.027 at 12^{th} week of age (Fig.7).

There was a significant increase in maternal antibody titres (P<0.01) from 0.610 ± 0.00 to 2.357 ± 0.226 during zero hours of birth to 24 hours after birth. There was a significant fall in maternal antibody from 24 to 72 hours after birth (Table 28).

4.2.3.2 Group II

The maternal antibody titres of kids in Group II against type C antigen are shown in Table 26. Immediately after birth a titre of 0.610 ± 0.00 was observed, which then increased to 2.033 ± 0.205 after taking colostrum at 24 hours after birth (Fig.7).

A significant increase in maternal antibody titres (P<0.01) from 0.610 ± 0.00 to 2.033 ± 0.205 was observed from zero hours of birth to 24 hours after birth. From 72 hours after birth the maternal antibody titres started to decline and became 0.610 ± 0.000 at 12th week of birth (Table 28).

4.2.3.3 Comparison of Type C Maternal Antibody Titres Between Groups

The comparison of type C maternal antibody titres of two groups are given in the Table 27. There was no significant difference in the mean type C maternal antibody titre during entire the study period (Fig.7a).

4.2.4 Type Asia-1 Maternal Antibody Titres

4.2.4.1 Group 1

The mean type Asia-1 maternal antibody titre of Group 1 is given in the Table 29. The mean type Asia-1 maternal antibody titre of Group 1 was 0.610 ± 0.0 immediately after birth. The highest mean antibody titre of 2.328 ± 0.23 was observed 24 hours after birth (Fig.8).

There was a significant rise in maternal antibody titres (P<0.01) from 0.610 ± 0.000 to 2.328 ± 0.23 during zero hours of birth to 24 hours after birth. A significant fall in maternal antibody titres from 72 hours of birth to 12th week of birth (1.964 ± 0.167 to 0.610 ± 0.0) (Table 32).

4.2.4.2 Group II

The maternal antibody titres of kids in Group II against type Asia-1 antigen are presented in Table 30. Immediately after birth a titre of 0.610 ± 0.000 was observed. The highest mean type Asia-1 maternal antibody titre of 2.022 ± 0.209 was observed at 24 hours after birth (Fig.8).

There was a significant increase in maternal antibody titres (P<0.01) from 0.610 ± 0.0 to 2.022 ± 0.209 was observed from zero to 24 hours after birth. A

significant fall in antibody titres was observed between 72 hours to first week, third to fourth week and eighth to 12th week (Table 32).

4.2.4.3 Comparison of Type Asia-1 Maternal Antibody Titres Between Groups

The comparison of type Asia-1 maternal antibody titres of two groups are given in the Table 31. There was no significant difference in the mean type Asia-1 maternal antibody titre during the entire study period (Fig.8a).

4.3 PROTECTION ATTAINED BY VACCINATION

For FMD type O antibody titres of 1.5 and above is taken as protective, for the protection of FMDV type A the antibody titres of one and above is taken as protective, for FMDV type C the antibody titres of one and above is taken as protective and for type Asia-1 the antibody titres of 1.4 and above is taken as protective (Srinivasan, 2001).

4.3.1 Group 1

4.3.1.1 Type O

Mean type O antibody titre of Group 1 animals reached to the protective level during 21st day post vaccination and maintained upto fifth month and titre became below the protective level at sixth month which again increased above the protective level by booster vaccination at seventh month and protective level maintained upto ninth month. Antibody titre was below the protective level during 11th and 12th month.

4.3.1.2 Type A

Mean type A antibody titre of Group 1 animals reached the protective level by seventh day post vaccination and maintained during the entire study period upto 12th month post vaccination.

4.3.1.3 Type C

Mean type C antibody titre of Group 1 animals attained the protective level by seventh day post vaccination and maintained during the entire study period upto 12th month post vaccination.

4.3.1.4 Type Asia-1

The mean type Asia-1 antibody titre reached the protective level by 21st day post vaccination and maintained during the entire study period upto 12th post vaccination.

4.3.2 Group II

4.3.2.1 Type O

The mean type O antibody titres reached the protective level by 21st day post vaccination and maintained upto sixth month. The titre was below the protective level during eighth and ninth month, which again increased to the protective level by booster vaccination at ninth month and maintained upto 12th month.

4.3.2.2 Type A

The mean type A antibody titre of Group II animals reached the protective level by seventh day post vaccination and maintained above the protective level during the entire period of study.

4.3.2.3 Type C

The mean type C antibody titre reached to the protective level by 14th day post vaccination and maintained above the protective level during the entire period of study.

4.3.2.4 Type Asia-1

The mean type Asia-1 antibody titre reached the protective level by 21st day post vaccination and maintained above the protective level during the entire period of study.

4.3.3 Maternal antibodies of Group I

4.3.3.1 Type O

In kids born from Group I animals the protective antibody titre was achieved at 24 hours after birth and maintained only upto one week of age. The mean type O maternal antibody titre was below the protective level during the rest of the study period.

4.3.3.2 Type A

The protective antibody titre was attained at 24 hours after birth and maintained upto four weeks of age.

4.3.3.3 Type C

The protective antibody titre was observed at 24 hours after birth after taking colostrum and maintained upto four weeks of age.

4.3.3.4 Type Asia-1

The protective antibody titre was obtained at 24 hours after birth and maintained only upto two weeks of age.

4.3.4 Maternal antibodies of group II

4.3.4.1 Type O

The protective antibody titre was observed as early as 24 hours after birth, which maintained only upto first week of age.

4.3.4.2 Type A

The protective antibody titres was achieved at 24 hours after birth and maintained above the protective level upto four weeks of age.

4.3.4.3 Type C

The protective antibody titre was observed at 24 hours after birth and maintained upto four weeks of age.

4.3.4.4 Type Asia 1

The protective antibody titre was obtained at 24 hours after birth and maintained only upto three weeks of age.

Table 1. The Type O FMD antibody titres of Group I animals

Ani no	Days Post Vaccination				Months											
	0	7	14	21	1	2	3	4	5	6	7	8	9	10	11	12
B1	0.610	0.969	1.121	1.885	1.881	1.675	1.596	1.483	1.302	1.298	1.696	1.637	1.756	1.200	1.128	1.420
B2	0.610	1.006	1.232	1.882	1.885	2.038	2.062	1.638	1.696	1.656	1.773	1.937	2.038	1.357	1.339	1.504
B3	0.610	0.701	0.969	2.427	2.431	2.800	2.401	1.923	1.726	1.184	1.726	1.575	1.427	1.418	1.374	1.356
B4	0.621	0.901	1.226	1.947	1.997	1.980	1.881	1.706	1.500	1.328	1.763	2.011	1.712	1.392	1.355	1.292
B5	0.610	0.726	0.999	1.927	1.953	2.178	2.112	1.886	1.348	1.212	1.765	1.923	1.666	1.626	1.414	1.347
B9	0.610	0.999	1.292	1.675	1.670	1.881	1.870	1.709	1.444	1.016	1.549	1.521	1.444	1.374	1.112	1.110
B11	0.610	1.102	1.346	2.800	2.775	2.800	2.425	1.937	1.881	1.890	2.038	2.178	1.937	1.801	1.726	1.575
B14	0.610	0.826	1.196	2.318	2.312	2.312	2.015	1.913	1.656	1.547	1.913	2.261	1.925	1.561	1.427	1.497
B16	0.610	0.901	0.999	1.997	1.889	2.273	2.036	1.854	1.511	1.461	1.917	1.854	1.449	1.249	1.194	1.394
B17	0.610	0.691	0.969	2.207	2.205	2.622	2.122	1.871	1.549	1.596	1.763	2.319	1.848	1.892	1.561	1.480
B19	0.610	0.901	1.216	1.957	1.969	1.956	1.808	1.707	1.481	1.318	1.979	2.010	1.707	1.351	1.363	1.227
B20	0.610	0.691	0.826	1.600	1.575	2.138	2.005	1.763	1.700	1.763	1.881	1.963	1.696	1.544	1.461	1.461
B22	0.610	1.121	1.196	2.427	2.421	1.654	1.649	1.544	1.347	1.331	1.924	2.273	1.816	1.595	1.577	1.363
B23	0.610	0.884	1.110	1.261	1.277	1.675	1.496	1.321	1.296	1.320	1.477	1.217	1.121	1.110	0.999	0.969
B235	0.610	1.002	1.155	2.014	2.012	2.273	2.103	1.787	1.787	1.765	1.924	1.625	1.412	1.337	1.121	1.161
Mean ± SE	0.611± 0.000	0.895± 0.037	1.123± 0.037	2.022± 0.097	2.017± 0.096	2.150± 0.097	1.972± 0.068	1.736± 0.046	1.548± 0.048	1.446± 0.064	1.806± 0.04	1.887± 0.082	1.664± 0.064	1.454± 0.055	1.343± 0.051	1.344± 0.043

Table 2. The Type O FMD antibody titres of Group II animals

Ani no	Days Post Vaccination				Months											
	0	7	14	21	1	2	3	4	5	6	7	8	9	10	11	12
A1	0.610	1.110	1.342	2.205	2.207	1.957	1.837	1.688	1.647	1.434	1.357	1.353	1.121	1.710	1.544	1.353
A2	0.619	1.015	1.292	2.455	2.444	1.992	1.979	1.912	1.881	1.889	1.796	1.790	1.696	2.765	2.432	2.001
A3	0.610	0.999	1.216	2.366	2.350	1.997	1.703	1.700	1.672	1.672	1.549	1.514	1.421	1.904	1.613	1.647
A4	0.610	1.456	1.464	2.456	2.427	1.992	1.969	1.906	1.888	1.968	1.992	2.018	2.133	2.800	1.688	2.112
A7	0.610	1.444	1.456	2.800	2.756	2.427	1.880	1.709	1.809	1.803	1.804	1.835	2.067	2.800	1.966	1.873
A8	0.610	1.213	1.244	2.800	2.800	2.775	2.625	2.400	2.244	2.067	1.825	2.025	1.883	2.800	2.800	2.518
A9	0.610	0.999	1.110	2.121	2.096	1.804	1.581	1.425	1.331	1.318	1.321	1.353	1.121	1.804	1.425	1.318
A11	0.610	1.126	1.212	2.291	2.199	2.006	1.703	1.598	1.456	1.358	1.301	1.306	1.121	2.098	1.500	1.418
A12	0.610	0.989	1.102	1.682	1.651	1.598	1.412	1.461	1.353	1.296	1.299	1.251	1.016	1.791	1.681	1.444
A13	0.610	1.109	1.204	2.400	2.386	1.765	1.768	1.710	1.507	1.422	1.374	1.301	1.121	2.775	1.581	1.627
A14	0.610	0.691	0.996	1.544	1.287	1.115	1.245	1.121	1.238	1.192	1.145	0.999	1.045	1.837	1.696	0.964
A17	0.610	0.726	0.969	1.549	1.500	1.521	1.564	1.544	1.461	1.444	1.096	1.151	1.001	1.332	0.610	0.787
A18	0.610	0.901	0.999	1.461	1.450	1.461	1.477	1.348	1.247	1.129	1.129	1.266	1.225	1.611	1.511	1.353
A20	0.610	1.110	1.205	1.656	1.636	1.543	1.500	1.418	1.425	1.425	1.450	1.353	1.225	1.651	1.444	1.425
A220	0.610	1.235	1.246	1.966	1.899	1.564	1.569	1.510	1.418	1.444	1.422	1.348	1.353	1.968	1.502	1.564
Mean± SE	0.611± 0.000	1.075± 0.056	1.204± 0.04	2.117± 0.116	2.073± 0.12	1.834± 0.11	1.721± 0.08	1.630± 0.08	1.572± 0.07	1.524± 0.07	1.457± 0.072	1.458± 0.08	1.370± 0.1	2.110± 0.136	1.666± 0.1252	1.560± 0.113

Table 3. Comparison of the mean Type O (Mean± SE) antibody titre of two groups

Gr	Days				Months											
	0	7	14	21	1	2	3	4	5	6	7	8	9	10	11	12
I	0.611± 7E-04	0.895± 0.037	1.123± 0.04	2.022± 0.097	2.017± 0.1	2.150± 0.1	1.972± 0.07	1.736± 0.05	1.548± 0.05	1.446± 0.06	1.806± 0.04	1.887± 0.08	1.664± 0.06	1.454± 0.056	1.343± 0.0519	1.344± 0.043
II	0.611± 6E-04	1.075± 0.056	1.204± 0.04	2.117± 0.116	2.073± 0.12	1.834± 0.11	1.721± 0.08	1.630± 0.08	1.572± 0.07	1.524± 0.07	1.457± 0.072	1.458± 0.08	1.370± 0.1	2.110± 0.136	1.666± 0.1252	1.560± 0.113
	NS	**	NS	NS	NS	*	*	NS	NS	NS	**	**	**	**	*	NS

* Significant at 5% level

** Significant at 1% level

NS- No significant difference between the groups.

