IMMUNE RESPONSE TO COMBINED FOOT AND MOUTH DISEASE, HAEMORRHAGIC SEPTICAEMIA AND BLACK QUARTER VACCINE AND THEIR RESPECTIVE MONOVALENT VACCINES IN CATTLE

By

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THESIS

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DECLARATION

I hereby declare that the thesis entitled **"IMMUNE RESPONSE TO COMBINED FOOT AND MOUTH DISEASE, HAEMORRHAGIC SEPTICAEMIA AND BLACK QUARTER VACCINE AND THEIR RESPECTIVE MONOVALENT VACCINES IN CATTLE"** 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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K. Rajkumar

Dedicated
To
My Parents and Guide

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Introduction

1. INTRODUCTION

Eighty per cent of India's population live in rural areas and depend on agricultural and animal husbandry activities for their livelihood. Our country has a huge cattle population around 200 million including cows and bullocks. The contribution of dairy sector to nation's economy is estimated to be approximately 50, 000 crore rupees annually (Ramaswamy, 2000).

Foot and Mouth Disease (FMD) is an economically important viral disease affecting cloven footed animals. There are about 5000 FMD out breaks reported every year in India which affect nearly three lakhs animals (Kumar, 1996). Though not a killer disease, FMD causes considerable reduction in the milk production and impairment of motive power. Together with indirect losses such as export embargo on animal products and by-products, repeat breeding etc, the disease inflicts astronomical impact on Indian economy.

Haemorrhagic Septicaemia (HS) and Black Quarter (BQ) is an acute, highly fatal, bacterial disease of cattle and buffaloes. HS is caused by certain serotypes of *Pasteurella multocida* notably serotypes 6:B (Asia strain) (OIE, 1992) and BQ by *Clostridium chauvoei*. In India it is thought that more than 40,000 animals die every year and the incidence of the disease peak in the rainy season (Seifer, 1992).

The strategy adopted for the control and prevention of these diseases varies from country to country depending upon the country's geographic location, technological, economical development and prevailing political attitude. Systematic large scale vaccination is the most appropriate method to bring down the incidence of these diseases wherever slaughter is not possible due to so many reasons. Hence, in India, vaccination is the only method which can be adopted for control and prevention of these diseases in cattle.

Vaccination of a large number of animals in a country like India involves tremendous man power and labour cost especially when monovalent vaccines are used which warrants high cost of vaccine and labour. It is therefore, a very important

point for consideration of combined vaccination than individual vaccination to reduce the cost.

Under this circumstance, the present work was conducted with the following objectives: to study

1. the efficacy of combined FMD, HS and BQ vaccine in comparison to respective individual vaccine.

2 a. the level of immunity to each disease by seromonitoring

b. the duration of immunity to each of these vaccines.

Review of Literature

2. REVIEW OF LITERATURE

2.1 Monovalent vaccine

2.1.1 FMD vaccine

Vallee *et al.* (1926) achieved inactivation of the FMD virus by formaldehyde and reported that the inactivated virus retained the immunogenicity.

Schmidt (1939) reported the immunopotentiating ability of alum hydroxide and it can be used as an adjuvant in FMD vaccine.

Waldmann *et al.* (1941) suggested that the FMD virus should be first adsorbed on alum hydroxide then inactivated with formaldehyde for using as a vaccine.

BHK- 21cells, a continuous cell line derived from baby hamster kidney cell was found to be supporting the growth of FMD virus and these cell line can be used for large scale production of FMD vaccine (Mowat and Chapman, 1962).

The most widely used emulsifying agent in the water-in-oil emulsion is Arlacel A special (Mannide mono-oleate) which is combined with a light mineral oil: Bayol F, Drakeol 6VR or Marcol 52 (Mc Kercher and Graves, 1977).

Oil-adjuvanted FMD vaccine failed to evoke an immune response in calves, but proved to be the preparation of choice for adult cattle (Solymom *et al.*, 1977).

Sutmoller (1977) reported that oil adjuvanted FMD vaccines had higher and long lasting protection levels when compared to aluminium hydroxide vaccines prepared from the same source of inactivated antigens.

Mowat (1978) stated that the first step in the development of inactivated FMD vaccines were taken 50 years ago when it was found that suspension of the virus treated with a dilute solution of formaldehyde could give some degree of protection against infection.

The relative cost effectiveness of the use of FMD oil-adjuvant vaccine for immunizing cattle against existing

aluminium hydroxide vaccine was studied by Astudillo and Auge-de-Mello (1980) and found oil-adjuvant vaccine as preferred one because of relatively fewer number of injections required and greater immunogenicity.

Rivenson *et al.* (1982) reported that oil-emulsion FMD vaccine containing 42.5 per cent Marcol 52, 6.55 per cent Arlacel 83 and 0.95 per cent Tween 80 gave better result when compared to a hydroxy saponin FMD vaccine prepared by adsorption on aluminium hydroxide fortified with 0.1 per cent saponin, five per cent glycerol and 1 in 30,000 thiomersal.

Roncha *et al.* (1983) recommended that vaccination of young cattle should be performed at least three times six months apart followed by re-vaccination using oil adjuvant FMD vaccine.

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

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

Rao *et al.* (1993) reported that marcol oil adjuvanted FMD vaccine produced better serological responses than paraffin-emulsion and aluminium hydroxide-saponin FMD vaccines. Booster vaccination after 21 days with both oil-emulsion vaccines produced no significant anamnestic response.

Spath *et al.* (1995) studied the immune response of oil-ajuvanted FMD vaccines in calves. Results obtained indicated that three to four months old calves with non-protective levels of colostral-derived antibodies responded with high antibody titres to vaccination.

Barnett *et al.* (1996) compared two novel oil-adjuvant, montanide ISA 25 and 206 (Seppic, Paris). The results indicated that the FMD 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 injection route.

Hunter (1996) assessed the performance of selected oil-adjuvants containing SAT serotypes of FMD virus in cattle, sheep, and goats. A commercial double oil emulsion vaccine elicited higher antibody titres and a more prolonged antibody response than conventional vaccines.

According to Doel (1999), potential variables in vaccination against FMD like use of oil-adjuvant for cattle are less critical when compared to elements like selection of appropriate strains and proper and timely administration.

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.

2.1.2 HS Vaccine

Depley (1948) developed a vaccine against *Pasteurella multocida,* which consisted of a suspension of bacteria lysed by saponin.

Rau and Govil (1950) used agar-grown *Pasteurella multocida* bacteria and found that a heavy concentration of organisms were required for a good immune response.

Bain (1954) developed an adjuvant vaccine containing mineral oil and lanolin, which avoided the severe tissue reactions encountered with other adjuvant vaccines.

Limited field trials and extensive laboratory experimentation have shown that Bain's vaccine confers solid immunity to *Pasteurella multocida* in cattle and bufflaoes up to 12 months (Dhanda and Lall, 1958).

Knox and Bain (1960) stated formalin is widely used to sterilize *Pasteurella multocida* vaccine and formalin binds the protein and polysaccharide elements in extracts of type 1 or type B.

An oil adjuvant vaccine prepared from the Pasteurella isolates protected against challenge with a highly virulent strain one month later and conferred a high level of immunity for up to a year under field conditions (Zaher *et al.*, 1976).

Mittal *et al.* (1977) developed multiple emulsion *Pasteurella multocida* vaccine. The newly developed vaccine is easy to inject, and is quite stable during storage.

Mittal *et al.* (1979) developed multiple emulsion vaccine by secondary emulsification of the adjuvant vaccine with Tween 80. The newly developed vaccine, which is a free flowing milky fluid, is easily injectable, and is quite stable during storage.

Alwis *et al.* (1978) assessed the performance of alum precipitated and oil adjuvant HS vaccine in cattle. Calves less than 3.5 months old responded poorly to both vaccines, whereas in calves of three and half to five months old, the alum precipitated vaccine gave immunity for three to four months and the oil adjuvant vaccine gave immunity for six to nine months.

An oil adjuvant vaccine of killed *Pasteurella multocida* commonly used in India for control and prevention of HS was compared with a multiple emulsion adjuvant vaccine. Multiple emulsion adjuvant vaccine was of thin consistency, much easier to inject and merited field trials in cattle (Mittal *et al.*, 1979).

Yadav and Ahooja (1983) studied the comparative efficacy of the oil-adjuvant and multi-emulsion oil-adjuvant vaccines against HS in cattle. Both multi-emulsion and oil-adjuvant vaccines were quite safe on field trial. The duration of immunity afforded by multiple emulsion vaccine was less compared to the conventional oil-adjuvant vaccine in a limited study carried up to one year with samples constituted with ratios of 3:3 and 3:2, but was comparable when mixed in the ratio of 3:1

In cattle, both alum-precipitated and aluminium hydroxide-adsorbed vaccines produced good response. There was no advantage gained with oil emulsion vaccines (Cameron and Bester, 1986).

Chandrasekaran *et al.* (1987) investigated the stability and potency of *Pasteurella multocida* oil adjuvant vaccine kept at different storage conditions and temperatures for various length of time. They recommended that the *Pasteurella multocida* oil adjuvant vaccine can be stored under refrigeration and may be used up to 15 months.

Muneer and Afzal (1989) prepared two oil-adjuvant vaccines of *Pasteurella multocida* Robert's type and evaluated for induction of immune response in buffalo calves. Adjuvant one was water-in-oil emulsion and adjuvant two was double emulsion. Both preparations induced sustained high antibody titres in buffalo calves beyond 230 days after vaccination.

Muneer *et al.* (1994) evaluated three oil-adjuvant vaccines of *Pasteurella multocida* 6:B with respect to the level and duration of the humoral immune response produced in buffalo calves. Preparation 1 was a water-in-oil emulsion containing Marcol 52, Montanide 888 and antigen at ratio of 6:1:3. Preparation 2 was a double emulsion containing Marcol 52, Arlacel A and Tween 80 in addition to antigen. Preparation 3 contained alpha-d-tocopheryl acetate, Montanide 888 and antigen. All the three preparations induced a similar sustained immune response beyond 270 days after vaccination.

Alwis *et al.* (1996) vaccinated the calves with HS oil adjuvant vaccine and challenged at 9^{th} and 12^{th} month by subcutaneous inoculation of a field isolate of *Pasteurella multocida* type 6:B. He concluded that protection produced by

the oil adjuvant vaccine to direct challenge at 9th and 12th month were 50per cent and 67per cent, respectively.

Shah *et al.* (1997) developed an oil adjuvant vaccine against HS caused by *Pasteurella multocida* serotypes B and E. Mineral oil and Mercol 52 were used as adjuvant together with Span 85 and Tween 85 as emulsifers. They demonstrated that the experimental oil adjuvant vaccine was superior to broth bacterin in providing protection against experimental HS in young buffalo calves beyond 250 days.

Pande *et al.* (2000) extracted *Pasteurella multocida* type A antigen by sonication and filtered through sephacryl S-200. The highest antibody titre was obtained with the protein of the first peak. Immunoblotting of the proteins of the first peak after SDS-PAGE gave 3 polypeptides such as 28, 59 and 37 KDa of which 59KDa protein was the predominant. In a mouse protection test, the protein of this peak induced protection against challenge.

2.1.3 BQ vaccine

Arloing *et al.* (1887) developed a dried, attenuated diseased muscle vaccine for Black Quarter (BQ).

Mc Ewen (1926) saw that formalin killed whole culture of *Clostridium chauvoei* were more effective immunizing agent than were germ free culture filtrate.

Since the discovery of the cause of blackleg, additional causative factors in the pathogenesis of the disease and method of immunization have been investigated sporadically (Robertson, 1929).

Sterne *et al.* (1951) developed a method of preparing bacterins by growing cultures in cellophane sacks. Vaccine made by this method was exceedingly potent, since cattle could be protected against 100 lethal dose of *Clostridium chauvoei* by injecting 0.04 ml. of formalized culture, suitably diluted.

The vegetative form of *Clostridium chouvoei* were killed by heating and the spores were sufficiently killed by heat that could be safely used (Hagan and Bruner 1961).

Stevenson and Stonger (1980) demonstrated that cellular antigens of *Clostridium chauvoei,* strain IRP-128 were

important in inducing of immunity against this bacterium in guinea pigs.

Awad *et al.* (1986) studied the efficacy of the three *Clostridium chauvoei* vaccines *via* alum precipitated, aluminium gel with saponin adsorbed vaccine, and oil adjuvant vaccine. The three vaccines gave equal protection to guinea pigs 21 days after immunization, while the oil adjuvant vaccine gave best protection after 10 days. In sheep, cattle and buffaloes, the oil adjuvant vaccine gave the strongest and most rapid agglutination response. Two doses of the vaccine produced protective levels of antibodies for one year in sheep, and nine month in cattle and buffaloes.

Farrag *et al.* (1987) studied maternal transmission of *Clostridium chauvoei* antibodies from vaccinated pregnant animals. He conducted trials on seven ewes and six buffaloes with *Clostridium chauvoei* oil adjuvant vaccine, two weeks before parturition. Agglutinating antibodies to *Clostridium chauvoei* were not present in newborn animals before sucking colostrum, but titres increased rapidly 12 hours after sucking. Agglutination titres were higher in colostral whey than in serum of all dams.