Table 4. Table of t- values between Days/Months for Type O

G	Days				Months									
	0&7	7&14	14&21	21&1	1&2	2&3	3&4	4&5	5&6	6&7	7&8	8&9	9&10	10&11	11&12
I	7.664 **	10.081 **	9.476 **	0.545	1.589	4.435 **	6.857 **	4.720 **	2.302 *	7.084 **	1.328	4.863 **	3.864 **	4.244 **	0.009
II	8.314 **	4.962 **	10.298 **	2.766 *	4.872 **	2.522 *	4.956 **	2.561 *	2.355 *	2.480 *	0.008	2.481 *	8.641 **	4.597 **	1.538

* Significant at 5% level

** Significant at 1% level

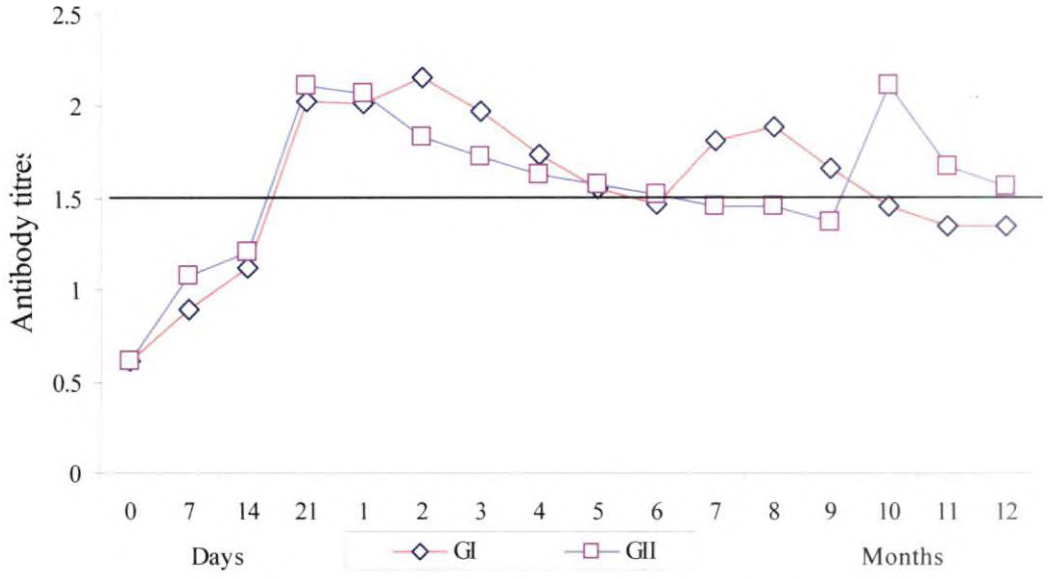

Fig.1. Seroconversion of type O antibody titres

(The black line indicates the level of antibody titre required for protection)

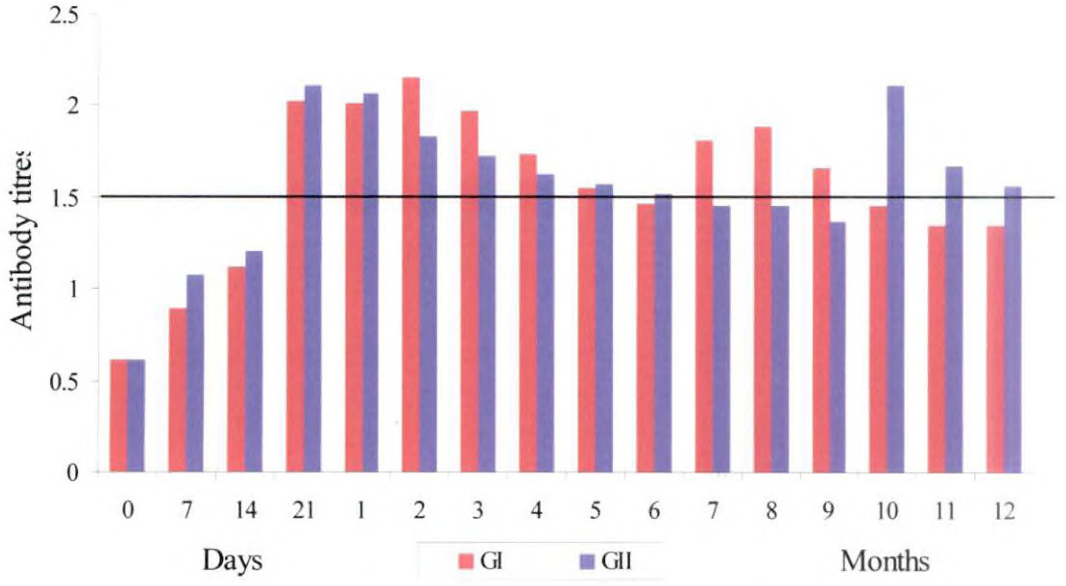

Fig.1a. Comparison of type O antibody titres of the two groups

(The black line indicates the level of antibody titre required for protection)

Table 5.The Type A FMD antibody titres of Group I animals

Ani no	Days Post Vaccination				Months											
	0	7	14	21	1	2	3	4	5	6	7	8	9	10	11	12
B1	0.610	0.896	1.201	2.103	2.101	2.438	1.788	1.801	1.656	1.525	1.801	1.801	1.607	1.541	1.455	1.642
B2	0.610	1.196	1.242	2.143	2.145	2.710	1.726	1.955	1.881	1.801	1.956	1.956	1.945	1.770	1.753	1.249
B3	0.610	1.112	1.156	1.676	1.678	2.025	1.501	1.637	1.605	1.600	1.773	2.025	1.965	1.801	1.771	1.686
B4	0.626	1.121	1.196	1.701	1.711	2.196	2.403	1.701	1.711	1.696	1.721	2.205	2.005	1.901	1.892	1.796
B5	0.610	0.996	1.110	2.176	2.176	2.400	2.212	1.636	1.558	1.336	1.928	1.965	1.708	1.701	1.547	1.205
B9	0.610	0.846	1.089	1.385	1.385	1.557	2.646	1.492	1.479	1.064	1.064	1.058	1.189	1.336	1.772	1.479
B11	0.610	0.926	1.105	1.557	1.561	2.164	1.401	1.912	1.766	1.770	1.772	2.164	1.912	1.915	2.132	1.532
B14	0.610	1.121	1.144	2.800	2.800	2.800	1.783	2.313	2.301	2.132	2.567	2.722	2.604	2.533	2.313	1.532
B16	0.610	1.096	1.203	2.161	2.196	2.411	1.245	1.915	1.824	1.801	1.851	1.915	1.388	1.319	1.361	1.552
B17	0.610	1.144	1.188	1.961	1.991	2.460	1.709	1.813	1.713	1.741	1.901	1.965	1.766	1.813	1.654	1.856
B19	0.610	0.999	1.147	1.965	1.991	2.211	1.656	1.711	1.522	1.449	1.713	1.909	1.522	1.562	1.505	1.702
B20	0.610	0.826	0.969	2.085	2.112	2.251	1.354	1.761	1.677	1.522	1.919	1.761	1.677	1.663	1.663	1.636
B22	0.610	1.132	1.215	2.138	2.141	2.445	1.656	1.703	1.504	1.500	2.089	2.139	1.915	1.720	1.703	1.672
B23	0.610	0.738	0.969	1.532	1.532	1.886	1.121	1.408	1.402	1.402	1.526	1.525	1.081	1.408	1.118	1.886
B235	0.610	1.152	1.161	2.169	2.200	2.421	1.634	1.804	1.677	2.034	2.212	1.861	1.851	1.806	1.772	1.686
Mean± SE	0.611± 0.001	1.020± 0.04	1.140± 0.02	1.970± 0.092	1.981± 0.092	2.292± 0.08	1.722± 0.108	1.771± 0.055	1.685± 0.056	1.625± 0.07	1.853± 0.084	1.931± 0.092	1.742± 0.1	1.719± 0.076	1.694± 0.08	1.607± 0.05

Table 6. The Type A FMD antibody titres of Group II animals

Ani no	Days Post Vaccination				Months											
	0	7	14	21	1	2	3	4	5	6	7	8	9	10	11	12
A1	0.610	1.212	1.256	1.966	1.966	1.788	1.788	1.685	1.523	1.220	1.036	1.199	1.099	1.788	1.685	1.685
A2	0.691	1.222	1.292	2.108	2.096	1.906	1.726	1.696	1.602	1.544	1.494	1.454	1.402	2.121	1.996	1.892
A3	0.610	1.192	1.211	2.101	2.101	1.866	1.501	1.501	1.454	1.461	1.378	1.252	1.100	2.101	1.903	1.933
A4	0.610	1.326	1.396	2.770	2.767	2.745	2.403	2.125	2.150	2.008	2.080	2.178	2.192	2.800	2.403	2.800
A7	0.610	1.241	1.244	2.800	2.800	2.746	2.212	1.910	1.992	1.713	2.800	2.800	1.925	2.800	2.447	2.588
A8	0.610	1.215	1.255	2.800	2.800	2.765	2.646	2.644	2.598	2.644	2.178	2.028	2.265	2.800	2.800	2.800
A9	0.610	0.969	1.196	1.543	1.561	1.501	1.401	1.385	1.121	1.120	1.001	0.999	0.969	1.385	1.322	1.392
A11	0.610	0.999	1.110	1.969	1.991	1.966	1.783	1.652	1.191	1.172	1.145	1.220	0.896	2.105	1.783	1.652
A12	0.610	0.726	1.101	1.339	1.339	1.301	1.245	1.241	1.240	1.246	1.196	1.196	1.245	1.746	1.413	1.243
A13	0.610	1.112	1.246	1.800	1.889	1.713	1.709	1.625	1.401	1.343	1.381	1.248	1.245	1.994	1.625	1.413
A14	0.610	0.842	1.095	1.966	1.966	1.801	1.656	1.357	1.325	1.058	1.006	1.184	1.109	2.077	1.903	1.357
A17	0.610	0.691	1.002	1.710	1.721	1.656	1.354	1.241	1.246	1.071	1.009	0.999	0.969	1.354	1.029	1.013
A18	0.610	0.901	1.204	1.918	1.921	1.959	1.656	1.509	1.418	1.456	1.437	1.408	1.311	1.656	1.509	1.514
A20	0.610	0.726	0.969	1.412	1.145	1.135	1.121	1.029	1.020	1.001	1.110	0.999	0.969	1.412	1.030	1.029
A220	0.610	1.009	1.198	1.932	1.946	1.901	1.634	1.612	1.543	1.471	1.359	1.222	1.110	1.930	1.613	1.634
Mean± SE	0.615± 0.005	1.026± 0.055	1.185± 0.03	2.009± 0.12	2.001± 0.126	1.917± 0.127	1.722± 0.108	1.614± 0.102	1.522± 0.11	1.435± 0.111	1.441± 0.135	1.426± 0.132	1.320± 0.115	2.005± 0.13	1.764± 0.13	1.730± 0.15

Table 7. Comparison of the mean Type A (Mean± SE) antibody titre of two groups

Gr	Days				Months											
	0	7	14	21	1	2	3	4	5	6	7	8	9	10	11	12
I	0.611± 0.001	1.020± 0.04	1.140± 0.02	1.970± 0.092	1.981± 0.092	2.292± 0.08	1.722± 0.108	1.771± 0.055	1.685± 0.056	1.625± 0.07	1.853± 0.084	1.931± 0.092	1.742± 0.1	1.719± 0.076	1.694± 0.08	1.607± 0.05
II	0.615± 0.005	1.026± 0.055	1.185± 0.03	2.009± 0.12	2.001± 0.126	1.917± 0.127	1.722± 0.108	1.614± 0.102	1.522± 0.11	1.435± 0.111	1.441± 0.135	1.426± 0.132	1.320± 0.115	2.005± 0.13	1.764± 0.13	1.730± 0.15
	NS	NS	NS	NS	NS	**	NS	NS	NS	NS	**	**	**	**	NS	NS

* Significant at 5% level

** Significant at 1% level

NS- No significant difference between the groups.

Table 8. Table of t- values between Days/Months for Type A

G	Days				Months										
	0&7	7&14	14&21	21&1	1&2	2&3	3&4	4&5	5&6	6&7	7&8	8&9	9&10	10&11	11&12
I	11.188 **	5.234 **	9.540 **	3.097 **	7.223 **	3.869 **	0.378	4.993 **	1.420	4.496 **	1.492	4.191 **	0.672	0.561	0.871
II	7.672 **	5.142 **	8.047 **	0.428	3.982 **	4.840 **	3.900 **	2.605 *	2.828 *	0.065	0.541	1.676	10.377 **	7.138 **	0.663

* Significant at 5% level

** Significant at 1% level

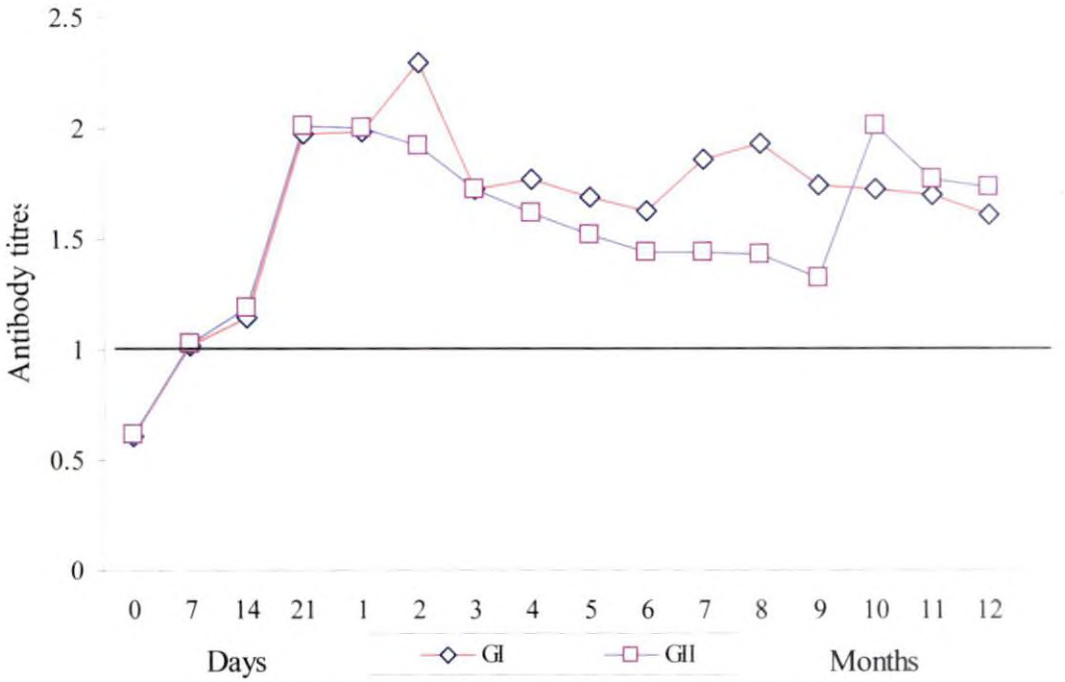

Fig.2. Seroconversion of type A antibody titres
(The black line indicates the level of antibody titre required for protection)

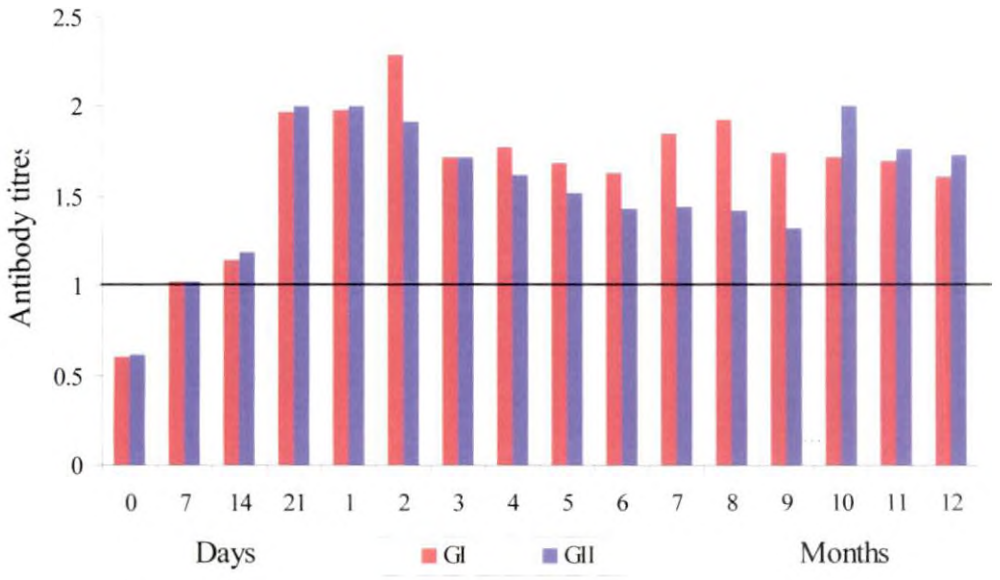

Fig.2a. Comparison of type A antibody titre of the two groups
(The black line indicates the level of antibody titre required for protection)

Table 9. The type C FMD antibody titres of Group I animals

Ani no	Days post Vaccination				Months											
	0	7	14	21	1	2	3	4	5	6	7	8	9	10	11	12
BI	0.610	1.012	1.213	2.403	2.401	2.438	2.156	1.801	1.656	1.525	1.801	1.801	1.607	1.541	1.455	1.642
B2	0.610	0.752	1.012	2.423	2.400	2.710	2.700	1.955	2.020	1.801	1.956	1.956	1.945	1.770	1.753	1.249
B3	0.610	0.901	1.110	1.676	1.678	2.025	1.721	1.637	1.605	1.600	1.773	2.025	1.965	1.801	1.771	1.686
B4	0.616	0.999	1.210	1.996	1.998	2.121	1.931	1.806	1.744	1.729	1.796	1.921	1.936	1.712	1.696	1.621
B5	0.610	0.910	1.121	2.271	2.281	2.400	2.275	1.636	1.558	1.336	1.928	1.965	1.708	1.701	1.547	1.205
B9	0.610	0.610	0.691	1.385	1.385	1.557	1.910	1.772	1.479	1.064	1.064	1.058	1.189	1.336	1.772	1.479
B11	0.610	1.224	0.999	1.557	1.561	2.164	2.101	1.912	1.766	1.770	1.772	2.164	1.912	1.915	2.132	1.532
B14	0.610	1.214	1.360	2.800	2.800	2.800	2.701	2.313	2.301	2.132	2.567	2.722	2.604	2.533	2.313	1.532
B16	0.610	1.116	1.218	2.361	2.400	2.411	2.400	1.915	1.824	1.801	1.851	1.915	1.388	1.319	1.361	1.552
B17	0.610	1.001	1.292	2.161	2.164	2.460	1.965	1.813	1.713	1.741	1.901	1.965	1.766	1.813	1.654	1.856
B19	0.610	0.999	1.196	2.177	2.177	2.211	1.909	1.711	1.522	1.449	1.713	1.909	1.522	1.562	1.505	1.702
B20	0.610	1.224	1.301	2.085	2.112	2.251	1.919	1.761	1.677	1.522	1.919	1.761	1.677	1.663	1.663	1.636
B22	0.610	1.121	1.324	2.438	2.441	2.445	2.139	1.703	1.504	1.500	2.089	2.139	1.915	1.720	1.703	1.672
B23	0.610	0.969	1.126	1.532	1.532	1.886	1.525	1.408	1.402	1.402	1.304	1.525	1.081	1.408	1.118	1.886
B235	0.610	1.244	1.356	2.169	2.200	2.421	2.034	1.804	1.677	2.034	2.212	1.861	1.851	1.806	1.772	1.686
Mean± SE	0.610± 0.000	1.020± 0.047	1.169± 0.045	2.096± 0.103	2.102± 0.103	2.287± 0.081	2.092± 0.084	1.796± 0.051	1.697± 0.059	1.627± 0.071	1.843± 0.089	1.912± 0.090	1.738± 0.095	1.707± 0.075	1.681± 0.074	1.596± 0.048

Table 10. The Type C FMD antibody titres of Group II animals

Ani no	Days Post Vaccination				Months											
	0	7	14	21	1	2	3	4	5	6	7	8	9	10	11	12
A1	0.610	0.912	1.244	1.966	1.966	1.788	1.788	1.685	1.656	1.220	1.036	1.199	1.099	1.788	1.685	1.685
A2	0.612	0.969	1.210	2.765	2.701	2.456	2.009	1.944	1.901	1.881	1.791	1.702	1.646	2.796	2.492	2.196
A3	0.610	1.112	1.212	2.101	2.101	1.866	1.501	1.501	2.020	1.461	1.378	1.252	1.100	2.101	1.903	1.933
A4	0.610	1.246	1.456	2.800	2.800	2.745	2.403	2.125	1.605	2.008	2.080	2.178	2.192	2.800	2.403	2.800
A7	0.610	1.061	1.296	2.800	2.800	2.746	2.701	1.910	1.558	1.713	2.800	2.800	1.925	2.800	2.447	2.588
A8	0.610	1.296	1.359	2.800	2.800	2.765	2.762	2.644	1.479	2.644	2.178	2.028	2.265	2.800	2.800	2.800
A9	0.610	0.721	0.969	1.543	1.561	1.501	1.401	1.385	1.766	1.120	1.001	0.999	0.969	1.385	1.322	1.392
A11	0.610	0.971	1.110	2.112	2.115	1.966	1.783	1.652	2.301	1.172	1.145	1.220	0.896	2.105	1.783	1.652
A12	0.610	0.752	0.999	1.339	1.339	1.301	1.245	1.241	1.824	1.246	1.196	1.196	1.245	1.746	1.413	1.243
A13	0.610	1.296	1.354	2.169	2.172	2.129	1.994	1.625	1.713	1.343	1.381	1.248	1.245	1.994	1.625	1.413
A14	0.610	1.006	1.214	1.966	1.966	1.801	1.656	1.357	1.522	1.058	1.006	1.184	1.109	2.077	1.903	1.357
A17	0.610	0.691	1.211	1.710	1.721	1.656	1.354	1.241	1.677	1.071	1.009	0.999	0.969	1.354	1.029	1.013
A18	0.610	0.969	1.321	1.918	1.921	1.959	1.656	1.509	1.504	1.456	1.437	1.408	1.311	1.656	1.509	1.514
A20	0.610	0.721	1.121	1.412	1.145	1.135	1.121	1.029	1.402	1.001	1.110	0.999	0.969	1.412	1.030	1.029
A220	0.610	0.999	1.327	1.932	1.946	1.901	1.634	1.612	1.677	1.471	1.359	1.222	1.110	1.930	1.613	1.634
Mean± SE	0.610± 0.000	0.981± 0.052	1.227± 0.035	2.089± 0.130	2.070± 0.135	1.981± 0.132	1.801± 0.129	1.631± 0.104	1.707± 0.060	1.458± 0.115	1.460± 0.137	1.442± 0.134	1.337± 0.117	2.050± 0.137	1.797± 0.138	1.750± 0.153

Table 11.Comparison of the mean Type C (Mean± SE) antibody titre of two groups

Gr	Days				Months											
	0	7	14	21	1	2	3	4	5	6	7	8	9	10	11	12
I	0.610± 0.000	1.020± 0.047	1.169± 0.045	2.096± 0.103	2.102± 0.103	2.287± 0.081	2.092± 0.084	1.796± 0.051	1.697± 0.059	1.627± 0.071	1.843± 0.089	1.912± 0.090	1.738± 0.095	1.707± 0.075	1.681± 0.074	1.596± 0.048
II	0.610± 0.000	0.981± 0.052	1.227± 0.035	2.089± 0.130	2.070± 0.135	1.981± 0.132	1.801± 0.129	1.631± 0.104	1.707± 0.060	1.458± 0.115	1.460± 0.137	1.442± 0.134	1.337± 0.117	2.050± 0.137	1.797± 0.138	1.750± 0.153
	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	*	**	*	*	NS	NS

* Significant at 5% level

** Significant at 1% level

NS- No significant difference between the groups

Table 12. Table of t- values between Days/Months for Type C

Gr	Days				Months										
	0&7	7&14	14&21	21&1	1&2	2&3	3&4	4&5	5&6	6&7	7&8	8&9	9&10	10&11	11&12
I	8.378 **	4.764 **	11.562 **	1.623	4.173 **	3.586 **	5.617 **	6.356 **	1.589	3.981 **	1.530	3.710 **	0.850	0.571	0.857
II	7.127 **	7.437 **	7.958 **	1.013	4.118 **	4.744 **	3.218 **	0.617	1.792	0.033	0.650	1.681	9.779 **	7.679 **	0.863

* Significant at 5% level

** Significant at 1% level

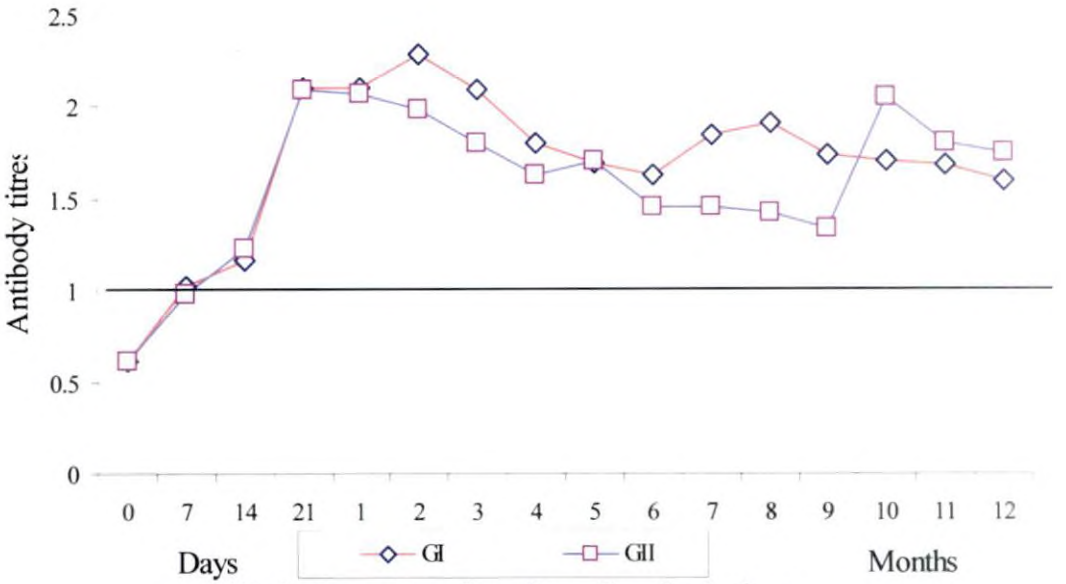

Fig.3. Seroconversion of type C antibody titres
(The black line indicates the level of antibody titre required for protection)