Tanaka *et al.* (1994) produced polyclonal rabbit anti-idiotypic antibodies against two monoclonal antibodies specific for flagella of *Clostridium chauvoei*. They suggested that an anti-idiotypic antibody containing an internal image of *Clostridium chauvoei* flagella could be used as a vaccine.

Troxel *et al.* (1999) studied the Clostridial vaccination efficacy on stimulating and maintaining an immune response in preweaned beef calves. The calves were vaccinated with Alpha-7 (A7, oil adjuvant) or Ultrabac 7 (UB7, AlOH adjuvant) at the age of 50.4 days. The calves were revaccinated with the same treatment on day 170. There was a tendency for higher *Clostridium chauvoei* antibody titres on day 191 for the A7-treated calves than for the UB7-treated calves. They concluded that calves vaccinated at 50 days of age and not again until weaning may not be adequately protected against clostridial diseases.

Kojima *et al.* (2000) found that the GST-flagellin fusion protein and the purified Flic of *Clostridium chauvoei* induced very little protective immunity in mice. From the result they suggested that a conformation – dependent epitope play

an important role in the development of immunity against blackleg.

Borrmann et al. (2001) reported that cell culture assay offered a valid *in vitro* alternative to the animal experiment for the titration of sera generated in the course of potency tests of Clostridial vaccine.

2.2 Combined vaccine

Sinha and Prasad (1973) reported that Haemorrhagic Septicaemia agar washed alum precipitated black quarter broth vaccine provided absolute protection in vaccinated calves against a fatal challenge infection of the homologous strains of *Pasteurella multocida* and *Clostridium chauvoei.*

A saponin vaccine containing 2.5 X 10^9 formalized *Pasteurella multocida* Roberts' type one per ml and formalized *Clostridium chauvoei* culture provided solid immunity in cattle in laboratory and large scale field trials. (Baharsefat *et al.*, 1976).

Srivastava *et al.* (1976) observed that combined *Pasteurella multocida* and *Clostridium chauvoei* oil adjuvant vaccine conferred a dependable grade of immunity which was comparable to that conferred by single vaccine against these diseases when used alone and which was superior to the combined *Pasteurella multocida* and *Clostridium chauvoei* alum precipitated vaccine.

Kadymov and Aliev (1978) concluded that there was little difference between animals given monovalent vaccines and those given the combined vaccine of *Pasteurella multocida* and *Clostridium chauvoei.*

Darie *et al.* (1979) carried out simultaneous vaccination of intensively reared lambs against Clostridial, anthrax and Foot and Mouth Disease. They concluded that there was no interference with the immunogenic effect of the different vaccines.

Combined vaccine against *Pasteurella multocida, Clostridium perfringens* and *Clostridium septicum* was carried out by Kadymov and Aliev (1979). They concluded that the

combined vaccine showed no difference in the survival rate between monovalent and polyvalent immunization.

Joseph and Hedger (1984) opined that simultaneous administration of Foot and Mouth Disease and Haemorrhagic Septicaemia inactivated vaccines produced no adverse effect and the serological response did not differ from the response to either vaccine given separately.

Gugiu *et al.* (1989) conducted comparative studies of vaccination of groups of seven cattle with the FMD vaccine on its own, or with the *Pasteurella multocida* vaccine showed that the protection against FMD was not reduced by association with anti-pasteurellosis vaccination.

Afzal and Muneer (1990) reported that the combined water–in-oil adjuvanted HS and FMD vaccine induced antibody titres equivalent to oil adjuvant vaccines of HS and FMD when done separately in different groups. He concluded that this combined vaccine could reduce the frequency and cost of vaccination.

Simultaneous or singular administration of FMD and RP vaccines induce satisfactory serological responses without any adverse effects (Srinivas *et al.*, 1996)

Combined vaccine prepared from cultures of *Clostridium chauvoei* and *Pasteurella multocida* with aluminium hydroxide as adjuvant, was tested on 2500 cattle and sheep. The vaccine was safe for use in animals and gave a high level of immunity in potency test on guineapigs, rabbits and mice (Ardehali *et al.*, 1997).

Reddy *et al.* (1997) studied serological response to combined vaccination of cattle against FMD, HS, and BQ and reported that serological response of cattle to all the three antigen either alone or combined together were similar and concluded that combined vaccine containing FMD, HS and BQ can be used to vaccinate animals.

2.3 Enzyme Linked Immunosorbant Assay (ELISA)

2.3.1 FMD ELISA

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 (0.693) between the two tests.

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

Hamblin *et al.* (1986b) found out that a titre of one in 16 virus neutralization was equivalent at 1 in 40 by ELISA as indicated by the overall regression between the ELISA and the VN data.

The antibody titre against Foot and Mouth disease after infection and vaccination was evaluated using ELISA. The antibody titres recorded by ELISA were similar following primary vaccinations and until five days after secondary vaccination (Hamblin *et al.*, 1987).

Many workers found out that there was a positive correlation existed between ELISA and Micro Neutralization Test titres with ELISA giving higher titres for antibodies against Foot and Mouth disease (Westbury *et al.*, 1988; Kalanidhi *et al.*, 1993; Rao *et al.*, 1993).

Maanen *et al.* (1989) compared liquid phase blocking ELISA and SNT for evaluating immunity in potency testing of FMD vaccines. The correlation co-efficient between LPB-ELISA and Serum Neutralization Test were 0.91 for type O and A, and 0.82 for type C.

A complex trapping-blocking (CTB) ELISA was described by Maanen and Maanen (1990) for FMD antibody detection. Results obtained from CTB-ELISA showed a positive correlation with VNT results.

The ELISA is specific, sensitive and quantitative, and also has the advantage that, it is more rapid to perform, is less variable, gives fewer false positive results and is not dependent on tissue culture systems over microneutralisation test (OIE Manual, 1992).

Periolo *et al.* (1993) assessed specific serum activity levels against four reference strains of Foot and Mouth Disease virus in animals vaccinated with quadrivalent oil-vaccines using LPB-ELISA. Serum LPB-ELISA titres were directly correlated with percentage of protected animals.

According to Gruia *et al.* (1995) the LPB-ELISA method eliminated the need for the maintenance of cell culture and live FMD viruses and the results of the test were obtained within 24 hours. The sensitivity and reproducibility of the test are comparable to virus neutralisation test.

Saha and Sen (1995) described the application of liquid phase blocking ELISA for detecting of FMD antibodies. The animals of an organized dairy farm were selected and screened for type O FMD antibody one month after vaccination with monovalent vaccine. A comparison was made between LPB-ELISA and sandwich competition ELISA. Higher titres were observed in LPB-ELISA (2 to 2.8) than sandwich competition ELISA (1.1 to 2.0).

Araujo *et al.* (1996) used liquid phase blocking sandwich ELISA for detection of antibodies against FMD virus in water buffalo sera. The antibody titres obtained in the blocking ELISA had a high positive correlation coefficient with VNT (0.9 for type 'O', and 0.82 for type A).

O' Donnel *et al.* (1996) applied liquid phase blocking sandwich ELISA for the detection of antibodies against FMD virus with a bio-engineered 3D protein. The assay was able to detect antibodies as early as five days post inoculation.

Armstrong (1997) employed ELISA for detecting FMD antibodies in cattle milk. Sample from convalescent cattle showed a high correlation between antibody levels in milk and serum.

The enzyme-linked Immunosorbent assay is marked by its rapidity, specificity and sensitivity for assessing the antibody titre against Foot and Mouth Disease (Dhanda and Gopalkrishnan, 1998).

Smitsaart *et al.* (1998) studied the herd immunity level induced in cattle by Foot and Mouth Disease oil 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 $\log_{10}=1.5$.

The anti FMDV antibodies in vaccinated mice were tested by Liquid Phase (LP) ELISA, Solid Phase (SP) ELISA and virus neutralization by Santos *et al.* (2000). They found that the anti FMDV antibody titres in mice were detected by ELISA as early as 14 days after vaccination.

2.3.2 HS ELISA

Klaassen *et al.* (1985) prepared lipopolysaccharide antigen, boiled-cell extract antigen, and boiled whole-bacterium antigen of *Pasteurella multocida* and used it in an Enzyme Linked Immunosorbent Assay to detect rabbit immunoglobulin G antibody to *Pasteurella multocida.* The sensitivity of each antigen preparation was compared by using sera from *Pasteurella multocida*-infected and uninfected rabbits and sera from two rabbits immunized with different serotypes of *Pasteurella*

multocida. In the ELISA, all the three antigen preparations detected high titres of antibodies in infected rabbits and markedly lower levels in uninfected rabbits. When whole-bacterium or boiled-cell extract antigens were used, the ELISA detected antibodies in sera from both immunized rabbits, but with lipopolysaccharide antigen, only antibody to the homologous serotype was detected. He also concluded that the boiled-cell extract was chosen as the best antigen preparation to be used in ELISA.

ELISA and immunoblotting techniques were used to detect the humoral immune response to *Pasteurella multocida*, in bovine sera. Elevated level of antibodies to a crude lipopolysaccharide preparation were found in vaccinated animals (Johnson *et al.*, 1989).

Erler *et al.* (1991) developed ELISA for determining antibodies to *Pasteurella multocida* and *Pasteurella haemolytica* using common salt extracts of the two organisms and found correlation between serological titre and protection against infection was established for *Pasteurella multocida* type B infection in calves.

Rabbit serum samples from eleven different research facilities were evaluated for the presence of immunoglobulin G against *Pasteurella multocida* by using an ELISA. The ELISA test showed a high degree of agreement (92.94per cent) with two other *Pasteurella multocida* ELISAs at different diagnostic facilities. This study confirms that an ELISA testing for serum antibodies against the *Pasteurella multocida* is a reliable diagnostic tool to screen colonies for *Pasteurella multocida* (Zaoutis *et al.*, 1991).

Chandrasekaran *et al.* (1993a) estimated serum antibody response of buffalo immunized with three conventional Haemorrhagic Septicaemia broth bacterin *via* alum-precipitated vaccine and oil adjuvant vaccine and one experimental double emulsion vaccine by ELISA, employing lipopolysaccharide and boiled or formalin-killed *Pasteurella multocida* type 6:B as antigens.

Johnson *et al.* (1993) studied the antibody responses of rabbits and cattle to vaccination with various *Pasteurella multocida* strains using ELISA, immunoblotting and passive mouse protection test.

The antibody response of cattle to one or two doses of an oil and lanolin-based Haemorrhagic Septicaemia bacterin was measured by ELISA using a lipopolysaccharide extract of formalin-killed *Pasteurella multocida*, Katha strain. ELISA antibody units were compared with the protective response in the passive mouse protection test. There was significant correlation between the HS antibody ELISA and Passive mouse protection test results. It is suggested that ELISA may be preferable to passive mouse protection test for the evaluation of HS vaccination programs (Natalia *et al.*, 1993).

Chandrasekaran *et al.* (1993b) investigated the relationship between active protection in buffalo immunized with different types of HS bacterins and antibody measured by the standard passive mouse protection test, the indirect haemagglutination test and ELISA.

Natalia and Patten (1993) stated that passive mouse protection test was compared to ELISA as alternative methods for investigating the response of cattle to vaccination with *Pasteurella multocida* and concluded that correlation between the two tests was significant.

Chandrasekaran *et al.* (1994) estimated immune response in buffaloes vaccinated with a non-adjuvanted broth vaccine by ELISA and suggested that there was a relationship between ELISA antibody titres and active protection in buffaloes.

An ELISA and gel diffusion precipitin test (GDPT) were used to determine the antibody levels in serum samples from the immunized rabbits. The ELISA detected an increase in specific Ig G levels against *Pasteurella multocida* and the GDPT detected antibodies against both serotypes in immunized rabbits (Opacka *et al.*, 1996).

Confer *et al.* (1996) vaccinated 29, five to eight month old calves subcutaneously or by aerosol exposure on days zero and seven with live or killed *Pasteurella multocida* or phosphate-buffered saline solution (control) and subsequently challenge exposed with virulent *Pasteurella multocida.* They quantified antibody responses to *Pasteurella multocida* A : 3 outer membrane using an ELISA.

Opacka *et al.* (1997a) tested the vaccine immunogenicity serologically in blood samples by ELISA. The ELISA detected an increased Ig G level after immunization.

Opacka *et al.* (1997b) measured the level of specific Ig G to *Pasteurella multocida* in the sera of rabbits vaccinated against pasteurellosis using ELISA. A statistically significant increase in Ig G level was found in rabbits challenged one month after immunization in comparison to non-vaccinated animals. It was concluded that the ELISA may be used to evaluate vaccine effectiveness, instead of experimental infection of animals.

Verma *et al.* (1997) immunized nineteen calves with a single four ml dose of multiple emulsion vaccine. Immune response was measured by indirect haemagglutination (IHA) and ELISA. Statistically, ELISA values were better than IHA values.

Opacka *et al.* (1998) vaccinated Boviseptivac oil-adjuvanted vaccine to six to twelve months old calves. The immunogenicity was determined by the agar gel diffusion preciptin test and ELISA. Titre of specific precipitins and Ig G to

Pasteurella multocida serotype two increased significantly after vaccination for at least three months.

2.3.3 BQ ELISA

Tadich *et al.* (1988) estimated serum concentration of epsilon antitoxin (*Clostridium perfringens*, type D) by ELISA.

Pasini and Alito (1989) detected *Clostridium chauvoei* antibodies in the serum of cattle vaccinated against blackleg using modified ELISA.

Hamaoka *et al.* (1990) evaluated immunity in mice vaccinated with blackleg vaccine by ELISA using culture fluid antigen obtained by precipitation at 0.3 saturation of ammonium sulfate, was used successfully to titrate the protective immunity induced by *Clostridium chauvoei* vaccine.

Jubb *et al.* (1993) used ELISA in measuring serum antibodies to type C and D botulinum toxins (A-ELISA). They concluded that ELISA were sensitive and specific in detecting

antibody to toxoid and could be used to monitor response to vaccination in cattle.

ELISA and indirect haemagglutination test (IHA) were suitable for measuring antibody responses in guineapigs immunized with various antigens of *Clostridium chauvoei* (Kar and Harbola, 1995).

ELISAs which measure serum antibody to type C and D botulinum toxins in cattle were used to diagnose type C botulism in a herd (Main and Gregory, 1996).

Raadsma *et al.* (1996) measured ELISA antibody titres to *Clostridium tetani* and *Clostridium chauvoei* two weeks after booster vaccination.

Tanaka *et al.* (1998) developed quantitative ELISA using purified flagella of *Clostridium chauvoei* as the antigen to measure the anti-flagellar titre in mice, and relationship between the anti-flagellar titre and the immunigenicity of blackleg vaccine. They suggested that the flagella based ELISA may be useful as an aid for evaluation of immunogenicity of blackleg vaccines.

Capture and competitive enzyme immunoassay techniques were promising substitutes for the neutralization test for vaccines in mice, but required further validation. The testing of hyperimmune sera by ELISA proved to be more difficult, and further research was needed (Ebert *et al.*, 1999).

Materials and Methods

3. MATERIALS AND METHODS

The study was carried out in the Department of Veterinary Epidemiology and Preventive Medicine, College of Veterinary and Animal Sciences, Mannuthy during the period of May 2000 to June 2001.

3.1 Materials

3.1.1 Glassware and reagents

In this study, Borosil brand of glassware, Laxbro plastics and analytical or guaranteed reagent grade of chemicals were used.

The materials were processed using standard methods (Hoskins, 1967) and sterilized either in hot air oven or autoclave depending upon the materials to be sterilized.

3.1.2 Experimental animals

Eighteen calves, which were above four months of age and not vaccinated against FMD, HS, and BQ were selected, for the

study from University Livestock Farm, Mannuthy and Cattle Breeding Farm, Thumburmuzhi. They were grouped into three of six animals in each and each group was immunized with different vaccines for the seroconversion studies.

3.1.3 Vaccines

Group I: Was immunized with Combined FMD, HS, BQ oil adjuvant vaccine

Group II: Was immunized with FMD oil-adjuvant vaccine and combined HS and BQ Aluminium hydroxide gel vaccine.

Group III: Was immunized with FMD oil-adjuvant vaccine, HS Aluminium hydroxide gel vaccine and BQ Aluminium hydroxide gel vaccine

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

3.1.4.2 Reagents

a. Coating Buffer (0.5 M carbonate–Bicarbonate Buffer) pH 9.6

Sodium carbonate	3.18 g
Sodium bicarbonate	5.86 g
Distilled water to make	2000ml

(The reagent was dissolved first in 500 ml distilled water and made upto 2000 ml).

b. Dulbecco's Phosphate Buffered Saline (DPBS) pH 7.2

i) Stock solution (5x)

Sodium chloride	40.0 g
Potassium chloride	1.0 g
Magnesium chloride ($MgCl_2.6H_2O$)	0.5 g
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	1000ml

(Calcium chloride was dissolved separately in distilled water and added)

ii) Working solution (1x)

DPBS stock solution (5x)	1000ml
Distilled water	4000ml

c. Dulbecco's Phosphate Buffered Saline–Tween-20 (DPBS-T)

Tween-20	0.5 ml
DPBS(1x)	1000ml

d. Citrate Buffer (Substrate buffer) pH 5.0

Citric acid	5.11 g
Disodium hydrogen orthophosphate	7.3 g
Distilled water to make	2000ml

e. (i) Substrate solution

Orthophenylene diamine dihydrochloride(Sigma)	30 mg
Citrate buffer	75 ml

(ii) Activated substrate solution

33 per cent Hydrogen peroxide	0.001ml
Substrate solution	2 ml

f. Reaction stopper solution (1M H_2SO_4)

Conc. Sulphuric acid	60 ml
Distilled water	2000ml

g. Blocking buffer

Normal Bovine Serum	10 ml
Normal Rabbit Serum	5 ml
DPBS-T	85 ml

3.1.4.3 Biologicals

a. Viral Antigen

BHK 21 cell adapted, aziridine inactivated O, A, C and Asia-1 Foot and Mouth disease virus antigens were supplied by M/S Indian Immunologicals Ltd, Hyderabad.

b. Anti '146s' Immune Rabbit Serum (IRS)

Type specific rabbit antisera against O, A, C and Asia-1 FMDV antigens were supplied by M/S Indian Immunologicals Ltd, Hyderabad.

c. Anti '146s' Immune Guinea Pig Serum (IGPS)

Type specific guinea pig antisera against O, A, C and Asia-1 FMD antigens were supplied by M/S Indian Immunologicals Ltd., Hyderabad.

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.

3.1.5 Indirect ELISA

3.1.5.1 ELISA Plates

Flat bottom 96 well ELISA plates (NUNC) were used as the test plates

3.1.5.2 Reagents

a. Coating Buffer (0.5M carbonate–Bicarbonate Buffer) pH 9.6

Sodium carbonate	3.18 g
Sodium bicarbonate	5.86 g
Distilled water to make	2000ml

(The reagent was first dissolved in 500 ml distilled water and made upto 2000 ml).

b. Dulbecco's Phosphate Buffered Saline-Tween20 Albumin (DPBS-TA)

DPBS (1x)	1000ml
Tween-20	0.5 ml
Bovine Serum Albumin	10 g

c. Citrate Buffer (Substrate buffer) pH 5.0

Citric acid	5.11 g
Disodium hydrogen orthophosphate	7.3 g
Distilled water to made	2000 ml

d. (i) Substrate solution

Orthophenylene diamine dihydrochloride (Sigma)	30 mg
Citrate buffer	75 ml

(ii) Activated substrate solution

33% Hydrogen peroxide	0.001ml
Substrate solution	2 ml

e. Reaction stopper solution (1M H_2SO_4)

Con. Sulphuric acid	60 ml
Distilled water	2000 ml

f. Blocking buffer

PBS-TA

3.1.5.3 Biologicals

a. Antigen

Outer membrane protein of *Pasteurella multocida* supplied by National Dairy Development Board, Anand and Supernatent sonicated *Clostridium chauvoei* antigen from Indian Immunologicals Ltd, Hyderabad were used.

b. Anti-bovine Horse Radish Peroxidase Conjugated IgG

Anti-bovine Horse Radish Peroxidase Conjugated IgG was used at a working dilution of 1 in 1000 in blocking buffer.

c. Positive serum

Passteurella multocida and *Clostridium chauvoei* positive serum were supplied by M/S Indian Immunologicals Ltd, Hyderabad.

3.2 Methods

3.2.1 Vaccination of animals

Group I

All the six calves of this group were vaccinated as follows:

Combined FMD, HS, BQ oil adjuvant vaccine

Primary vaccination	:	at 4 months of age
First booster dose	:	at 13 months of age
Dose	:	3 ml
Route of vaccination	:	Deep intramuscular

Group II

All the six calves of this group were vaccinated as follows:

FMD oil-adjuvant vaccine

Primary vaccination	:	at 4 months of age
First booster dose	:	at 13 months of age
Dose	:	2 ml
Route of vaccination	:	Deep intramuscular

Combined HS, BQ Aluminium hydroxide gel vaccine

Primary vaccination	:	at 6 months of age
First booster dose	:	at 12 months of age
Dose	:	3 ml
Route of vaccination	:	Subcutaneous

Group III

All the six calves of this group were vaccinated as follows:

FMD oil-adjuvant vaccine

Primary vaccination	:	at 4 months of age
First booster dose	:	at 13 months of age
Dose	:	2 ml
Route of vaccination	:	Deep intramuscular

HS Aluminium hydroxide gel vaccine

Primary vaccination	:	at 5 months of age
First booster dose	:	at 11 months of age
Dose	:	2 ml
Route of vaccination	:	Subcutaneous

BQ Aluminium hydroxide gel vaccine

Primary vaccination	:	at 6 months of age
First booster dose	:	at 12 months of age
Dose	:	2 ml
Route of vaccination	:	Subcutaneous

3.2.2 Collection of serum samples

Blood was collected from all twelve before vaccination. The serum was separated and inactivated at 56°C for 30 minutes in water bath. The samples were stored at –20°C, which formed the pre-vaccination samples.

All the calves were bled at monthly intervals post vaccination for a period of one year from the date of primary vaccination upto the age of 16th month. Serum samples were separated and inactivated at 56°C for 30 minutes in water bath. All the serum samples were stored at –20°C.

3.2.3 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).

3.2.3.1 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 4
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 2000
A	:	1 in 2000
C	:	1 in 2000
Asia-1	:	1 in 1500

IRS dilutions were made in coating buffer.

c. Immune Guinea Pig Serum (IGPS)

O : 1 in 2000

A : 1 in 2000

C : 1 in 2000

Asia-1 : 1 in 2000

IGPS dilutions were made in blocking buffer.

d. Anti guinea pig HRPO conjugated IgG (Sigma)

Working dilution 1 in 2000 (in blocking buffer)

3.2.3.2 Test procedure

3.2.3.2.1 Coating of test plates

All the four types of IRS were made into corresponding working dilutions with coating buffer (0.5 M carbonate bicarbonate buffer).

Flat bottom 96 well ELISA plates (NUNC) were used for coating with IRS. Then 50 μl of IRS was added at working dilution to all the 96 wells. The plates were sealed and kept at room temperature in a moist chamber overnight for coating.

3.2.3.2.2 Preparation of carrier plates

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

'U' bottom 96 well microtitre plates (TARSON) were used. Fifty μl of DPBS-T was added to all the 96 wells. Added 50 μl of test serum samples in 1 to 10 wells of first row, i.e. sample I in 1A, Sample II in 2A etc. (ten samples on a single plate). Two-fold dilutions were made column-wise (A to H wells of 1 to 10 columns).

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

3.2.3.2.3 Transfer of serum-antigen mixture to test plates

The IRS coated plates were washed five times with DPBS-T and tapped to dry. The contents of carrier plates were transferred 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.

3.2.3.2.4 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. Fifty μl of corresponding IGPS was added at working dilution to all the wells. The plates were sealed and incubated at 37°C for one hour with intermittent shaking.

3.2.3.2.5 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. Fifty μl of conjugate was added at working dilution

to all the wells. Sealed the plates and incubated at 37°C for one hour with intermittent shaking.

3.2.3.2.6 Addition of substrate

The test plates were washed five times with DPBS-T and tapped to dry. Fifty μl of activated substrate solution was added to all the wells. The plates were kept in darkness for 15 minutes.

3.2.3.2.7 Addition of stopper solution

After 15 minutes, plates were taken out and added 50 μl of 1M H_2SO_4 to all the wells.

3.2.3.2.8 Reading of the plates

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

3.2.3.2.9 Controls

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

3.2.3.3 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 end point of each serum dilution obtaining from the mean O.D value of antigen control and expressed as $\log_{10}$ of the serum dilution.

3.2.4 Indirect ELISA

Indirect ELISA for the assessment of serum neutralizing antibody titre against *Pasteurella multocida* was carried out as per Natalia et al. (1993) and *Clostridium chauvoei* was carried out as per Pasine and Aliţo, (1989) with slight modification.

3.2.4.1 Standardisation of reagents

The working dilution of antigens test serum and Anti-bovine HRPO conjugated IgG were assessed by checker board titration procedure.

The working dilution of different reagents are as follows:

a. Antigen

Pasteurella multocida	1 in 1000
Clostridium chauvoei	1 in 1000

b. Test serum

Test serum	1 in 1000 in PBS-TA

c. Antibovine HRPO conjugated IgG

Working dilution	1 in 1000 (in blocking buffer)

3.2.4.2 Test procedure

3.2.4.2.1 Coating of test plates

Flat bottom 96 well ELISA plates were used for coating with corresponding bacterial antigen. Antigen were made

into corresponding working dilution with coating buffer. Fifty µl of corresponding bacterial antigen was added at working dilution to all the 96 wells. Sealed the plates and kept at room temperature in a moist chamber overnight for coating.

3.2.4.2.2 Addition of test serum to the test plates

Washed the antigen coated plates five times with DPBS-T and tapped to dry.