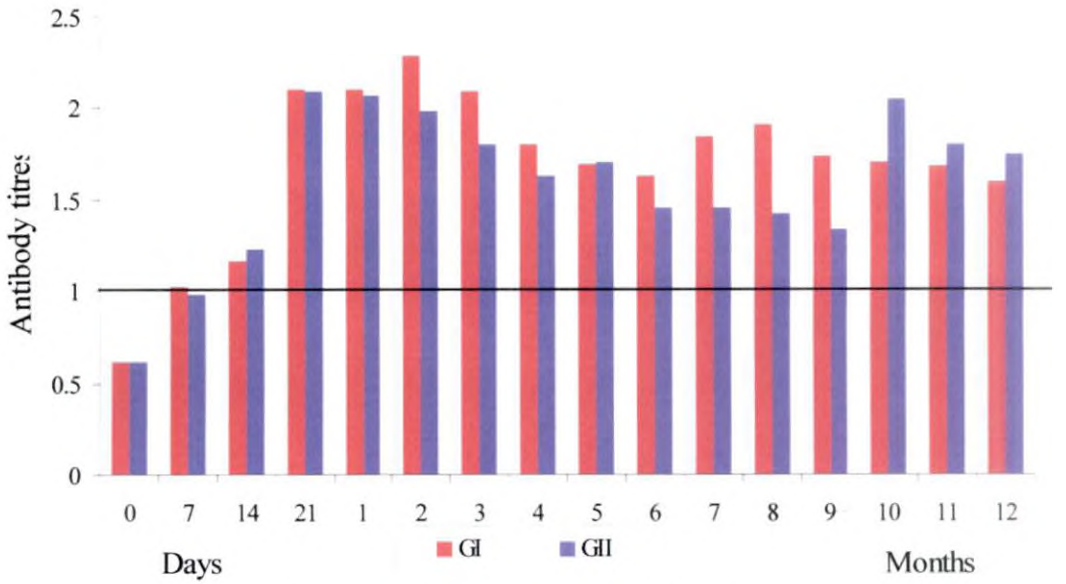

Fig.3a. Comparison of type C antibody titre of the two groups
(The black line indicates the level of antibody titre required for protection)

Table 13.The Type ASIA-1 FMD antibody titres of Group I animals

Ani no	Days Post Vaccination				Months											
	0dpv	7dpv	14dpv	21dpv	1	2	3	4	5	6	7	8	9	10	11	12
BI	0.610	1.056	1.134	2.135	2.121	2.182	1.802	1.717	1.564	1.544	1.717	2.067	1.713	1.433	1.802	1.395
B2	0.610	0.691	0.999	1.960	1.969	2.372	2.001	1.650	1.492	1.421	1.723	1.991	1.998	1.489	1.791	1.585
B3	0.610	0.969	1.219	1.802	1.804	1.881	1.802	1.696	1.570	1.568	1.791	2.159	1.801	1.697	1.596	1.504
B4	0.619	1.002	1.184	2.196	2.201	2.224	1.991	1.782	1.656	1.535	1.701	1.996	1.792	1.696	1.626	1.594
B5	0.610	0.726	0.876	1.456	1.496	1.597	1.565	1.494	1.444	1.402	2.008	1.969	1.597	1.500	1.332	1.383
B9	0.610	0.821	0.969	1.708	1.713	1.713	1.600	1.386	1.325	1.212	1.341	1.408	1.352	1.329	1.560	1.121
B11	0.610	0.912	1.110	2.331	2.321	2.401	2.102	1.726	1.801	1.800	1.960	2.137	1.726	1.601	1.708	1.671
B14	0.610	0.901	1.055	2.036	1.991	2.137	1.998	1.846	1.800	1.701	1.846	1.859	1.907	1.723	1.713	1.960
B16	0.610	1.002	1.144	1.956	1.991	1.996	1.836	1.723	1.742	1.705	2.001	1.831	1.525	1.410	1.288	1.524
B17	0.610	1.121	1.121	2.182	2.001	2.121	2.051	1.830	1.623	1.600	1.830	2.800	1.612	1.652	1.369	1.509
B19	0.610	0.999	1.186	1.998	1.996	2.101	1.914	1.705	1.721	1.759	1.796	1.845	2.800	1.513	1.494	1.482
B20	0.610	1.110	1.203	1.881	1.802	1.977	1.880	1.701	1.596	1.492	1.561	1.735	1.504	1.492	1.249	1.389
B22	0.610	1.196	1.201	1.697	1.696	1.701	1.711	1.702	1.702	1.696	1.809	1.997	1.581	1.755	1.892	1.811
B23	0.610	0.691	0.969	1.561	1.597	1.600	1.596	1.442	1.492	1.421	1.493	1.457	1.382	1.256	1.296	1.340
B235	0.610	1.155	1.186	2.770	2.656	2.770	2.708	2.770	2.764	2.770	2.181	1.711	1.488	1.420	1.486	1.571
Mean± SE	0.611± 0.001	0.957± 0.043	1.104± 0.027	1.978± 0.085	1.957± 0.077	2.052± 0.084	1.904± 0.072	1.745± 0.081	1.686± 0.084	1.642± 0.090	1.784± 0.055	1.931± 0.084	1.719± 0.091	1.531± 0.039	1.547± 0.054	1.523± 0.052

Table 14.The Type ASIA-1 FMD antibody titres of Group II animals

Ani no	Days Post Vaccination				Months											
	0	7	14	21	1	2	3	4	5	6	7	8	9	10	11	12
A1	0.610	0.969	1.119	2.656	2.303	2.121	1.881	1.585	1.364	1.121	1.246	1.188	1.244	2.025	1.484	1.440
A2	0.611	1.212	1.296	2.775	2.780	2.742	2.411	2.105	1.892	1.866	1.794	1.786	1.656	2.801	2.796	2.702
A3	0.610	1.110	1.196	2.800	2.800	2.656	2.800	2.444	2.421	2.426	1.912	1.611	1.483	2.078	2.216	2.104
A4	0.610	1.210	1.346	2.800	2.800	2.700	2.121	1.726	1.690	1.576	2.302	2.800	2.262	2.800	2.800	2.800
A7	0.610	0.999	1.211	2.800	2.800	2.721	2.303	1.980	2.175	1.713	2.800	1.601	1.918	2.800	2.800	2.653
A8	0.610	0.916	1.355	2.800	2.800	2.651	2.700	2.656	2.800	2.644	2.800	2.800	2.774	2.800	2.800	2.800
A9	0.610	0.999	1.110	2.122	2.182	1.912	1.880	1.664	1.501	1.446	1.247	1.121	1.201	1.664	2.159	1.923
A11	0.610	1.006	1.106	2.096	2.039	1.923	1.726	1.575	1.277	1.172	1.211	1.250	1.110	2.099	1.829	1.726
A12	0.610	0.756	0.969	1.482	1.500	1.444	1.492	1.444	1.389	1.212	1.112	1.191	1.200	1.370	1.483	1.459
A13	0.610	0.912	1.192	2.354	2.302	1.806	1.664	1.421	1.386	1.343	1.364	1.386	1.404	1.924	1.864	1.541
A14	0.610	0.691	1.255	1.611	1.621	1.597	1.492	1.343	1.177	1.058	1.124	1.271	1.110	2.069	1.829	1.519
A17	0.610	0.726	0.999	1.583	1.576	1.402	1.400	1.494	1.272	1.086	1.110	1.119	1.001	1.272	1.291	1.069
A18	0.610	0.999	1.121	2.202	2.201	1.952	1.713	1.585	1.494	1.491	1.496	1.480	1.575	2.039	1.585	1.488
A20	0.610	0.821	0.999	1.576	1.500	1.419	1.406	1.346	1.345	1.386	1.364	1.121	1.110	1.406	1.370	1.276
A220	0.610	1.156	1.244	1.713	1.788	1.656	1.601	1.542	1.502	1.338	1.386	1.374	1.121	2.063	1.902	1.674
Mean± SE	0.610± 0.000	0.965± 0.043	1.168± 0.031	2.225± 0.136	2.199± 0.133	2.047± 0.133	1.906± 0.119	1.727± 0.102	1.646± 0.122	1.525± 0.122	1.618± 0.151	1.540± 0.142	1.478± 0.129	2.081± 0.136	2.014± 0.143	1.878± 0.152

Table 15.Comparison of the mean Type Asia-1 (Mean± SE) antibody titre of two groups

Gr	Days				Months											
	0	7	14	21	1	2	3	4	5	6	7	8	9	10	11	12
I	0.611± 0.001	0.957± 0.043	1.104± 0.027	1.978± 0.085	1.957± 0.077	2.052± 0.084	1.904± 0.072	1.745± 0.081	1.686± 0.084	1.642± 0.090	1.784± 0.055	1.931± 0.084	1.719± 0.091	1.531± 0.039	1.547± 0.054	1.523± 0.052
II	0.610± 0.000	0.965± 0.043	1.168± 0.031	2.225± 0.136	2.199± 0.133	2.047± 0.133	1.906± 0.119	1.727± 0.102	1.646± 0.122	1.525± 0.122	1.618± 0.151	1.540± 0.142	1.478± 0.129	2.081± 0.136	2.014± 0.143	1.878± 0.152
	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	*	NS	**	**	*

* Significant at 5% level
** Significant at 1% level
NS- No significant difference between the groups

Table 16. Table of t- values between Days/Months for Type Asia-1

G	Days				Months										
	0&7	7&14	14&21	21&1	1&2	2&3	3&4	4&5	5&6	6&7	7&8	8&9	9&10	10&11	11&12
I	8.136 **	6.093 **	11.704 **	1.330	3.591 **	4.601 **	5.350 **	2.686 *	3.483 **	2.251 *	1.806	1.896	2.140	0.319	0.462
II	8.300 **	5.628 **	8.987 **	0.988	4.991 **	5.210 **	4.922 **	2.316 *	3.744 **	0.962	0.843	1.260	6.973 **	1.038	4.976 **

* Significant at 5% level
** Significant at 1% level

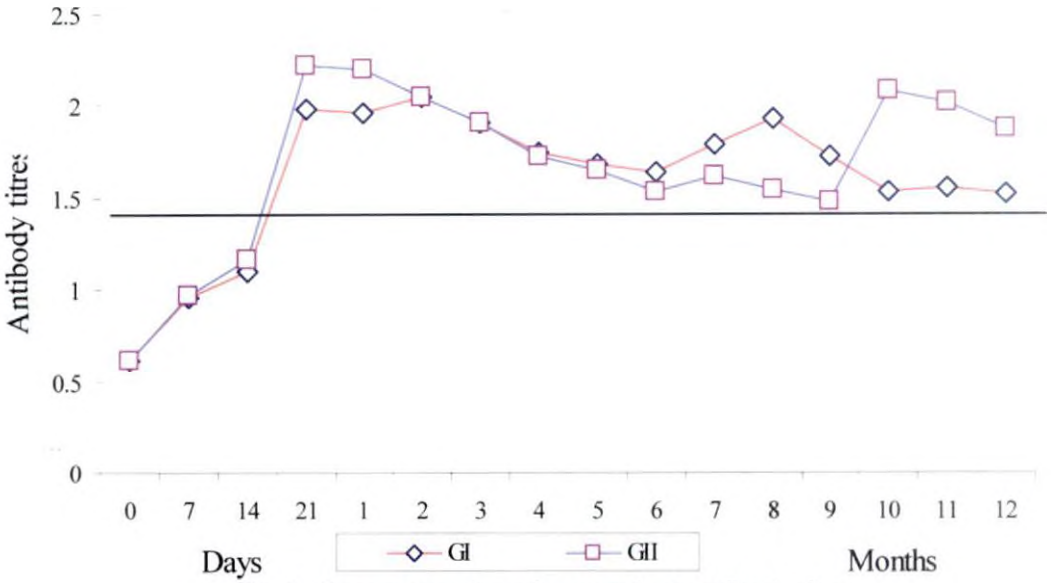

Fig.4. Seroconversion of type Asia-1 antibody titres
(The black line indicates the level of antibody titre required for protection)