Fifty µl of diluted test serum sample (in PBST-TA) was added to all the wells, in sample one in 1A, sample two in 2A etc., except in 11^{th} and 12^{th} columns. Sealed the plates and incubated at 37^0C for one hour with intermittent shaking.

3.2.4.2.3 Addition of conjugate

Antibovine HRPO conjugated IgG was made into a working dilution of one in 1000 with blocking buffer (PBS-TA).

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.

3.2.4.2.4 Addition of substrate

Washed the test plates five time with PBS-T and tapped to dry. Added 50 µl if activated substrate solution to all the wells. The plates were kept in darkness for 15 minutes.

3.2.4.2.5 Addition of stopper solution

After 15 minutes, plates were taken out and added 50 µl of 1M H_2SO_4 to all the wells.

3.2.4.2.6 Reading of the plates

The Optical Density (O.D.) values were assessed using a multi-scan spectrophotometer at a wavelength of 492 nm.

3.2.4.2.7 Controls

A11, B11, C11, D11 well of each plates were taken as the positive controls where known positive serum was added.

E11, F11, G11, H11 well of each plate was taken as the negative control where known negative serum was added. And 12th columns were taken as blank.

3.2.4.2.8 Interpretation of Readings

The serum antibody titres for HS and BQ were calculated using the following formula.

(OD of test sera – OD of background) × factor dilution of sera.

ELISA units were expressed titre/ml.

3.2.5 Statistical analysis

Statistical analysis of the result obtained were done as per Snedecor and Cochran (1994).

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 (Plate 1) and Indirect ELISA (Plate 2) for the estimation of serum neutralizing antibody titres against *Pasteurella multocida* and *Clostridium chauvoei* antigen.

4.1 Seroconversion following vaccination in three different group

4.1.1 Seroconversion of FMDV type O antigen

4.1.1.1 Group I

The type 'O' antibody titres of all the animals belonging to Group I from zero to 12th month is given in Table (1) and Fig. (1 & 1a). After the primary vaccination the highest mean type O antibody titre of 2.222 ± 0.15 was observed during fourth month and subsequently declined to 1.626 ± 0.1 at ninth month. After booster vaccination at 9th month the peak antibody

titre 2.214 ± 0.01 was obtained at the end of the study at 12th month.

4.1.1.2 Group II

The antibody titres against type 'O' FMD antigen in group II are presented in Table (2) and Fig. (1 & 1a). The highest mean antibody titre after the primary vaccination was 2.197 ± 0.12 during fourth month thereafter the titre declined to 1.529 ± 0.21 at 9th month. The peak mean antibody titre of 2.028 ± 0.11 was obtained at 12th month after the booster.

4.1.1.3 Group III

The mean titres of type 'O' FMDV antibody of all the animals in Group III is given in Table (3) and Fig. (1 & 1a). Following primary vaccination the highest mean antibody titre obtained was 2.158 ± 0.12 during the first month Subsequently it fell to a level of 1.3 ± 0.15 at 9th month. After booster vaccination at 9th month the mean antibody titre increased to a

maximum of 2.267 ± 0.11 after one month and thereafter it declined to 2.058 ± 0.15 at 12th month.

4.1.1.4 Comparison of type O FMDV antibody titres between groups

The comparison of FMDV type 'O' antibody titres between three groups in each month is given in Table (4) and Fig. (1 & 1a). There was no significant difference in antibody titres between Group I, II and III in the entire study period, except during the first month where there was significant difference ($P \leq 0.05$) in antibody titres between Group I and III.

4.1.2 Seroconversion of FMDV type-A antibodies

4.1.2.1 Group I

The type 'A' antibodies to FMDV type A antigen of Group I animals are presented in Table (5) and Fig. (2 & 2a). Following primary vaccination, the highest mean titre of 1.941 ± 0.09 was obtained during second month Subsequently it declined to 1.34 ± 0.1 at 9th month. After booster vaccination at 9th month peak mean antibody titre of 2.224 ± 0.12 was obtained

after two months, and thereafter it declined to 1.826 ± 0.13 at 12th month.

4.1.2.2 Group II

The FMDV type 'A' antibody titres of Group II animals are provided in Table (6) and Fig. (2 & 2a). Following the primary vaccination, highest mean titre was recorded during first month (1.827 ± 0.09) which declined at (1.294 ± 0.13) 9th month. After booster vaccination at 9th month the peak mean antibody titre (2.101 ± 0.11) was obtained after a month, and thereafter it declined to 1.864 ± 0.05 at 12th month.

4.1.2.3 Group III

The FMDV type 'A' antibody titres of Group III animals are presented in Table (7) and Fig. (2 & 2a). Following the primary vaccination the highest mean antibody titre was noted during fourth month (1.831 ± 0.06) which fell to a level of 0.889 ± 0.18 at 9th month. After booster vaccination at 9th month antibody titre rose to a maximum of 2.153 ± 0.14 within a

month and thereafter it declined to 1.986 ± 0.11 at the end of the study.

4.1.2.4 Comparison of type A FMDV antibody titres between groups

The comparison of FMDV type 'A' antibody titres of the animals of all the three groups are given in Table (8) and Fig. (2 & 2a). A significant difference was observed between Group I and II during second ($P \leq 0.05$) and third month ($P \leq 0.05$). During rest of the period there was no significant difference between the groups.

4.1.3 Seroconversion of FMDV type C antibodies

4.1.3.1 Group I

The antibody titres of serum samples collected from Group I animals against FMDV type 'C' antigen are given in Table (9) and Fig.(3 & 3a). Following primary vaccination highest mean antibody titre (1.888 ± 0.09) was recorded during sixth month, which fell to level of 1.033 ± 0.13 at 9th month. After booster vaccination at 9th month, the peak mean antibody titre

was recorded at 11th month (2.395 ± 0.12) and thereafter it declined to 2.257 ± 0.04 at 12th month.

4.1.3.2 Group II

The FMDV type 'C' antibody titres of Group II animals are presented in Table (10) and Fig. (3 & 3a). Highest mean antibody titre following primary vaccination was noted during sixth month (2.428 ± 0.1) which fell to low level of 1.253 ± 0.24 at 9th month. After booster vaccination at 9th month the mean antibody titres increased to a peak of 2.52 ± 0.1 at 12th month.

4.1.3.3 Group III

FMDV type 'C' antibody titres of Group III animals are presented in Table (11) and Fig. (3 & 3a). After primary vaccination highest mean titre of 2.5 ± 0.09 was obtained during sixth month. At 9th month the titre was 1.09 ± 0.09. After booster vaccination at 9th month the highest antibody titre of 2.56 ± 0.13 was obtained after three months.

4.1.3.4 Comparison of type C FMDV antibody titres between groups

The comparison of FMDV type 'C' antibody titres of all the three groups in different months are given in Table (12) and Fig. (3 & 3a). There was no significant difference between type 'C' FMDV antibody titres between three groups except during sixth month ($P \leq 0.01$) where there was significant difference between Group I and II, and Group I and III.

4.1.4 Seroconversion of FMDV type Asia-1 antibodies

4.1.4.1 Group I

The FMDV type Asia-1 antibodies of Group I animals are presented in Table (13) and Fig. (4 & 4a). Highest mean antibody titre following primary vaccination was 2.297 ± 0.1 during fourth month. The titre fell to a level of 1.428 ± 0.08 at 9^{th} month, then subsequently after booster vaccination, at 9^{th} month, the mean antibody titre increased to a maximum of 2.248 ± 0.14 at the end of the study.

4.1.4.2 Group II

The FMDV type Asia-1 antibody titres of Group II animals during the study period are given in Table (14) and Fig.

(4 & 4a). Following primary vaccination, highest mean antibody titre was observed during third month (2.271 ± 0.11) which has fallen to a level of 1.339 ± 0.24 at 9th month. After booster vaccination at 9th month, the peak mean antibody titre was obtained at 11th month (2.341 ± 0.12) and thereafter it declined to 2.294 ± 0.11 at 12th month.

4.1.4.3 Group III

The FMDV type Asia-1 antibody titres of group III animals are shown in Table (15) and Fig. (4 & 4a). The highest mean titre following primary vaccination was noted within a month (2.184 ± 0.12) which fell to a level of 1.229 ± 0.09 at 9th month. After booster vaccination at 9th month the highest antibody titre of 2.055 ± 0.09 was obtained at 12th month.

4.1.4.4 Comparison of FMDV type Asia-1 antibody titres between groups

The comparison of FMDV type Asia-1 antibodies between three groups from zero to twelfth month are given in Table (16) and Fig (4 & 4a). There was no significant difference in antibody titres between Group I, II and III in the entire study

period except during the fourth month where there was a significant difference between Group I and II, and Group I and III ($P \leq 0.05$).

4.1.5 Seroconversion of Pasteurella multocida antibodies

4.1.5.1 Group I

Pasteurella multocida antibody titres of all the animals belonging to Group I from zero to 12th month are given in Table (17) and Fig. (5 & 5a). The highest mean antibody titre of 135.7 ±26.5 was obtained during ninth month of age. The titre was 100.4 ± 12 at 13th month of age. After booster vaccination at 13th month of age the antibody titre reached a peak of 107.3 ± 11 at 16th month of age.

4.1.5.2 Group II

Pasteurella multocida antibody titres of Group II animals are presented in Table (18) and Fig. (5 & 5a). Highest mean antibody titre was obtained after sixth month of primary vaccination (110.2 ± 26). Following booster vaccination at 12th month of age the mean antibody titre increased to a maximum of

161.4 ± 20.0 after two months and thereafter it declined to 131.9 ± 16.0 at end of study.

4.1.5.3 Group III

Pasteurella multocida antibody titres of all the animals belonging to Group III from zero to 12th month are given in Table (19) and Fig. (5 & 5a). After primary vaccination the highest mean antibody titre obtained was at ninth month of age (140.1 ± 17.1). Following the booster vaccination at 11th month of age antibody titre rose to 191.7 ± 19 after three months and thereafter it declined to 135.3 ± 10 at the end of the study.

4.1.5.4 Comparison of antibody titres to Pasteurella multocida between groups

The comparison of *Pasteurella multocida* antibody titres between three groups are given in Table (20) and Fig. (5 & 5a). A significant difference in antibody titre between Group I and II, and Group I and III were observed during second ($P \leq 0.01$), ninth ($P \leq 0.01$), tenth ($P \leq 0.01$) and eleventh month ($P \leq 0.05$).

4.1.6 Seroconversion of clostridium chauvoei antigens

4.1.6.1 Group I

Clostridium chauvoei antibody titres of Group I animals are given in Table (21) and Fig. (6 & 6a). Following the primary vaccination highest mean titre was recorded during 12th month of age (105.2 ± 22). The titre fell to 77.2 ± 9 at 13th month of age following booster vaccination at 13th month of age the antibody titre raised to 156.1 ± 19 after one month and thereafter the antibody titre declined to 81 ± 17 at 16th month of age.

4.1.6.2 Group II

The antibody titres against *Clostridium chauvoei* antigen in Group II animals are presented in Table (22) and Fig. (6 & 6a). Following primary vaccination at 6th month of age the highest mean antibody titre of 80.6 ± 4.4 was obtained after one month. Following booster vaccination at 12th month of age the antibody titre increased to a maximum of 162.2 ± 11 within a month and thereafter the antibody titre declined to 97 ± 10 during the end of the study.

4.1.6.3 Group III

The antibody titres of animals in Group III against *Clostridium chauvoei* antigen are given in Table (23) and Fig. (6 & 6a). The titre rose to 75.2 ± 19.5 after three month following primary vaccination at six month of age. After booster vaccination at 12th month of age the highest mean antibody titre (176.5 ± 4) was obtained within a month and thereafter it declined to 86.2 ± 19 at end of study.

4.1.6.4 Comparison of Clostridium chauvoei antibody titres between groups

The comparison of *Clostridium chauvoei* antibody titres of the animals of all the three groups are presented in Table (24) and Fig. (6 & 6a). A significant difference was observed between Group I and II, Group I and III during second ($P \leq 0.05$) and ninth month ($P \leq 0.01$).