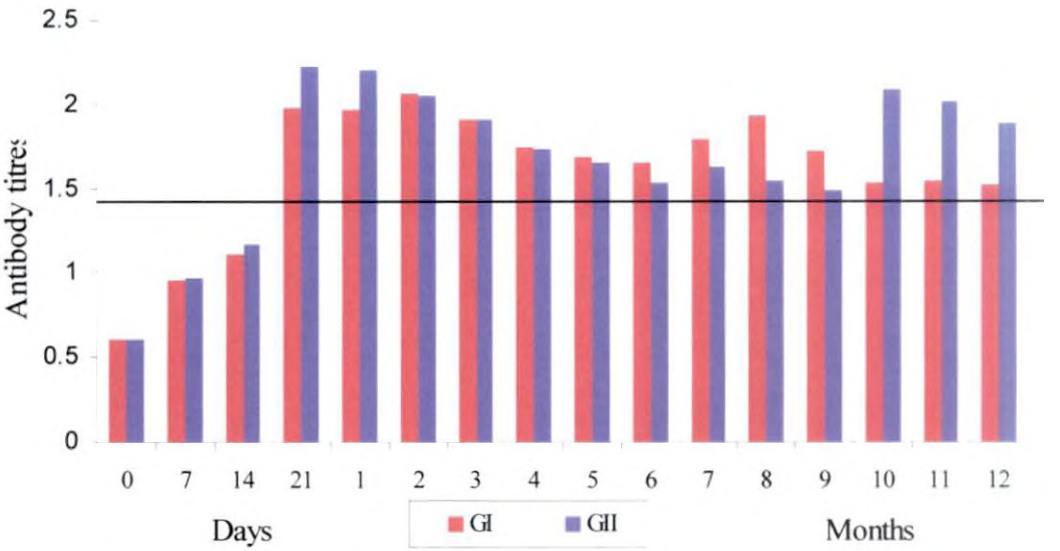

Fig.4a. Comparison of type Asia-1 antibody titres of the two groups
(The black line indicates the level of antibody titre required for protection)

Table 17. The Type O Maternal antibody titres of Group I kids

Kid no	Hours			Weeks					
	0	24	72	1	2	3	4	8	12
BK1	0.610	2.071	1.181	1.203	1.040	1.110	1.101	0.610	0.610
BK5	0.610	2.800	1.919	1.794	1.542	1.245	1.225	1.227	0.610
BK16	0.611	2.245	2.069	1.488	1.397	1.356	1.245	1.151	0.610
BK20	0.610	1.661	1.514	1.587	1.521	1.675	1.306	1.161	0.794
BK14	0.610	2.800	2.173	2.064	1.332	1.217	1.225	0.999	0.904
BK17	0.610	1.773	1.379	1.569	1.397	1.209	0.610	0.610	0.610
BK19	0.610	1.911	1.594	1.539	1.485	1.397	1.120	0.999	0.610
Mean± SE	0.610± 0.000	2.180± 0.18	1.690± 0.14	1.606± 0.101	1.388± 0.06	1.316± 0.07	1.119± 0.09	0.965± 0.1	0.678± 0.05

Table 18. The Type O Maternal antibody titres of Group II kids

Kid no	Hours			Weeks					
	0	24	72	1	2	3	4	8	12
AK16	0.610	1.597	1.562	1.567	1.488	1.237	1.209	1.093	0.610
AK20	0.610	1.649	1.982	1.530	1.578	1.546	1.206	1.114	0.610
AK13	0.610	1.544	1.499	1.626	1.487	1.766	1.251	1.017	0.610
AK18	0.610	1.462	1.384	1.450	1.456	1.416	1.116	0.996	0.611
AK7	0.610	2.800	1.694	1.626	1.544	1.487	1.427	1.251	0.610
AK8	0.611	2.800	2.800	1.649	1.501	1.462	1.327	1.209	0.610
AK9	0.610	1.501	1.410	1.327	0.786	0.715	0.610	0.610	0.610
Mean± SE	0.610± 0.000	1.908± 0.23	1.762± 0.189	1.539± 0.044	1.406± 0.1	1.376± 0.13	1.164± 0.1	1.041± 0.08	0.610± 0.000

Table 19. Comparison of the mean Type O (Mean± SE) Maternal antibody titre of kids of two groups

G	Hours			Weeks					
	0	24	72	1	2	3	4	8	12
I	0.610± 0.000	2.180± 0.18	1.690± 0.14	1.606± 0.101	1.388± 0.06	1.316± 0.07	1.119± 0.09	0.965± 0.1	0.678± 0.05
II	0.610± 0.000	1.908± 0.23	1.762± 0.189	1.539± 0.044	1.406± 0.1	1.376± 0.13	1.164± 0.1	1.041± 0.08	0.610± 0.000
	NS	NS	NS	NS	NS	NS	NS	NS	NS

NS- No significant difference between the groups

Table 20. Table of t- values between Days/Months for Type O Maternal antibodies

G	Hours			Weeks				
	0&24	24&72	72&1	1&2	2&3	3&4	4&8	8&12
I	8.929**	4.147**	0.901	2.442	1.257	2.276	2.403	2.971**
II	5.590**	0.863	1.307	1.828	0.509	3.148**	4.478**	5.386**

* Significant at 5% level
** Significant at 1% level

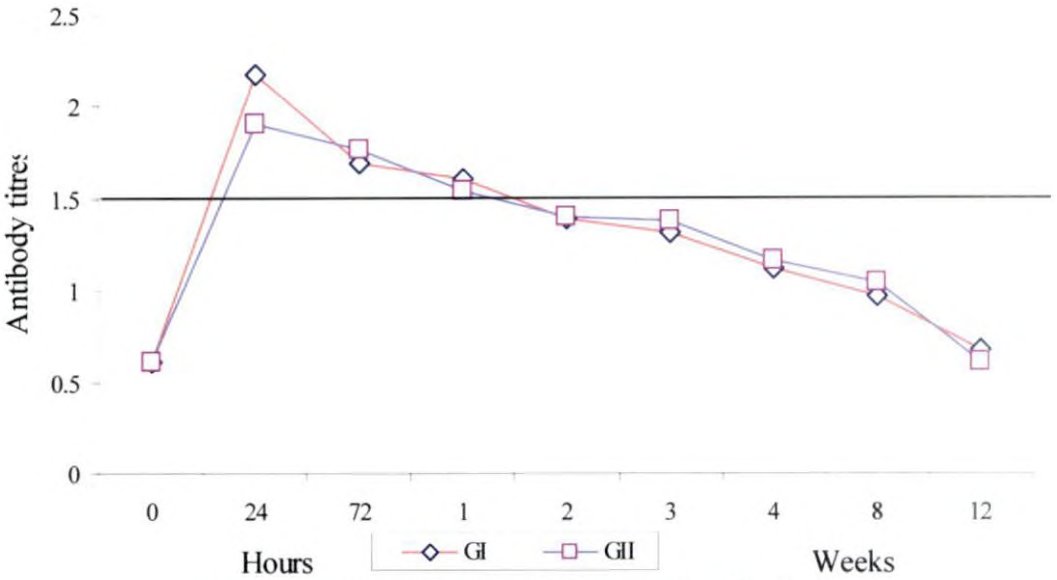

Fig.5. Seroconversion of type O maternal antibody titre
(The black line indicates the level of antibody titre required for protection)

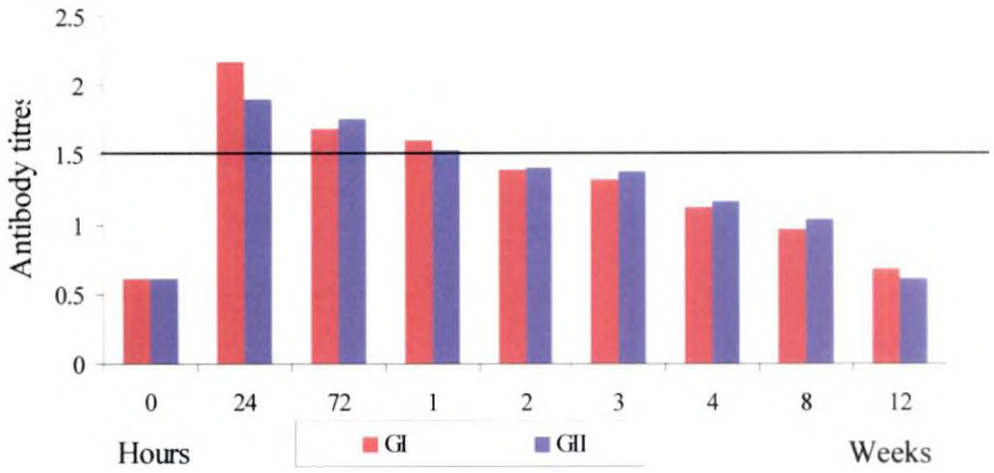

Fig.5a. Comparison of type O maternal antibody titre of the two groups
(The black line indicates the level of antibody titre required for protection)

Table 21.The Type A Maternal antibody titres of Group I kids

Kid no	Hours			Weeks					
	0	24	72	1	2	3	4	8	12
BK1	0.610	2.161	1.926	1.796	1.792	1.521	1.305	0.610	0.610
BK5	0.610	2.770	2.121	1.602	1.500	1.386	1.110	0.705	0.610
BK16	0.610	2.800	2.696	1.608	1.512	1.246	1.112	0.610	0.610
BK20	0.611	1.881	1.701	1.616	1.344	1.321	1.121	0.805	0.610
BK14	0.610	2.966	2.401	1.596	1.444	1.210	0.969	0.610	0.610
BK17	0.610	2.800	1.901	1.730	1.492	1.354	1.110	0.610	0.796
BK19	0.610	1.196	1.235	1.254	1.114	1.110	0.999	0.610	0.610
Mean± SE	0.610± 0.000	2.368± 0.25	1.997± 0.18	1.600± 0.065	1.457± 0.077	1.307± 0.05	1.104± 0.041	0.651± 0.029	0.637± 0.027

Table 22.The Type A Maternal antibody titres of group II kids

Kid no	Hours			Weeks					
	0	24	72	1	2	3	4	8	12
AK16	0.610	1.626	1.661	1.595	1.444	1.305	1.041	0.812	0.610
AK20	0.610	1.636	1.536	1.492	1.396	1.363	1.071	0.610	0.610
AK13	0.610	1.966	1.822	1.800	1.756	1.500	1.371	0.610	0.610
AK18	0.610	1.696	1.661	1.569	1.505	1.356	1.212	0.610	0.610
AK7	0.611	2.800	2.800	2.411	1.860	1.704	1.492	0.811	0.610
AK8	0.610	2.800	2.800	2.249	1.802	1.544	1.100	0.809	0.611
AK9	0.610	1.559	1.444	1.401	1.212	0.986	0.826	0.610	0.610
Mean± SE	0.610± 0.000	2.012± 0.21	1.961± 0.22	1.788± 0.148	1.568± 0.091	1.394± 0.085	1.159± 0.084	0.696± 0.041	0.610± 0.000

Table 23. Comparison of the mean Type A (Mean± SE) Maternal antibody titre of kids of two groups

G	Hours			Weeks					
	0	24	72	1	2	3	4	8	12
I	0.610± 0.000	2.368± 0.25	1.997± 0.18	1.600± 0.065	1.457± 0.077	1.307± 0.05	1.104± 0.041	0.651± 0.029	0.637± 0.027
II	0.610± 0.000	2.012± 0.21	1.961± 0.22	1.788± 0.148	1.568± 0.091	1.394± 0.085	1.159± 0.084	0.696± 0.041	0.610± 0.000
	NS	NS	NS	NS	NS	NS	NS	NS	NS

NS- No significant difference between the groups

Table 24. Table of t- values between Days/Months for Type A Maternal antibodies

G	Hours			Weeks				
	0&24	24&72	72&1	1&2	2&3	3&4	4&8	8&12
I	7.141**	2.894**	2.509**	4.200**	3.573*	8.905**	9.403**	0.340
II	6.697**	1.986	2.175	2.929*	5.739**	5.619**	5.469**	2.121

* Significant at 5% level
** Significant at 1% level

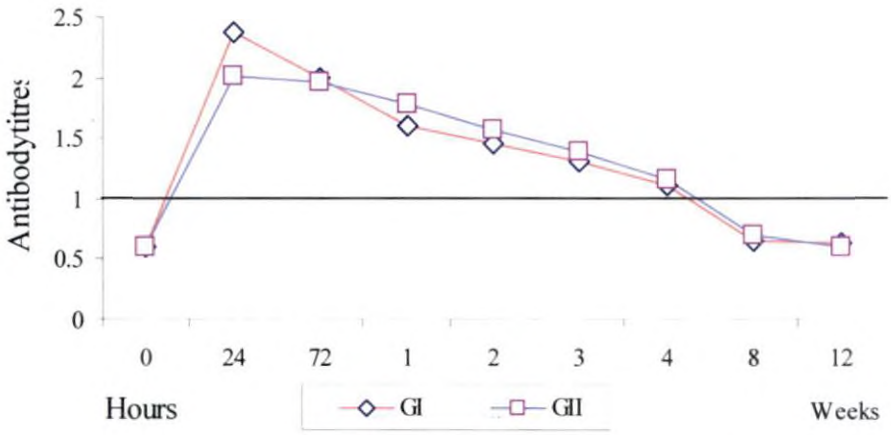

Fig.6. Seroconversion of type A maternal antibody titre
(The black line indicates the level of antibody titre required for protection)

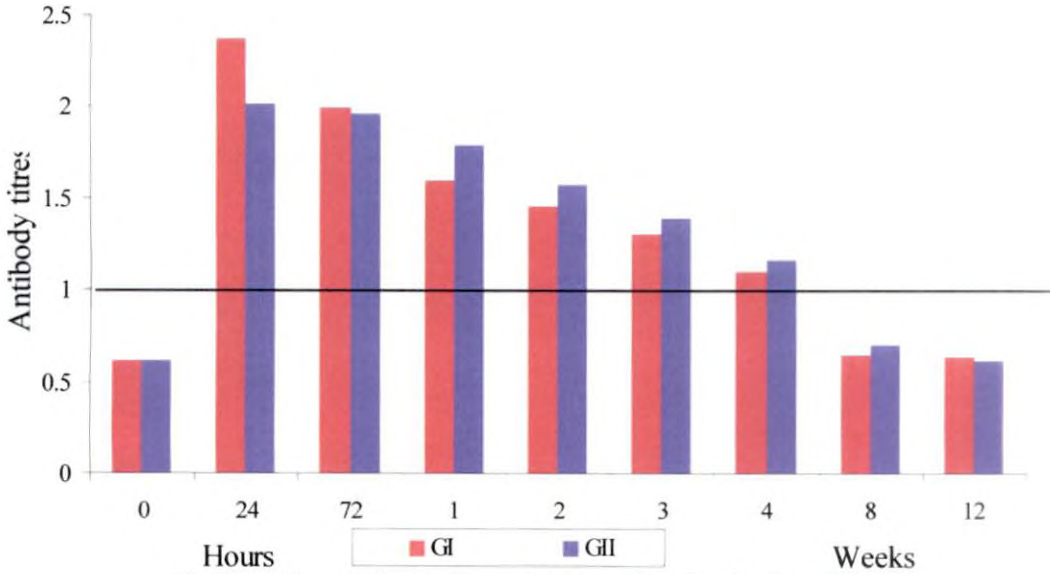

Fig.6a. Comparison of type A naternal antibody titre of the two groups
(The black line indicates the level of antibody titre required for protection)

Table 25. The Type C Maternal antibody titres of Group I kids

Kid no	Hours			Weeks					
	0	24	72	1	2	3	4	8	12
BK1	0.610	2.251	1.946	1.867	1.792	1.508	1.205	0.610	0.610
BK5	0.610	2.780	2.021	1.516	1.477	1.293	1.110	0.705	0.610
BK16	0.610	2.800	2.724	1.508	1.506	1.110	0.969	0.610	0.610
BK20	0.610	1.913	1.663	1.516	1.293	1.289	1.075	0.805	0.610
BK14	0.610	2.711	2.446	1.570	1.477	1.140	0.872	0.610	0.610
BK17	0.610	2.800	1.810	1.730	1.486	1.354	1.110	0.610	0.796
BK19	0.610	1.244	1.235	1.254	1.140	1.110	0.999	0.610	0.610
Mean± SE	0.610± 0.000	2.357± 0.226	1.978± 0.186	1.566± 0.073	1.453± 0.076	1.258± 0.056	1.049± 0.042	0.651± 0.029	0.637± 0.027

Table 26. The Type C Maternal antibody titres of group II kids

Kid no	Hours			Weeks					
	0	24	72	1	2	3	4	8	12
AK16	0.610	1.636	1.684	1.597	1.436	1.335	1.071	0.812	0.610
AK20	0.610	1.672	1.526	1.478	1.367	1.393	1.035	0.610	0.610
AK13	0.610	2.033	1.866	1.822	1.762	1.499	1.271	0.610	0.610
AK18	0.610	1.686	1.661	1.597	1.504	1.344	1.121	0.610	0.610
AK7	0.610	2.800	2.800	2.401	1.866	1.704	1.551	0.811	0.610
AK8	0.610	2.800	2.800	2.251	1.822	1.504	1.101	0.809	0.610
AK9	0.610	1.603	1.439	1.407	1.208	0.942	0.610	0.610	0.610
Mean± SE	0.610± 0.000	2.033± 0.205	1.968± 0.221	1.793± 0.147	1.566± 0.095	1.389± 0.089	1.109± 0.107	0.696± 0.041	0.610± 0.000

Table 27. Comparison of the mean Type C (Mean± SE) Maternal antibody titre of kids of two groups

G	Hours			Weeks					
	0	24	72	1	2	3	4	8	12
I	0.610± 0.000	2.357± 0.226	1.978± 0.186	1.566± 0.073	1.453± 0.076	1.258± 0.056	1.049± 0.042	0.651± 0.029	0.637± 0.027
II	0.610± 0.000	2.033± 0.205	1.968± 0.221	1.793± 0.147	1.566± 0.095	1.389± 0.089	1.109± 0.107	0.696± 0.041	0.610± 0.000
	NS	NS	NS	NS	NS	NS	NS	NS	NS

NS- No significant difference between the groups

Table 28. Table of t- values between Days/Months for Type C Maternal antibodies

G	Hours			Weeks				
	0&24	24&72	72&1	1&2	2&3	3&4	4&8	8&12
I	7.736**	2.779*	2.300	3.307	3.428*	8.033**	8.799**	0.340
II	6.917**	1.887	2.204	3.287*	4.005**	8.442**	4.297**	2.121

* Significant at 5% level
** Significant at 1% level

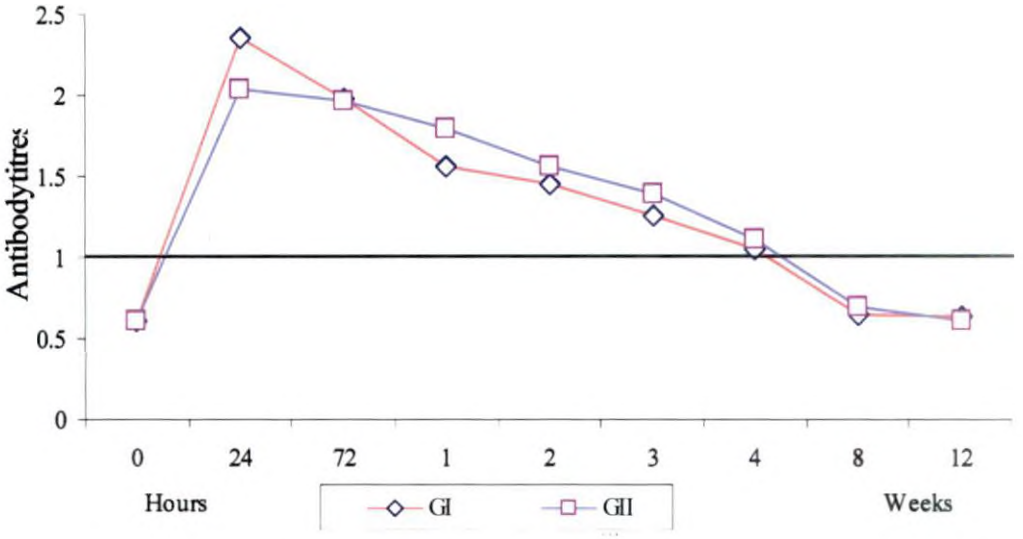

Fig.7. Seroconversion of type C maternal antibody titre
(The black line indicates the level of antibody titre required for protection)

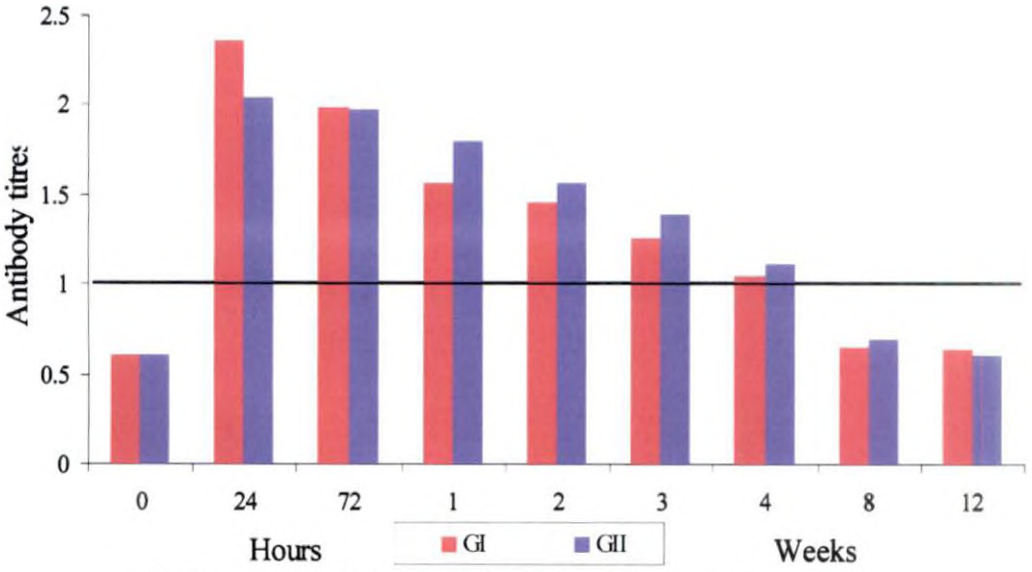

Fig.7a. Comparison of type C maternal antibody titre of the two groups
(The black line indicates the level of antibody titre required for protection)

Table 29. The Type ASIA-1 Maternal antibody titres of Group I kids

Kid no	Hours			Weeks					
	0	24	72	1	2	3	4	8	12
BK1	0.610	1.923	1.854	1.818	1.609	1.256	1.070	0.610	0.610
BK5	0.610	2.800	1.871	1.494	1.447	1.308	1.283	0.880	0.610
BK16	0.610	2.800	2.800	1.736	1.577	1.296	1.112	0.960	0.610
BK20	0.610	1.651	1.699	1.657	1.635	1.447	1.354	1.180	0.610
BK14	0.610	2.800	2.242	1.926	1.840	1.577	1.461	0.845	0.610
BK17	0.610	2.800	1.859	1.595	1.546	1.421	1.308	0.780	0.610
BK19	0.610	1.524	1.421	1.218	1.210	1.110	0.999	0.969	0.610
Mean± SE	0.610± 0.000	2.328± 0.23	1.964± 0.167	1.635± 0.09	1.552± 0.073	1.345± 0.0571	1.227± 0.064	0.889± 0.067	0.610± 0.000

Table 30. The Type ASIA-1 Maternal antibody titres of Group II kids

Kid no	Hours			Weeks					
	0	24	72	1	2	3	4	8	12
AK16	0.610	1.665	1.622	1.609	1.572	1.471	1.177	1.296	0.610
AK20	0.610	1.941	1.668	1.609	1.658	1.765	1.486	1.193	0.610
AK13	0.610	1.862	1.973	1.694	1.560	1.635	1.452	1.283	0.610
AK18	0.610	1.551	1.545	1.420	1.367	1.452	1.268	1.113	0.610
AK7	0.610	2.800	2.800	2.245	1.973	1.367	1.463	0.610	0.610
AK8	0.610	2.800	2.800	2.425	1.941	1.369	1.001	0.999	0.998
AK9	0.610	1.532	1.402	1.383	1.251	1.208	1.258	0.610	0.610
Mean± SE	0.610± 0.000	2.022± 0.209	1.973± 0.22	1.769± 0.153	1.617± 0.1	1.467± 0.07	1.301± 0.0675	1.015± 0.111	0.665± 0.055

Table 31.Comparison of the mean Type Asia-1 (Mean± SE) Maternal antibody titre of kids of two groups

G	Hours			Weeks					
	0	24	72	1	2	3	4	8	12
I	0.610± 0.000	2.328± 0.23	1.964± 0.167	1.635± 0.09	1.552± 0.073	1.345± 0.0571	1.227± 0.064	0.889± 0.067	0.610± 0.000
II	0.610± 0.000	2.022± 0.209	1.973± 0.22	1.769± 0.153	1.617± 0.1	1.467± 0.07	1.301± 0.0675	1.015± 0.111	0.665± 0.055
	NS	NS	NS	NS	NS	NS	NS	NS	NS

NS- No significant difference between the groups

Table 32. Table t- values between Days/Months for Type Asia-1 Maternal antibodies

G	Hours			Weeks				
	0&24	24&72	72&1	1&2	2&3	3&4	4&8	8&12
I	7.581**	2.206	2.492*	2.936*	5.822**	5.662**	4.071**	4.187**
II	6.751**	1.058	2.067*	2.265	1.291	2.495*	2.171	2.783*