Table 1. The antibody titres to FMDV Type O in group I

Age of calf in month	4th	5th	6th	7th	8th	9th	10th	11th	12th	13th	14th	15th	16th
Month of sample collection / Ani. No	0	1	2	3	4	5	6	7	8	9	10	11	12
C004	0.600	1.867	1.803	1.860	2.190	2.065	1.588	1.368	1.530	1.975	2.325	2.091	2.409
C008	0.600	1.800	2.106	2.700	1.962	1.907	1.722	1.534	1.566	1.804	2.086	2.151	2.007
C010	0.600	1.621	1.845	1.946	1.813	1.872	1.676	1.166	1.492	1.804	1.726	1.635	2.115
C021	0.600	1.829	2.070	1.790	2.700	1.682	1.400	1.505	0.638	1.335	1.966	1.372	2.169
655	0.600	1.855	1.990	2.553	2.646	1.801	1.608	1.118	1.926	1.361	1.899	2.077	2.523
657	0.600	1.914	2.175	2.053	2.022	1.934	1.761	0.600	1.187	1.481	1.806	1.927	2.061
Mean ± SE	0.600 ± 0.01	1.814 ± 0.04	1.998 ± 0.06	2.150 ± 0.15	2.222 ± 0.15	1.876 ± 0.05	1.625 ± 0.05	1.215 ± 0.14	1.389 ± 0.17	1.626 ± 0.10	1.968 ± 0.08	1.875 ± 0.12	2.214 ± 0.01

Table 2. The antibody titres to FMDV Type O in group II

Age of calf in month	4th	5th	6th	7th	8th	9th	10th	11th	12th	13th	14th	15th	16th
Month of sample collection / Ani. No	0	1	2	3	4	5	6	7	8	9	10	11	12
C002	0.600	1.978	2.049	1.648	1.927	2.293	0.600	1.596	1.570	1.076	1.818	1.879	1.783
C009	0.600	1.788	2.700	2.136	1.947	1.998	1.840	1.603	1.990	0.740	2.700	1.703	2.164
C024	0.600	2.159	2.254	2.527	1.939	1.486	1.966	1.691	1.992	1.585	1.892	1.815	2.365
C023	0.600	1.786	1.836	2.118	2.326	1.571	0.711	1.620	1.800	1.975	1.778	1.945	1.161
658	0.600	1.858	1.799	1.994	2.343	0.600	1.668	1.102	1.776	1.995	1.943	2.352	1.998
663	0.600	2.062	1.969	2.700	2.700	1.507	1.578	2.264	1.318	1.804	1.928	2.018	2.700
Mean ± SE	0.600 ± 0.01	1.938 ± 0.06	2.101 ± 0.13	2.187 ± 0.15	2.197 ± 0.12	1.575 ± 0.23	1.393 ± 0.24	1.646 ± 0.15	1.741 ± 0.10	1.529 ± 0.21	2.009 ± 0.14	1.952 ± 0.09	2.028 ± 0.11

Table 3. The antibody titres to FMDV Type O in group III

Age of calf in month	4th	5th	6th	7th	8th	9th	10th	11th	12th	13th	14th	15th	16th
Month of sample collection / Ani. No	0	1	2	3	4	5	6	7	8	9	10	11	12
C032	0.600	1.970	2.366	2.700	2.36	1.561	1.527	1.389	1.356	1.818	1.923	1.933	2.700
C033	0.600	2.163	2.056	2.218	1.976	1.636	0.860	1.628	1.494	1.331	2.310	1.935	2.241
C035	0.600	2.027	1.768	2.405	2.113	1.571	2.300	1.506	1.611	1.346	2.329	1.938	1.585
C036	0.600	2.700	2.021	1.933	2.176	1.291	1.242	1.571	0.735	1.488	2.700	2.332	1.997
670	0.600	2.258	1.952	1.522	1.648	2.211	0.791	1.603	1.560	1.063	2.341	2.373	1.986
669	0.600	1.831	1.878	1.968	1.373	1.355	1.079	2.700	1.956	0.739	2.003	2.700	1.839
Mean ± SE	0.600 ± 0.01	2.158 ± 0.12	2.006 ± 0.08	2.124 ± 0.16	1.941 ± 0.14	1.604 ± 0.13	1.300 ± 0.22	1.732 ± 0.19	1.452 ± 0.16	1.300 ± 0.15	2.267 ± 0.11	2.201 ± 0.04	2.058 ± 0.15

Table 4. Comparison of the mean FMDV type O (Mean ± SE) antibody titres of three groups

Age of calf in month	4th	5th	6th	7th	8th	9th	10th	11th	12th	13th	14th	15th	16th
Month of sample collection / Groups	0	1	2	3	4	5	6	7	8	9	10	11	12
Group I	0.600 ± 0.01	1.814 ± 0.04^{A}	1.998 ± 0.06	2.150 ± 0.15	2.222 ± 0.15	1.876 ± 0.05	1.625 ± 0.05	1.215 ± 0.14	1.389 ± 0.17	1.626 ± 0.10	1.968 ± 0.08	1.875 ± 0.12	2.214 ± 0.01
Group II	0.600 ± 0.01	1.938 ± 0.06^{AB}	2.101 ± 0.13	2.187 ± 0.15	2.197 ± 0.12	1.575 ± 0.23	1.393 ± 0.24	1.646 ± 0.15	1.741 ± 0.10	1.529 ± 0.21	2.009 ± 0.14	1.952 ± 0.09	2.028 ± 0.11
Group III	0.600 ± 0.01	2.158 ± 0.12^{B}	2.006 ± 0.08	2.124 ± 0.16	1.941 ± 0.14	1.604 ± 0.13	1.30 ± 0.22	1.732 ± 0.19	1.452 ± 0.16	1.30 ± 0.15	2.267 ± 0.11	2.201 ± 0.04	2.058 ± 0.15
Cd	NS	0.2527*	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

Values in same column bearing same superscript do not differ significantly
NS – No significant difference between the groups
* Significant at 5% level

Table 5. The antibody titres to FMDV Type A in group I

Age of calf in month	4th	5th	6th	7th	8th	9th	10th	11th	12th	13th	14th	15th	16th
Month of sample collection / Ani. No	0	1	2	3	4	5	6	7	8	9	10	11	12
C004	0.600	1.543	2.060	1.809	1.725	1.209	1.425	1.454	1.531	1.039	1.620	2.700	2.258
C008	0.600	1.524	1.985	1.914	1.767	1.576	1.469	1.820	0.683	1.237	1.327	2.199	1.815
C010	0.600	2.037	1.560	2.266	1.905	1.645	1.559	0.957	1.547	1.406	2.045	2.421	1.823
C021	0.600	1.407	2.069	1.999	1.890	1.799	0.600	1.418	1.472	1.140	1.881	2.137	2.080
655	0.600	1.971	2.218	1.920	2.064	1.977	1.497	1.819	1.111	1.451	1.851	1.803	1.628
657	0.600	1.739	1.756	1.472	1.916	1.488	1.554	1.764	1.911	1.788	1.655	2.089	1.353
Mean ± SE	0.600 ± 0.01	1.703 ± 0.10	1.941 ± 0.09	1.896 ± 0.10	1.877 ± 0.04	1.615 ± 0.10	1.350 ± 0.15	1.538 ± 0.13	1.344 ± 0.19	1.343 ± 0.10	1.729 ± 0.10	2.224 ± 0.12	1.826 ± 0.13

Table 6. The antibody titres to FMDV Type A in group II

Age of calf in month	4th	5th	6th	7th	8th	9th	10th	11th	12th	13th	14th	15th	16th
Month of sample colle-ction / Ani. No	0	1	2	3	4	5	6	7	8	9	10	11	12
C002	0.600	1.903	1.842	1.306	1.504	2.700	1.639	1.509	1.623	0.966	1.789	2.003	2.045
C009	0.600	1.967	1.606	1.752	1.508	1.529	1.555	1.749	1.393	1.435	1.905	1.881	1.907
C024	0.600	2.057	1.514	1.462	2.023	1.639	1.027	1.834	1.003	1.770	2.282	1.949	1.857
C023	0.600	1.978	1.721	0.702	1.391	1.663	2.192	0.600	1.223	1.263	1.996	2.700	1.666
658	0.600	1.494	0.666	1.643	1.721	1.405	1.324	1.929	1.390	1.433	2.563	2.000	1.804
663	0.600	1.567	0.643	1.366	1.749	1.014	1.600	2.066	1.103	0.899	2.073	1.832	1.908
Mean ± SE	0.600 ± 0.01	1.827 ± 0.09	1.332 ± 0.21	1.371 ± 0.15	1.649 ± 0.09	1.658 ± 0.22	1.556 ± 0.15	1.615 ± 0.21	1.222 ± 0.07	1.294 ± 0.13	2.101 ± 0.11	2.060 ± 0.13	1.864 ± 0.05

Table 7. The antibody titres to FMDV Type A in group III

Age of calf in month	4th	5th	6th	7th	8th	9th	10th	11th	12th	13th	14th	15th	16th
Month of sample collection / Ani. No	0	1	2	3	4	5	6	7	8	9	10	11	12
C032	0.600	2.060	1.639	1.040	1.843	1.242	1.677	0.900	1.437	0.621	1.671	1.828	2.298
C033	0.600	1.446	1.358	1.833	1.924	1.401	1.430	1.745	1.784	0.891	2.159	2.018	2.004
C035	0.600	1.082	2.031	1.470	2.002	1.131	1.028	1.983	0.925	0.864	2.325	1.920	1.571
C036	0.600	1.784	1.895	1.534	1.951	1.454	1.250	1.582	1.402	0.600	2.100	2.161	2.069
670	0.600	1.924	1.853	2.042	1.700	1.804	1.393	1.365	1.690	0.609	1.964	2.018	2.254
669	0.600	1.967	1.673	1.410	1.568	0.943	1.144	1.830	1.440	1.749	2.700	2.700	1.725
Mean ± SE	0.600 ± 0.01	1.710 ± 0.15	1.741 ± 0.09	1.554 ± 0.14	1.831 ± 0.06	1.329 ± 0.12	1.320 ± 0.09	1.567 ± 0.15	1.448 ± 0.13	0.889 ± 0.18	2.153 ± 0.14	2.107 ± 0.12	1.986 ± 0.11

Table 8. Comparison of the mean FMDV type A (Mean ± SE) antibody titres of three groups

Age of calf in month	4th	5th	6th	7th	8th	9th	10th	11th	12th	13th	14th	15th	16th
Month of sample collection / Groups	0	1	2	3	4	5	6	7	8	9	10	11	12
Group I	0.600 ± 0.01	1.703 ± 0.10	1.941 ± 0.09^{A}	1.896 ± 0.10^{A}	1.877 ± 0.04	1.615 ± 0.10	1.350 ± 0.15	1.538 ± 0.13	1.344 ± 0.19	1.343 ± 0.10	1.729 ± 0.10	2.224 ± 0.12	1.826 ± 0.13
Group II	0.600 ± 0.01	1.827 ± 0.09	1.332 ± 0.21^{B}	1.371 ± 0.15^{B}	1.649 ± 0.09	1.658 ± 0.22	1.556 ± 0.15	1.615 ± 0.21	1.222 ± 0.07	1.294 ± 0.13	2.101 ± 0.11	2.060 ± 0.13	1.864 ± 0.05
Group III	0.600 ± 0.01	1.710 ± 0.15	1.741 ± 0.09^{AB}	1.554 ± 0.14^{AB}	1.831 ± 0.06	1.329 ± 0.12	1.320 ± 0.09	1.567 ± 0.15	1.448 ± 0.13	0.889 ± 0.18	2.153 ± 0.14	2.107 ± 0.12	1.986 ± 0.11
Cd	NS	NS	0.4502*	0.4047*	NS	NS	NS	NS	NS	NS	NS	NS	NS

Values in same column bearing same superscript do not differ significantly
NS – No significant difference between the groups
* Significant at 5% level

Table 9. The antibody titres to FMDV Type C in group I

Age of calf in month	4th	5th	6th	7th	8th	9th	10th	11th	12th	13th	14th	15th	16th
Month of sample collection / Ani. No	0	1	2	3	4	5	6	7	8	9	10	11	12
C004	1.460	1.530	1.490	2.460	1.070	2.088	1.837	1.500	1.136	1.290	1.990	2.800	2.264
C008	1.130	2.180	1.560	2.80	1.070	1.935	1.919	1.078	1.053	0.604	1.470	2.096	2.231
C010	0.970	1.770	1.670	0.600	0.600	2.075	2.026	1.005	0.604	1.189	1.600	2.364	2.419
C021	1.360	0.600	0.600	0.600	0.600	0.640	1.533	0.604	1.231	1.312	1.720	2.692	2.270
655	1.170	1.870	1.760	1.670	1.280	1.160	1.819	1.239	1.125	0.994	1.230	2.235	2.118
657	1.140	1.710	1.710	1.520	1.380	1.140	2.235	0.693	1.339	0.657	1.060	2.208	2.265
Mean ± SE	1.222 ± 0.07	1.610 ± 0.22	1.465 ± 0.17	1.608 ± 0.37	1.000 ± 0.13	1.503 ± 0.25	1.888 ± 0.09	1.005 ± 0.14	1.077 ± 0.10	1.003 ± 0.13	1.512 ± 0.14	2.395 ± 0.12	2.257 ± 0.04

Table 10. The antibody titres to FMDV Type C in group II

Age of calf in month	4th	5th	6th	7th	8th	9th	10th	11th	12th	13th	14th	15th	16th
Month of sample collection / Ani. No	0	1	2	3	4	5	6	7	8	9	10	11	12
C002	1.190	1.770	1.570	1.520	1.670	2.359	2.659	1.522	1.563	2.363	1.600	2.709	2.475
C009	0.600	1.800	1.520	1.600	1.370	2.363	2.765	1.046	1.061	1.132	1.690	2.800	2.800
C024	1.220	1.740	1.600	1.660	1.450	2.042	2.378	1.081	1.536	1.356	1.100	2.286	2.210
C023	1.380	1.800	1.830	1.590	1.400	0.910	2.404	0.893	1.227	0.604	1.650	2.772	2.800
658	1.410	1.660	1.470	1.500	0.600	0.600	2.036	1.354	0.604	0.949	0.600	2.337	2.310
663	1.290	1.740	2.630	0.600	0.600	0.600	2.360	0.604	0.971	1.147	1.400	2.122	2.531
Mean ± SE	1.182 ± 0.12	1.752 ± 0.02	1.770 ± 0.17	1.412 ± 0.16	1.182 ± 0.18	1.477 ± 0.35	2.428 ± 0.10	1.08 ± 0.13	1.157 ± 0.15	1.253 ± 0.24	1.340 ± 0.17	2.500 ± 0.12	2.520 ± 0.10