* Significant at 5% level
** Significant at 1% level

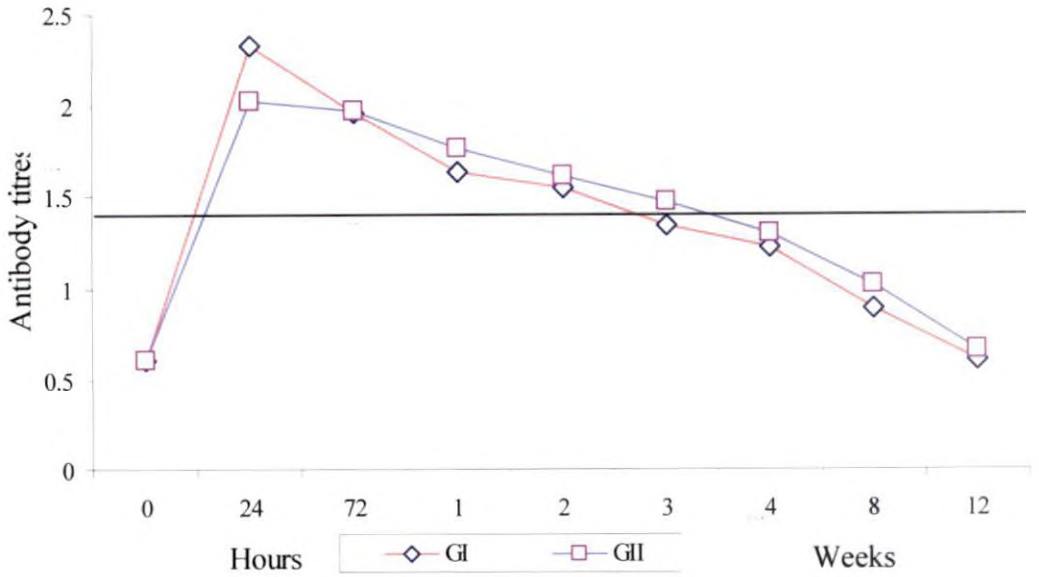

Fig.8. Seroconversion of type Asia-1 maternal antibody titre
(The black line indicates the level of antibody titre required for protection)

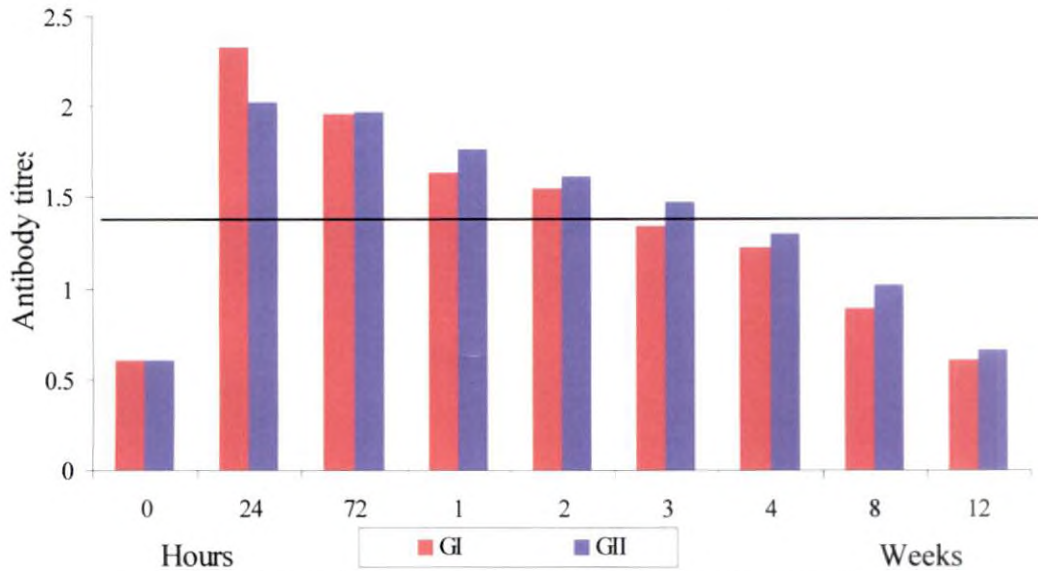

Fig.8a. Comparison type Asia-1 maternal antibody titre of the two groups
(The black line indicates the level of antibody titre required for protection)

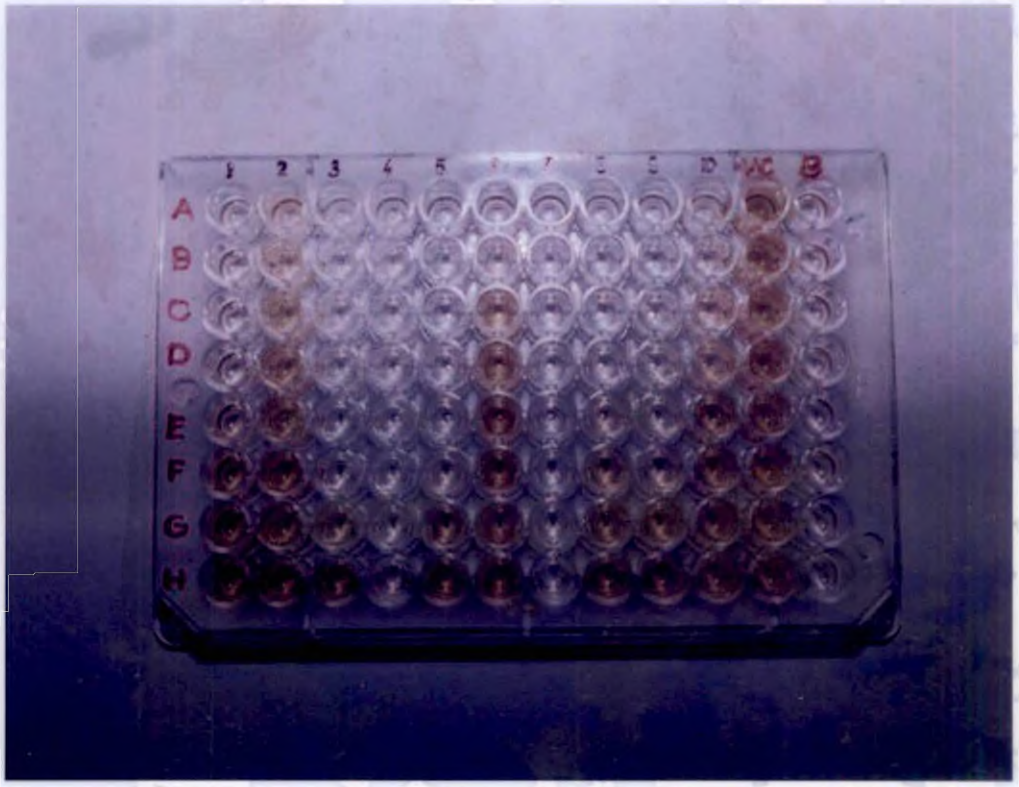

Plate 1. Liquid phase Blocking Enzyme Linked Immunosorbent Assay (LPB-ELISA) Test Plate

Columns 1 to 10

Samples 1 to 10 in two-fold dilutions from A to H

Column VC
Virus control

Column B
Column Blank

Discussion

5. DISCUSSION

5.1 SEROCONVERSION FOLLOWING VACCINATION IN DIFFERENT GROUPS

5.1.1 Seroconversion of Type O Antibodies

5.1.1.1 Group 1

A rise in antibody titres of Group 1 animals was detected as early as seven days post vaccination and it reached the protective antibody titres within the first 21 days of vaccination. The antibody titres further increased and attained the maximum level after the first booster vaccination. This result is in accordance with the observation made by Nair and Sen (1993a) who studied the antibody response of sheep to aluminium hydroxide gel foot and mouth disease virus type O and Asia-1 vaccines and found that the antibody titres were detected at seven days post vaccination and reached maximum between 21 and 28 days post vaccination and then declined. A booster vaccination further increased the antibody level after two to four weeks. Sharma and Murthy (1985) conducted vaccination trials in sheep with FMD polyvalent vaccine and reported the appearance of neutralizing antibody between eight and ten days with peak titre between 21 and 35 days post vaccination. The satisfactory neutralizing antibody titres could be observed in animals with booster dose given four months after primary vaccination.

The significantly higher antibody titres in Group 1 animals during second month and eight month was because of anamnestic response produced by the booster vaccination given during the first and seventh month. This observation agrees with the opinion of Tizard (2000) who described that repeated injection of antigen produced immune response with shorter lag period and for a longer duration than single inoculation.

The protective titre was reached by 21 days after vaccination and was maintained during the study period except during sixth, tenth, 11th and 12th month, which might be due to poor antigenicity of type O antigen. Pay and Hingley (1987) stated that FMDV type O antigen was inherently weak antigen. This low titre of antibodies below the protective level makes the vaccinated animal susceptible to infection during these periods.

5.1.1.2 Group II

In Group II seroconversion was detected at seven days post vaccination and reached the protective antibody titres within the first 21 days of vaccination. This finding is in accordance with the observation made by Barnett and Cox (1999) who studied the antibody response of sheep vaccinated with A22 Iraq antigen formulated as an oil emulsion or as an aluminium hydroxide saponin vaccine, and monitored over a six month period and observed a rapid antibody response which peaked seven to 21 days post vaccination regardless of adjuvant.

In Group II animals the peak antibody titre was reached at 21 days post vaccination and it declined there after. This finding does not agree with the findings of Patil *et al.* (2002b) who reported that the oil adjuvant vaccine elicited high neutralizing immune response in goats for all the four serotypes and the peak antibody titres were observed at 90 days post vaccination.

After booster vaccination at ninth month, the antibody titres increased. This observation concurs with the opinion of Tizard (2000). The antibody titre was below the protective level during the seventh, eighth and ninth month. This may be due to the poor antigenicity of type O as stated by Pay and Hingley (1987). This may be the reason for outbreaks due to type O among vaccinated animals.

5.1.1.3 Comparison of Type O Antibody Titres Between Groups

Group I and Group II animals did not show any significant difference in mean type O FMD antibody titre throughout the study period except during the period of booster vaccination. This finding corroborates with the observation of Nair and Sen (1993b) and Barnett and Cox (1999) where as Patil *et al.* (2002b) who reported that oil adjuvant vaccines were superior to gel vaccines in goats.

5.1.2 Seroconversion of Type A Antibodies

5.1.2.1 Group I

A rise in the antibody titres of Group I animals was detected at seven days post vaccination and reached the protective antibody titres within the first seven days of vaccination. The maximum antibody titre was observed at second month after the first booster vaccination and it declined thereafter. This finding is in accordance with the observation made by Sharma and Murthy (1985) and Nair and Sen (1993a).

The significantly higher antibody titres in Group I animals during second month and eight month was because of the anamnestic response produced by the booster vaccination which agrees the findings of Srinivas *et al.* (1996) who stated that the booster vaccination increases the antibody titre considerably.

The protective titre of Group I animals was maintained throughout the study period. In contrast to this finding Bipin (2001) observed a low titre below the protective level in calves in the fifth month after vaccination with aluminium hydroxide gel FMD vaccine.

5.1.2.2 Group II

The seroconversion in Group II animals was detected at seven days post vaccination and reached the protective titre within the first seven days post

vaccination which is in accordance with the observation of Barnett and Cox (1999).

In Group II animals antibody titres reached the peak at 21 days post vaccination and it declined thereafter. But peak antibody titres following vaccination with oil adjuvant vaccine were detected at 90 days post vaccination by Patil *et al.* (2002b).

Increasing antibody titre was recorded after the booster vaccination at ninth month. All the animals responded well after the primary vaccination. The antibody titre of Group II animals was maintained above the protective level throughout the study period. This finding concurs with the findings of Rajkumar and Saseendranath (2003) who found that the oil-adjuvant FMD vaccine elicited protective antibody titres in calves during the entire study period of 12 months.

5.1.2.3 Comparison of Type A Antibody Titres Between Groups

There was no significant difference between the two groups in mean type A FMD antibody titre throughout the study period except during second, seventh, eighth, ninth and tenth month. This difference in antibody titres may be due to the subsequent booster vaccinations during this period. This results correlates with the findings of Nair and Sen (1993b) and Barnett and Cox (1999). But a significant difference between the two adjuvant groups in immune response was reported by Patil *et al.* (2002b) who observed an increased and prolonged immune response with oil adjuvant vaccine.

5.1.3 Seroconversion of Type C Antibodies

5.1.3.1 Group I

Seroconversion of Group I animals was detected at seven days post vaccination and reached the protective antibody titre within the first seven days of vaccination itself. The maximum antibody titre was obtained during the second month of study after the booster vaccination. This finding is in

accordance with the findings of Sharma and Murthy (1985) and Nair and Sen (1993a).

The significantly higher antibody titres was observed in Group I animals during second month and eight month because of the secondary response produced by the booster vaccination, which correlates with the findings of Tizard (2000).

The antibody titre of Group I animals was maintained above the protective level throughout the study period. This finding agrees the findings of Bipin (2001) who observed that the protective type C antibody titre was maintained throughout the study period of 12 months in calves following vaccination using aluminium hydroxide gel FMD vaccine.

5.1.3.2 Group II

Seroconversion of Group II animals was detected at seven days post vaccination and reached the protective titre within the 14 days of vaccination. Similar observations were also made by Barnett and Cox (1999). The maximum antibody titre of the Group II animals was observed at 21 days post vaccination and the antibody titres declined thereafter. This finding corroborates with the findings of Ouldridge *et al.* (1982) who indicated that the primary response to oil adjuvanted vaccine developed in pigs by eight days post vaccination and the neutralizing antibody titre peaked between 14 to 21 days and persisted at relatively high level for at least 148 days.

Increasing antibody titre was recorded after booster vaccination at ninth month. The protective antibody titre was maintained upto ninth month before the booster vaccination. This findings agree with the findings of Anand Rao *et al.* (1993) who reported that serum neutralizing antibody titres in calves administered oil emulsion vaccine remained at satisfactory level on ninth month post vaccination.

All the animals in the Group II responded well after the primary vaccination and protective antibody titre was maintained throughout the study period. This finding agrees the findings of Anand Rao *et al.* (1993).

5.1.3.3 Comparison of Type C Antibody Titres Between Groups

There was no significant difference in mean type C FMD antibody titres throughout the study period between Group I and II except during seventh, eighth, ninth and tenth month of vaccination because of booster vaccination. This finding correlates the finding of Nair and Sen (1993b), Barnett and Cox (1999) and does not agree with the findings of Patil *et al.* (2002b). Hunter (1996) assessed the performance of oil adjuvanted SAT serotypes of FMD vaccine in cattle, sheep and goats and found that a commercial double oil emulsion vaccine elicited higher antibody titres and a more prolonged antibody response than conventional vaccines.

5.1.4 Seroconversion of Type Asia-1 Antibodies

5.1.4.1 Group 1

In this group, seroconversion was detected at seven days post vaccination and reached the protective titre within the first 21 days. The maximum antibody titre was observed during the second month of study after the booster vaccination and there after declined. This finding is in accordance with the findings made by and Sharma and Murthy (1985) and Nair and Sen (1993a).

The significantly higher antibody titre in Group 1 animals during second month and eighth month was because of the booster vaccination (Srinivas *et al.*, 1996).

The antibody titre of Group 1 animals was maintained above the protective level throughout the study period. But a fall in the antibody titre below the protective level was observed by Bipin (2001) in calves vaccinated with aluminium hydroxide gel FMD vaccine during the fifth month.

5.1.4.2 Group II

In Group II seroconversion was detected at seven days post vaccination and attained the protective titre within the first 21 days post vaccination. This finding is in accordance with the findings of Ouldridge *et al.* (1982) and Barnett and Cox (1999).

The maximum antibody titre of the Group II animal was observed at 21 days post vaccination and the antibody titre declined thereafter. This finding does not agree with the findings of Patil *et al.* (2002b) who observed the peak titres during 90 days post vaccination.

Increasing antibody titre was recorded after the booster vaccination at ninth month. The protective antibody titre was maintained upto nine month before the booster vaccination. All the animals in the Group II responded well after the primary vaccination and protective antibody titre was maintained throughout the study period. This finding is in accordance with the findings of Anand Rao *et al.* (1993).

5.1.4.3 Comparison of Type Asia-1 Antibody Titres Between Groups

There was no significant difference in mean type Asia-1 antibody titre throughout the study period between the two groups except during the period of booster vaccination. This observation agrees with the findings of Nair and Sen (1993b) and Barnett and Cox (1999) where as this finding does not agree with the observation made by Hunter (1996) and Patil *et al.* (2002b).

5.2 MATERNAL ANTIBODIES

5.2.1 Type O Maternal Antibody Titres

5.2.1.1 Group 1

The mean type O maternal antibody titre of Group 1 was 0.610 ± 0.0 immediately after birth. Graves (1963) mentioned that the calves born from FMD vaccinated cows were devoid of antibody but after ingestion of colostrum they acquired protective antibodies. The highest mean type O maternal antibody titre was observed at 24 hours after birth and then it declined. This finding is in accordance with the findings of Nair (1995) who stated that lambs fed with colostrum showed a high rise of neutralizing antibody titre from 12^{th} to 48 hours and satisfactory antibody titre could be maintained upto four weeks of age.

The protective titre of Group 1 kids was maintained only upto the first week of age.

5.2.1.2 Group II

The mean type O maternal antibody titre of Group II was 0.610 ± 0.000 immediately after birth. The highest maternal antibody titre was observed during 24 hours after birth and it declined thereafter. The protective titre of Group II kids was maintained only upto the first week of age. This observation does not agree with the observation of Spath *et al.* (1995) who reported that calves born from vaccinated dams had high maternal antibodies upto two months of age.

5.2.1.3 Comparison of Type O Maternal Antibodies Between Two Groups

There was no significant difference in mean type O maternal antibodies. Both the group of kids had protective antibody titre only upto the first week of age. This finding is in contradictory with finding of Sadir *et al.* (1984) who stated that maternal antibody could confer a good protection to newborn calves for at least 60 days. But McCullough *et al.* (1992b) observed that the maternal

antibody level, though below the protective levels, could still prevent the calves from getting clinical disease.

5.2.2 Type A Maternal Antibody Titres

5.2.2.1 Group I

The mean type A maternal antibody titres of Group I was 0.610 ± 0.00 immediately after birth. The highest mean type A maternal antibody titre was observed at 24 hours after birth then it declined to original value at 12th week after birth. The protective antibody titre of Group I kids was maintained upto four weeks. This observation is in accordance with the observation of Nair (1995).

5.2.2.2 Group II

The Group II kids had the maternal antibody titre of 0.610 ± 0.0 immediately after birth. The highest mean type A maternal antibody titre was observed at 24 hours after birth and became 0.610 ± 0.00 at 12th week after birth. The protective antibody titre of Group II kids was maintained up to four weeks of age after birth. According to Spath *et al.* (1995), calves born from vaccinated dams had high maternal antibody titres upto two months of age. But in this study the protective titre was maintained only upto four weeks of age.

5.2.2.3 Comparison of Type A Maternal Antibodies Between Groups

Group I and Group II kids did not show any significant difference during the entire study period. Both the groups maintained the protective antibody titre upto four weeks of age. This finding correlates with the findings of Nair (1995).

5.2.3 Type C Maternal Antibody Titres

5.2.3.1 Group I

The mean type C maternal antibody titre of Group I kids was 0.610 ± 0.000 immediately after birth. The highest mean type C maternal antibody titre was observed at 24 hours after birth then it declined at 12th week after birth. The protective antibody titre of Group I kids was maintained upto four weeks. This finding is in accordance with the observation of Nair (1995). Shankar and Uppal (1986) studied the immune response of young calves to vaccination with type C foot and mouth disease vaccine and reported that the prevaccination titre of most of the calves born to FMD vaccinated cows showed varying degree of maternal antibody with the SN indices ranging from 0.0 to 3.0, while the calves born to unvaccinated cows showed negligible levels.

5.2.3.2 Group II

In this study, the mean type C antibody titre at the time of birth was 0.610 ± 0.0. The highest mean type C antibody titre was recorded at 24 hours after birth and it declined at 12th week after birth. The protective antibody titre of Group II kids was maintained upto four weeks of age. But Spath *et al.* (1995) observed satisfactory level of maternal antibodies upto 60 days.

5.2.3.3 Comparison of Type C Maternal Antibodies Between Groups

There was no significant difference in mean type C maternal antibodies between groups. Both the group kids maintained the protective titre upto four weeks of age, irrespective of the vaccines used.

5.2.4 Type Asia-1 Maternal Antibody Titre

5.2.4.1 Group I

The mean type Asia-1 maternal antibody titre of Group I was 0.610 ± 0.00 immediately after birth. The highest mean type Asia-1 maternal antibody titre

was observed at 24 hours after birth then it became 0.610 ± 0.000 at 12th week after birth. This observation is in accordance with the observation of Nair (1995), but duration of protective immunity in Group I kids was maintained only upto two weeks after birth.

5.2.4.2 Group II

In this study, the mean type Asia-1 antibody titre was *0.610 ± 0.000* immediately after birth. The highest mean type Asia-1 antibody titre was recorded at 24 hours after birth and became 0.665 ± 0.055 at twelfth week after birth. The protective antibody titre of Group II kids was maintained upto three weeks. But Spath *et al.* (1995) observed satisfactory level of maternal antibodies in calves upto 60 days.

5.2.4.3 Comparison of Type Asia-1 Maternal Antibodies Between Groups

There was no significant difference in mean type Asia-1 maternal antibodies between two groups. Group I kids maintained the protective antibody titre upto two weeks of age where as the Group II kids maintained up to three weeks of age. Duration of protective immunity due to maternal antibodies could be maintained for three weeks by using oil adjuvant vaccine, where as the gel vaccine provided protective maternal antibodies only upto two weeks.

Summary

6. SUMMARY

The comparative seroconversions of two different foot-and-mouth disease vaccines in goats were studied at Kerala Agricultural University Goat and Sheep Farm, Mannuthy. Thirty unvaccinated goats of four month age were selected and grouped into two of fifteen animals each. Group I animals were vaccinated with inactivated quadrivalent aluminium hydroxide gel saponin adjuvant FMD vaccine and Group II animals were vaccinated with inactivated quadrivalent FMD oil adjuvanted vaccine as per the manufacture's schedule. The antibody titre against foot and mouth disease virus types O, A, C and Asia-1 were assessed by liquid phase blocking ELISA.

Mean type O antibody titres of two groups were found below the protective level before the first vaccination. Group I animals showed the protective level of type O antibody titres throughout the study period except sixth, tenth, eleventh and twelfth month. Group II animals showed the protective level of type O antibody titres throughout the study period except seventh, eighth and ninth month. Both the groups responded well to respective booster vaccination.

Primary vaccination of both the groups resulted in an increase in mean type A antibody titres above the protective level. Group I and Group II animals maintained the protective antibody titres throughout the study period. Responses to booster vaccination were evident in both the groups.

Both the groups maintained protective mean type C antibody titre throughout the study period. Booster vaccination produced an increase in antibody titres of Group I and Group II.

Group I and Group II animals maintained protective mean type Asia-1 antibody titre during the entire study period. All the vaccination including

primary and booster vaccination produced increase in antibody titre than the previous month in both the groups.

In kids born from Group I and Group II animals, the protective level of type O maternal antibodies was maintained only upto the first week of age where as the protective levels of type A and type C maternal antibodies were maintained upto four weeks of age.

Mean type Asia-1 maternal antibody titre were maintained at the protective level only upto two weeks of age in Group I kids, where as in Group II kids the protective level was maintained upto three weeks of age.

From this study, it is concluded that both the vaccines *i.e.* oil adjuvanted vaccine and aluminium hydroxide gel saponin adjuvanted vaccine are equally good in eliciting satisfactory immune response but the difference is there in number of times of immunization. Group I animals received three times injection, whereas Group II animals received only two times injection. So the Group II vaccine reduces labour cost of injection, number of visit and stress to the animals to a very great extend. Therefore oil adjuvanted vaccine may be preferred when compared to aluminium hydroxide gel vaccine. The maternal antibody titres of kids born from vaccinated animals of both the groups were maintained upto one to four weeks of age regardless of the adjuvant used in the vaccine.

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COMPARATIVE SEROCONVERSION OF FOOT-AND-MOUTH DISEASE VACCINES IN GOATS

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ABSTRACT

The comparative seroconversions of two different foot-and-mouth disease vaccines in goats were studied. Group I animals were vaccinated with inactivated quadrivalent aluminium hydroxide gel saponin adjuvanted FMD vaccine. Group II animals were vaccinated with inactivated quadrivalent FMD oil-adjuvant vaccine as per the manufacture's schedule. Vaccinations were done in unvaccinated goats above four month of age. The antibody titre against foot and mouth disease virus types O, A, C and Asia-1 were assessed by liquid phase blocking ELISA.

Group I animals showed the protective titre of type O antibody titres throughout the period except sixth, tenth, eleventh and twelfth month. Group II animals showed the protective level throughout the study period except seventh, eighth and ninth month.

Both the groups showed the protective titre of type A, C and Asia-1 antibody titres throughout the study period up to 12 months. All the vaccination including primary and booster vaccinations produce increase in antibody titre.

Both the groups did not show any significant variation in antibody titres against FMDV type O, A, C and Asia-1 except the time of booster vaccination.

Kids born from both the groups showed the protective level of type O maternal antibody only upto one week of age where as protective level for type A and C maternal antibodies upto four weeks of age.

Group I kids maintained the protective level of type Asia-1 upto two weeks of age where as Group II maintained upto three weeks of age.

From this observation it is concluded that

1. Both the group of vaccine provides sufficient protective titre for FMDV type O, A, C and Asia-1.

2. Aluminium hydroxide gel vaccine performance is equally good as that of oil adjuvanted vaccine.

3. Oil-adjuvanted vaccine reduces labour cost for injection, number of visit and stress to the animals to a very great extent. Therefore oil-adjuvanted vaccine may be preferred when compared to gel vaccine.

4. The maternal antibody protect the kids which were born to vaccinated does one to four weeks of age, regardless of the adjuvant used in the vaccine.