Table 11. The antibody titres to FMDV Type C in group III

Age of calf in month	4th	5th	6th	7th	8th	9th	10th	11th	12th	13th	14th	15th	16th
Month of sample collection / Ani. No	0	1	2	3	4	5	6	7	8	9	10	11	12
C032	1.530	1.640	1.660	0.600	0.600	2.503	2.435	1.257	1.120	1.093	0.600	2.238	2.476
C033	1.350	1.590	1.280	0.600	0.600	2.093	2.268	1.123	1.027	1.132	1.780	2.800	2.800
C035	1.520	0.600	1.830	1.670	1.730	1.620	2.800	1.500	1.349	1.435	1.510	2.208	2.800
C036	0.600	0.600	1.770	1.260	0.600	0.600	2.800	1.743	1.284	1.067	1.390	2.729	2.524
670	0.600	1.660	1.550	1.420	1.670	0.600	2.316	1.054	1.495	0.777	1.280	1.122	1.971
669	0.600	0.600	0.600	1.790	1.000	1.770	2.407	1.174	1.639	1.067	2.460	2.800	2.800
Mean ± SE	1.033 ± 0.20	1.115 ± 0.23	1.448 ± 0.18	1.223 ± 0.21	1.033 ± 0.22	1.532 ± 0.32	2.500 ± 0.09	1.305 ± 0.10	1.313 ± 0.09	1.090 ± 0.09	1.503 ± 0.25	2.312 ± 0.27	2.56 ± 0.13

Table 12. Comparison of the mean FMDV type C (Mean ± SE) antibody titres of three groups

Age of calf in month	4th	5th	6th	7th	8th	9th	10th	11th	12th	13th	14th	15th	16th
Month of sample collection / Groups	0	1	2	3	4	5	6	7	8	9	10	11	12
Group I	1.222 ± 0.07	1.610 ± 0.22	1.465 ± 0.17	1.608 ± 0.37	1.000 ± 0.13	1.503 ± 0.25	1.888 ± 0.09^{A}	1.005 ± 0.14	1.077 ± 0.10	1.003 ± 0.13	1.512 ± 0.14	2.395 ± 0.12	2.257 ± 0.04
Group II	1.182 ± 0.12	1.752 ± 0.02	1.77 ± 0.17	1.412 ± 0.16	1.182 ± 0.18	1.477 ± 0.35	2.428 ± 0.10^{B}	1.080 ± 0.13	1.157 ± 0.15	1.253 ± 0.24	1.340 ± 0.17	2.500 ± 0.12	2.520 ± 0.10
Group III	1.033 ± 0.20	1.115 ± 0.23	1.448 ± 0.18	1.223 ± 0.21	1.033 ± 0.22	1.532 ± 0.32	2.500 ± 0.09^{B}	1.305 ± 0.10	1.313 ± 0.09	1.090 ± 0.09	1.503 ± 0.25	2.312 ± 0.27	2.560 ± 0.13
Cd	NS	NS	NS	NS	NS	NS	0.298**	NS	NS	NS	NS	NS	NS

Values in same column bearing same superscript do not differ significantly
NS – No significant difference between the groups
** Significant at 1% level

Table 13. The antibody titres to FMDV Type Asia-1 in group I

Age of calf in month	4th	5th	6th	7th	8th	9th	10th	11th	12th	13th	14th	15th	16th
Month of sample collection / Ani. No	0	1	2	3	4	5	6	7	8	9	10	11	12
C004	0.600	2.278	1.848	2.217	2.386	1.663	1.598	1.810	1.536	1.046	2.325	1.872	1.961
C008	0.600	2.104	2.084	1.805	2.700	2.461	1.701	1.971	1.555	1.584	2.086	1.721	2.700
C010	0.600	1.733	1.491	1.833	2.415	1.704	1.539	0.731	1.474	1.616	1.726	2.625	2.700
C021	0.600	1.959	1.540	2.086	1.995	1.730	1.235	1.675	0.645	1.431	1.966	2.082	2.216
655	0.600	2.456	2.700	1.930	2.104	1.429	1.591	1.923	1.941	1.512	1.899	1.653	1.961
657	0.600	1.808	1.398	2.043	2.184	1.921	1.660	1.526	1.150	1.382	1.806	2.400	1.954
Mean ± SE	0.600 ± 0.01	2.056 ± 0.11	1.843 ± 0.20	1.985 ± 0.06	2.297 ± 0.10	1.818 ± 0.14	1.554 ± 0.06	1.606 ± 0.18	1.383 ± 0.17	1.428 ± 0.08	1.968 ± 0.08	2.058 ± 0.15	2.248 ± 0.14

Table 14. The antibody titres to FMDV Type Asia-1 in group II

Age of calf in month	4th	5th	6th	7th	8th	9th	10th	11th	12th	13th	14th	15th	16th
Month of sample collection / Ani. No	0	1	2	3	4	5	6	7	8	9	10	11	12
C002	0.600	1.837	1.974	1.913	1.616	0.750	1.772	1.356	1.502	0.640	1.818	2.341	1.968
C009	0.600	2.700	1.804	2.324	1.336	1.376	1.975	1.769	1.578	1.065	2.700	2.135	2.326
C024	0.600	1.908	2.700	2.342	2.029	1.664	1.728	1.462	1.626	1.606	1.892	1.968	2.385
C023	0.600	1.764	1.962	2.700	1.898	1.565	2.700	0.750	2.700	1.550	1.778	2.700	2.700
658	0.600	1.819	2.036	2.377	1.829	1.208	1.524	1.488	1.510	0.899	2.272	2.700	2.402
663	0.600	2.074	1.968	1.972	1.668	1.559	1.609	1.439	1.480	2.274	2.033	2.207	1.988
Mean ± SE	0.600 ± 0.01	2.017 ± 0.14	2.074 ± 0.12	2.271 ± 0.11	1.729 ± 0.09	1.353 ± 0.13	1.884 ± 0.17	1.377 ± 0.13	1.732 ± 0.19	1.339 ± 0.24	2.082 ± 0.14	2.341 ± 0.12	2.294 ± 0.11

Table 15. The antibody titres to FMDV Type Asia-1 in group III

Age of calf in month	4th	5th	6th	7th	8th	9th	10th	11th	12th	13th	14th	15th	16th
Month of sample collection / Ani. No	0	1	2	3	4	5	6	7	8	9	10	11	12
C032	0.600	2.017	1.852	2.119	1.789	1.618	1.639	1.084	1.478	1.276	1.712	2.006	2.105
C033	0.600	2.226	1.073	2.170	1.873	1.736	1.637	1.866	0.600	1.358	1.968	2.035	2.175
C035	0.600	2.063	1.971	1.970	2.281	1.979	1.378	1.511	1.864	1.131	1.928	1.654	1.727
C036	0.600	2.700	2.137	1.974	1.958	1.478	1.682	1.571	1.155	0.835	1.808	2.130	2.152
670	0.600	2.268	2.010	1.956	0.896	2.700	1.736	1.639	0.728	1.564	1.801	2.314	2.367
669	0.600	1.830	2.700	2.328	1.258	1.512	0.600	2.700	0.914	1.213	2.700	2.117	1.805
Mean ± SE	0.600 ± 0.01	2.184 ± 0.12	1.957 ± 0.21	2.086 ± 0.06	1.675 ± 0.20	1.837 ± 0.18	1.445 ± 0.17	1.728 ± 0.22	1.123 ± 0.19	1.229 ± 0.09	1.986 ± 0.14	2.042 ± 0.08	2.055 ± 0.09

Table 16. Comparison of the mean FMDV type Asia-1 (Mean ± SE) antibody titres of three groups

Age of calf in month	4th	5th	6th	7th	8th	9th	10th	11th	12th	13th	14th	15th	16th
Month of sample collection / Groups	0	1	2	3	4	5	6	7	8	9	10	11	12
Group I	0.600 ± 0.01	2.056 ± 0.11	1.843 ± 0.20	1.985 ± 0.06	2.297 ± 0.10^{A}	1.818 ± 0.14	1.554 ± 0.06	1.606 ± 0.18	1.383 ± 0.17	1.428 ± 0.08	1.968 ± 0.08	2.058 ± 0.15	2.248 ± 0.14
Group II	0.060 ± 0.01	2.017 ± 0.14	2.074 ± 0.12	2.271 ± 0.11	1.729 ± 0.09^{B}	1.353 ± 0.13	1.884 ± 0.17	1.377 ± 0.13	1.732 ± 0.19	1.339 ± 0.24	2.082 ± 0.14	2.341 ± 0.12	2.294 ± 0.11
Group III	0.600 ± 0.01	2.184 ± 0.12	1.957 ± 0.21	2.086 ± 0.06	1.675 ± 0.20^{B}	1.837 ± 0.18	1.445 ± 0.17	1.728 ± 0.22	1.123 ± 0.19	1.229 ± 0.09	1.986 ± 0.14	2.042 ± 0.08	2.055 ± 0.09
Cd	NS	NS	NS	NS	0.4384*	NS	NS	NS	NS	NS	NS	NS	NS

Values in same column bearing same superscript do not differ significantly
NS – No significant difference between the groups
* Significant at 5% level

Table 17. The antibody titres to Pasteurella multocida antigen in group I(Units /ml)

Age of calf in month	4th	5th	6th	7th	8th	9th	10th	11th	12th	13th	14th	15th	16th
Month of sample collection / Ani. No	0	1	2	3	4	5	6	7	8	9	10	11	12
C004	25.9	112.6	123.7	112.6	86.2	160.6	150.7	87.1	122.8	76.8	118.7	84.3	111.5
C008	31.0	53.5	73.8	129.7	72.5	80.0	136.8	127.0	82.3	78.2	71.2	104.6	138.6
C010	5.3	79.0	126.3	94.2	49.4	111.1	100.0	67.9	88.9	71.0	106.2	104.3	135.7
C021	31.0	73.6	134.2	40.9	143.0	246.6	222.5	149.3	109.8	144.1	129.4	113.0	100.8
655	14.8	68.5	83.6	69.9	64.7	67.9	68.8	41.0	53.5	124.2	62.6	11.4	66.7
657	29.5	116.7	171.2	171.1	129.7	148.2	91.1	136.2	136.5	108.3	104.9	92.6	90.5
Mean ± SE	22.9 ± 4.0	83.9 ± 8.1	118.8 ± 14.0	103.1 ± 18.7	90.9 ± 15.1	135.7 ± 26.5	128.3 ± 22.4	95.7 ± 11.8	99.8 ± 12.0	100.4 ± 12.0	98.8 ± 10.0	85.0 ± 15.0	107.3 ± 11.0

Table 18. The antibody titres to Pasteurella multocida antigen in group II(Units /ml)

Age of calf in month	4th	5th	6th	7th	8th	9th	10th	11th	12th	13th	14th	15th	16th
Month of sample collection / Ani. No	0	1	2	3	4	5	6	7	8	9	10	11	12
C002	-	-	68.2	118.8	94.0	56.9	108.8	111.9	83.4	96.2	110.7	109.6	124.7
C009	-	-	57.4	146.8	101.5	122.2	105.5	132.2	91.1	203.1	210.8	179.5	194.2
C024	-	-	29.6	94.0	93.8	79.5	123.7	57.6	63.0	180.7	167.7	144.2	109.0
C023	-	-	66.7	41.6	90.8	86.8	73.9	71.6	81.2	155.3	151.4	92.2	110.0
658	-	-	43.4	67.4	34.7	105.0	40.7	83.1	234.8	121.6	102.7	179.9	89.3
663	-	-	114.1	98.7	48.2	185.1	122.3	117.9	107.8	155.6	223.3	197.1	164.0
Mean ± SE	-	-	63.2 ± 11.8	94.6 ± 12.2	77.2 ± 11.4	105.9 ± 18.3	95.8 ± 13.0	103.9 ± 15.5	110.2 ± 26.0	152.1 ± 16.0	161.4 ± 20.0	150.4 ± 17.0	131.9 ± 16.0

Table 19. The antibody titres to Pasteurella multocida antigen in group III (Units /ml)

Age of calf in month	4th	5th	6th	7th	8th	9th	10th	11th	12th	13th	14th	15th	16th
Month of sample collection / Ani. No	0	1	2	3	4	5	6	7	8	9	10	11	12
C032	-	42.1	35.0	40.8	54.0	191.0	141.8	111.6	95.8	185.5	183.2	156.0	110.7
C033	-	33.9	30.6	82.8	81.6	177.4	186.2	163.4	143.8	209.5	182.0	143.9	172.4
C035	-	5.1	19.3	133.9	114.0	74.5	81.8	90.5	87.9	140.3	187.2	134.6	123.3
C036	-	40.0	27.5	122.8	137.7	145.2	132.3	99.2	88.3	172.6	221.9	141.3	149.9
670	-	69.0	80.1	180.9	113.4	120.3	131.9	111.2	178.1	187.4	256.9	101.0	141.9
669	-	29.5	92.4	100.7	174.4	132.3	43.9	47.4	171.7	150.9	118.9	168.4	131.8
Mean ± SE	-	36.6 ± 8.0	47.5 ± 12.6	110.3 ± 19.5	112.5 ± 17.1	140.1 ± 17.1	119.6 ± 20.4	101.4 ± 17.5	127.6 ± 17.0	174.4 ± 10.0	191.7 ± 19.0	140.9 ± 9.0	135.3 ± 10.0

Table 20 Comparison of the *Pasteurella multocida* (Mean ± SE) antibody titres of three groups

Age of calf in month	4th	5th	6th	7th	8th	9th	10th	11th	12th	13th	14th	15th	16th
Month of sample collection / Groups	0	1	2	3	4	5	6	7	8	9	10	11	12
Group I	22.9 ± 4.0	83.9 ± 8.1^{A}	118.8 ± 14.0^{A}	103.1 ± 18.7	90.9 ± 15.1	135.7 ± 26.5	128.3 ± 22.4	95.7 ± 11.8	99.8 ± 12.0	100.4 ± 12.0^{A}	98.8 ± 10.0^{A}	85.0 ± 15.0^{A}	107.3 ± 11.0
Group II	-	-	63.2 ± 11.8^{B}	94.6 ± 12.2	77.2 ± 11.4	105.9 ± 18.3	95.8 ± 13.0	103.9 ± 15.5	110.2 ± 26.0	152.1 ± 16.0^{B}	161.4 ± 20.0^{B}	150.4 ± 17.0^{B}	131.9 ± 16.0
Group III	-	36.6 ± 8.0^{B}	47.5 ± 12.6^{B}	110.3 ± 19.5	112.5 ± 17.1	140.1 ± 17.1	119.6 ± 20.4	101.4 ± 17.5	127.6 ± 17.0	174.4 ± 10.0^{B}	191.7 ± 19.0^{B}	140.9 ± 9.0^{B}	135.3 ± 10.0
Cd	-	-	39.29**	NS	NS	NS	NS	NS	NS	39.11**	51.7**	43.3*	NS

Values in same column bearing same superscript do not differ significantly
NS – No significant difference between the groups
* Significant at 5% level
** Significant at 1% level

Table 21. The antibody titres to Clostridium chauvoei antigen in group I (Units /ml)

Age of calf in month	4th	5th	6th	7th	8th	9th	10th	11th	12th	13th	14th	15th	16th
Month of sample collection / Ani. No	0	1	2	3	4	5	6	7	8	9	10	11	12
C004	10.2	50.8	54.7	66.0	41.9	71.1	72.6	41.4	119.4	47.8	129.6	66.6	60.6
C008	11.3	22.6	32.2	77.3	65.2	45.2	57.0	20.0	73.1	70.2	228.9	166.6	106.6
C010	13.1	30.6	57.5	43.5	54.2	42.8	50.4	30.1	95.1	57.9	198.2	160.7	154.5
C021	9.3	43.8	49.5	44.1	120.4	113	136.4	112.2	115.2	112.0	137.3	87.8	49.0
655	5.4	0.8	14.5	20.8	28.3	31.0	31.4	40.6	32.3	88.6	103.7	51.7	47.7
657	16.1	55.3	59.3	129.8	51.0	66.3	46.5	97.3	196.3	86.4	138.6	99.1	67.6
Mean ± SE	10.9 ± 1.0	34.0 ± 8.1	44.6 ± 7.3	63.6 ± 15.5	60.2 ± 13.0	61.6 ± 11.8	65.7 ± 15.1	65.6 ± 13.8	105.2 ± 22.0	77.2 ± 9.0	156.1 ± 19.0	105.4 ± 20.0	81.0 ± 17.0

Table 22. The antibody titres to Clostridium chauvoei antigen in group II(Units /ml)

Age of calf in month	4th	5th	6th	7th	8th	9th	10th	11th	12th	13th	14th	15th	16th
Month of sample collection / Ani. No	0	1	2	3	4	5	6	7	8	9	10	11	12
C002	-	-	32.0	86.5	67.7	58.8	66.7	62.4	122.5	155.6	215.9	88.0	113.6
C009	-	-	31.4	95.5	44.9	94.1	69.0	104.5	101.4	169.2	142.3	90.9	122.1
C024	-	-	10.4	66.4	36.4	37.6	53.0	37.4	56.0	110.8	70.4	46.4	57.8
C023	-	-	43.1	88.6	84.6	55.8	43.6	61.6	121.1	190.5	140.4	62.4	83.3
658	-	-	14.4	72.0	37.1	41.4	33.5	46.5	205.3	174.7	143.8	180.1	88.6
663	-	-	40.0	74.6	96.1	130.4	86.9	106.4	117.7	172.2	187.7	131.1	116.4
Mean ± SE	-	-	28.5 ± 5.3	80.6 ± 4.4	61.1 ± 10.6	69.7 ± 14.6	58.8 ± 7.7	69.8 ± 11.8	119.2 ± 20.0	162.2 ± 11.0	150.1 ± 20.0	99.8 ± 20.0	97.0 ± 10.0

Table 23. The antibody titres to Clostridium chauvoei antigen in group III (Units /ml)

Age of calf in month	4th	5th	6th	7th	8th	9th	10th	11th	12th	13th	14th	15th	16th
Month of sample collection / Ani. No	0	1	2	3	4	5	6	7	8	9	10	11	12
C032	-	-	21.6	33.6	49.3	168.8	75.9	78.7	174.9	173.2	176.9	110.5	97.4
C033	-	-	26.7	24.0	64.1	62.2	91.3	59.1	113.6	187.5	167.3	99.1	58.1
C035	-	-	13.7	37.9	50.1	30.8	45.1	58.7	84.9	165.7	2.1	98.9	145.2
C036	-	-	12.7	41.9	50.0	51.1	53.1	61.7	76.5	192.7	1.7	143.3	131.4
670	-	-	18.7	125.0	50.1	74.5	92.4	81.2	18.6	166.7	216.1	98.2	58.0
669	-	-	21.1	27.7	124.0	63.7	30.0	42.4	127.2	173.1	108.3	90.9	27.2
Mean ± SE	-	-	19.1 ± 2.0	48.4 ± 15.5	64.6 ± 12.2	75.2 ± 19.5	64.6 ± 10.6	63.6 ± 5.7	132.6 ± 22.0	176.5 ± 4.0	174.8 ± 16.0	106.8 ± 7.0	86.2 ± 19.0

Table 24. Comparison of the *Clostridium chauvoei* (Mean ± SE) antibody titres of three groups

Age of calf in month	4th	5th	6th	7th	8th	9th	10th	11th	12th	13th	14th	15th	16th
Month of sample collection / Groups	0	1	2	3	4	5	6	7	8	9	10	11	12
Group I	10.9 ± 1.0	34.0 ± 8.1	44.6 ± 7.3^{A}	63.6 ± 15.5	60.2 ± 13.0	61.6 ± 11.8	65.7 ± 15.1	65.6 ± 13.8	105.2 ± 22.0	77.2 ± 9.0^{A}	156.1 ± 19.0	105.4 ± 20.0	81.0 ± 17.0
Group II	-	-	28.5 ± 5.3^{B}	80.6 ± 4.4	61.1 ± 10.6	69.7 ± 14.6	58.8 ± 7.7	69.8 ± 11.8	119.2 ± 20.0	162.2 ± 11.0^{B}	150.1 ± 20.0	99.8 ± 20.0	97.0 ± 10.0
Group III	-	-	19.1 ± 2.0^{B}	48.4 ± 15.5	64.6 ± 12.2	75.2 ± 19.5	64.6 ± 10.6	63.6 ± 5.7	132.6 ± 22.0	176.5 ± 4.0^{B}	174.8 ± 16.0	106.8 ± 7.0	86.2 ± 19.0
Cd	-	-	16.04*	NS	NS	NS	NS	NS	NS	26.6**	NS	NS	NS

Values in same column bearing same superscript do not differ significantly
NS – No significant difference between the groups
* Significant at 5% level
** Significant at 1% level

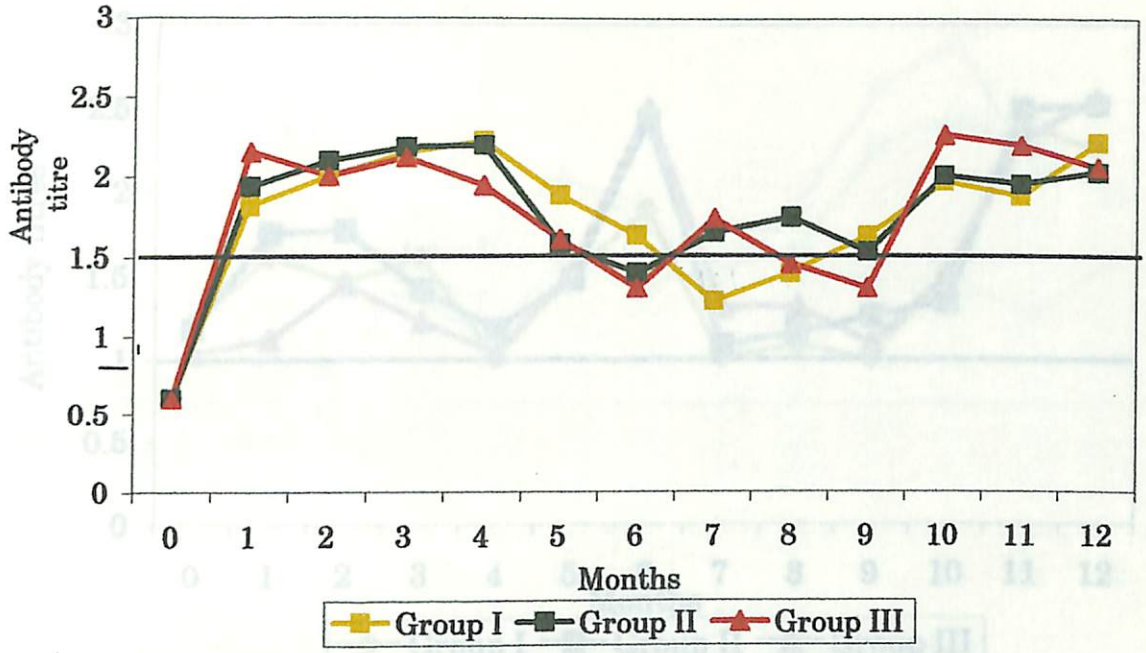

Fig1.Comparison of the mean FMDV type O antibody titres of three groups

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

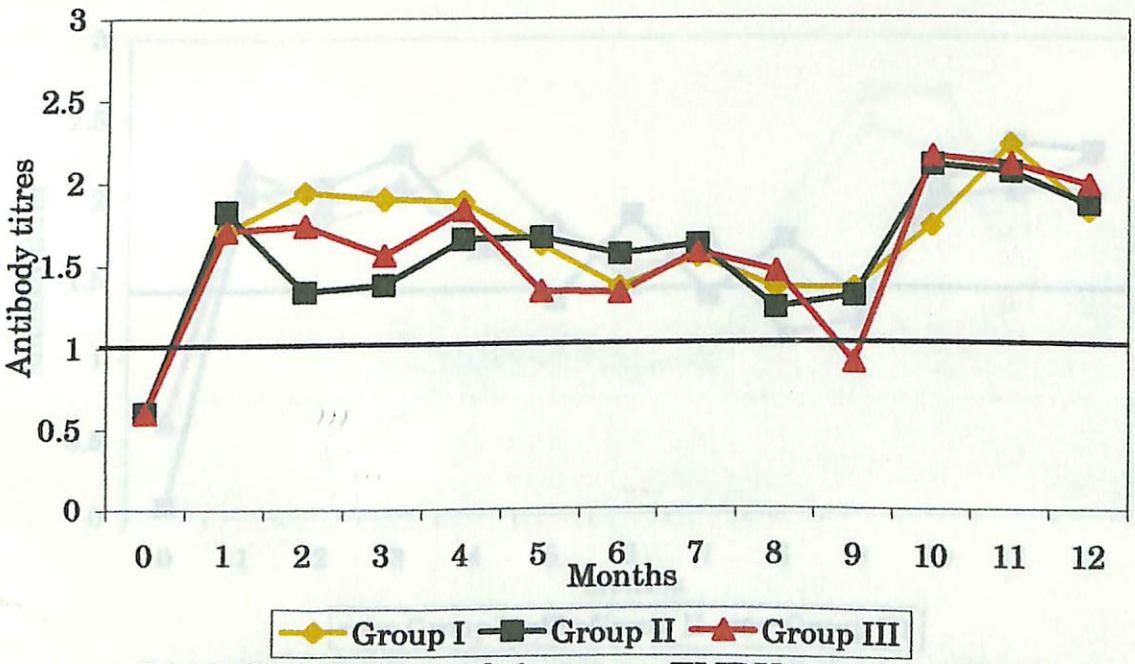

Fig.2 Comparison of the mean FMDV type A antibody titres of three group

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

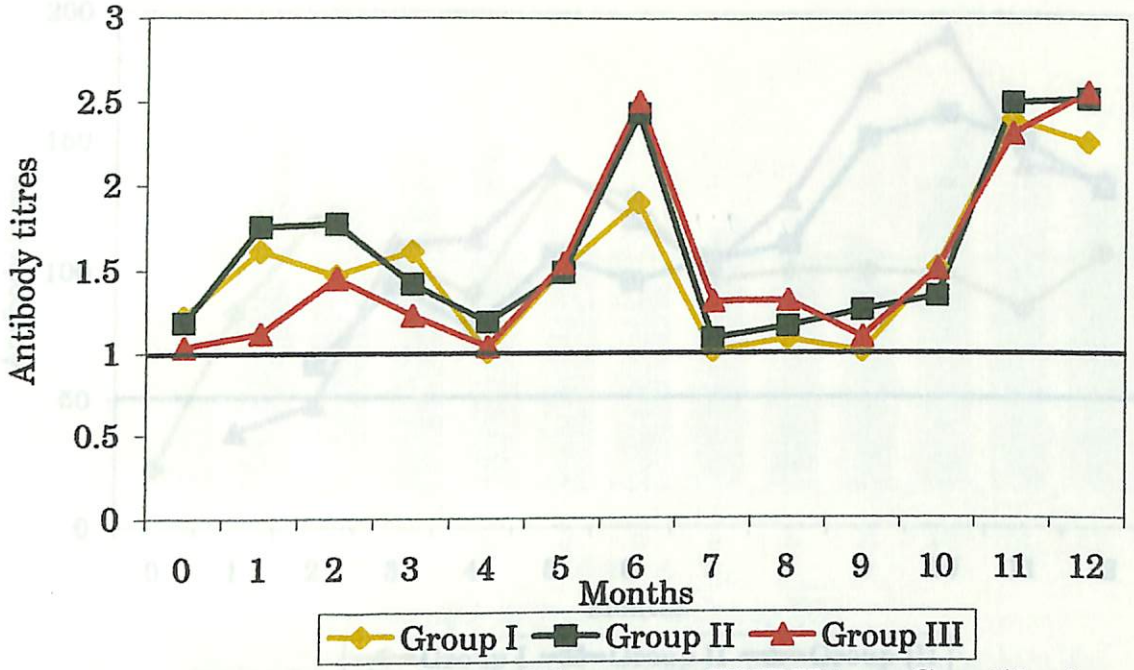

Fig.3 Comparison of the mean FMDV type C antibody titres of three group

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

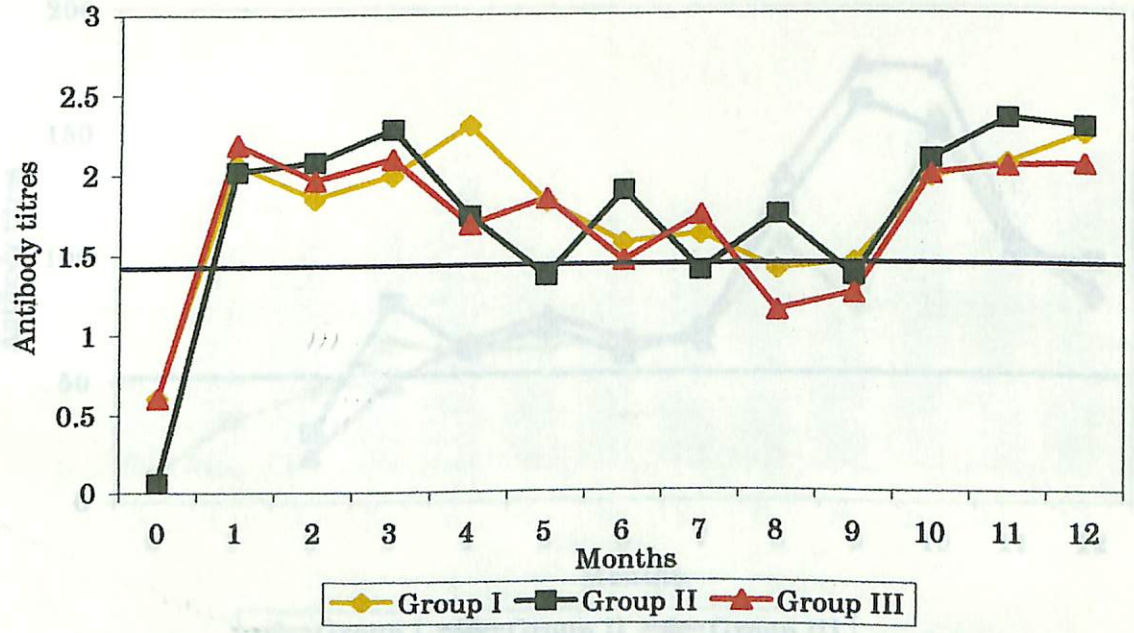

Fig.4 Comparison of the mean FMDV type Asia-1 antibody titres of three group

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

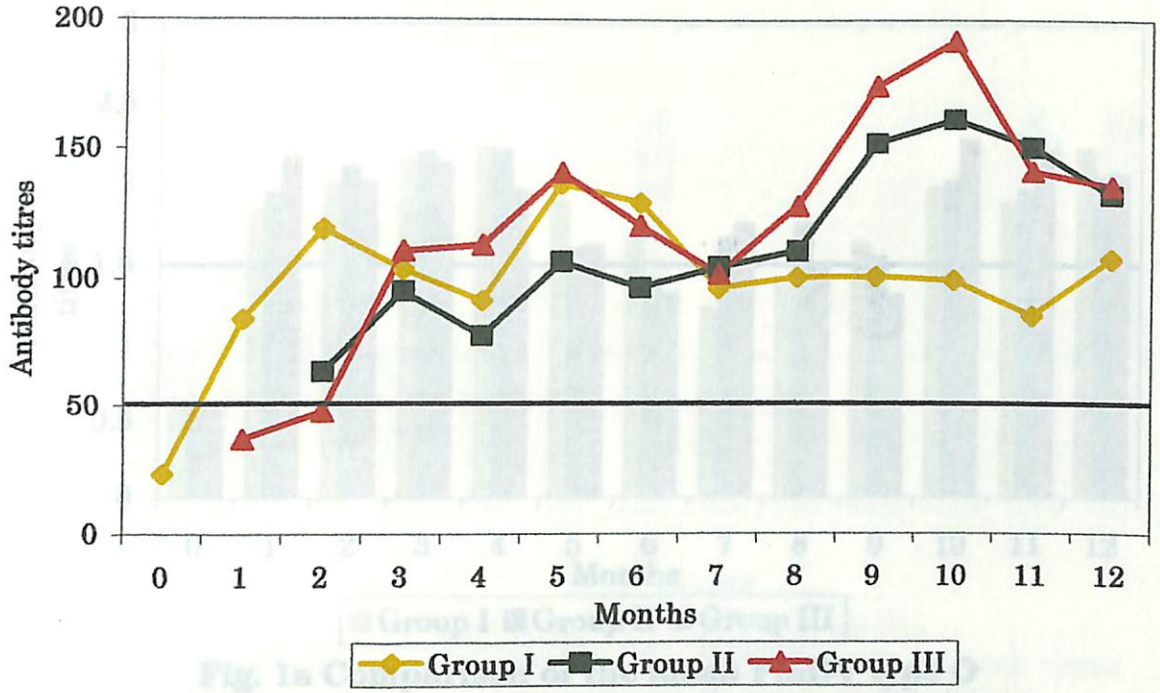

Fig. 5 Comparison of the mean *Pasteurella multicida* antibody titres of three groups

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

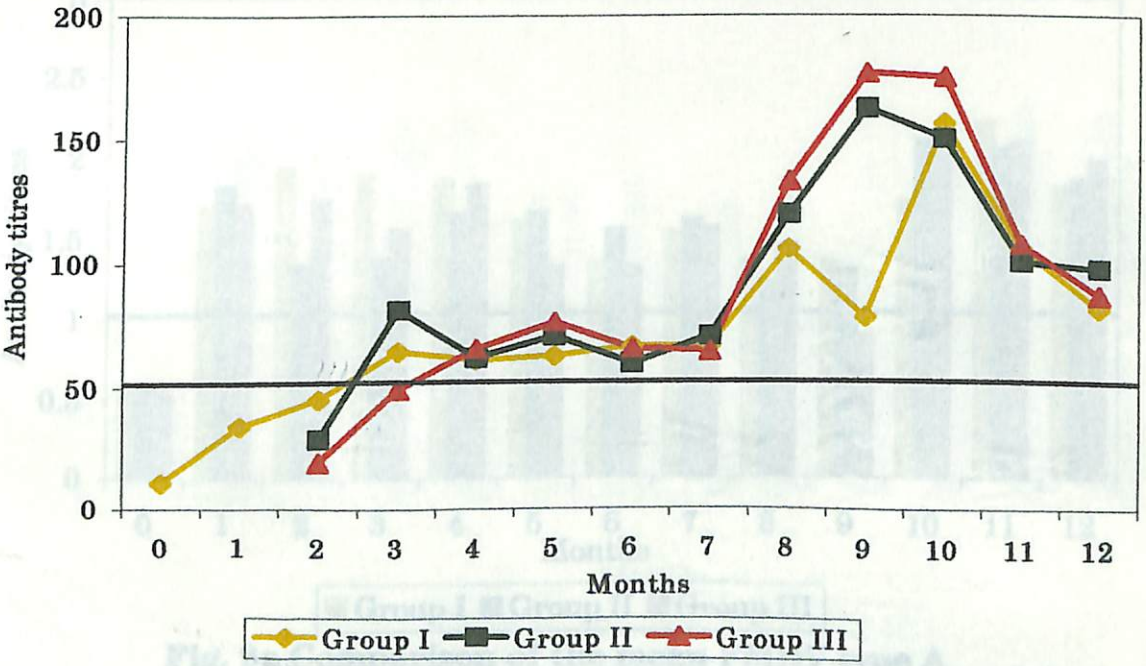

Fig. 6 Comparison of the mean *Clostridium chauvoei* antibody titres of three groups

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

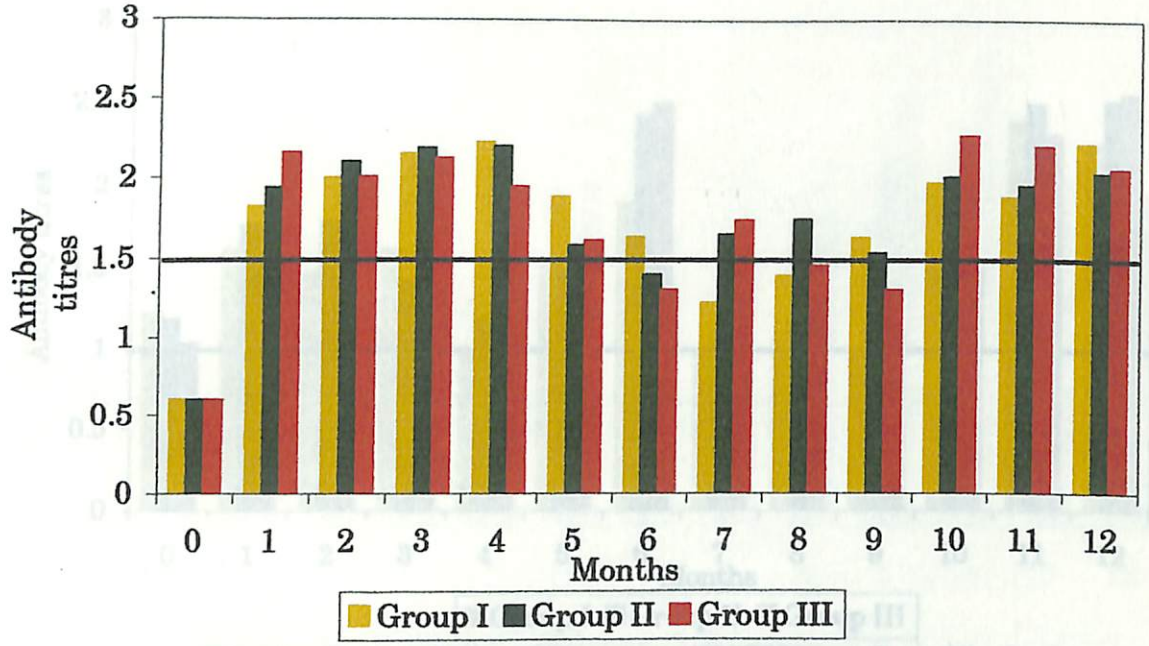

Fig. 1a Comparison of the mean FMDV type O antibody titres of three group

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

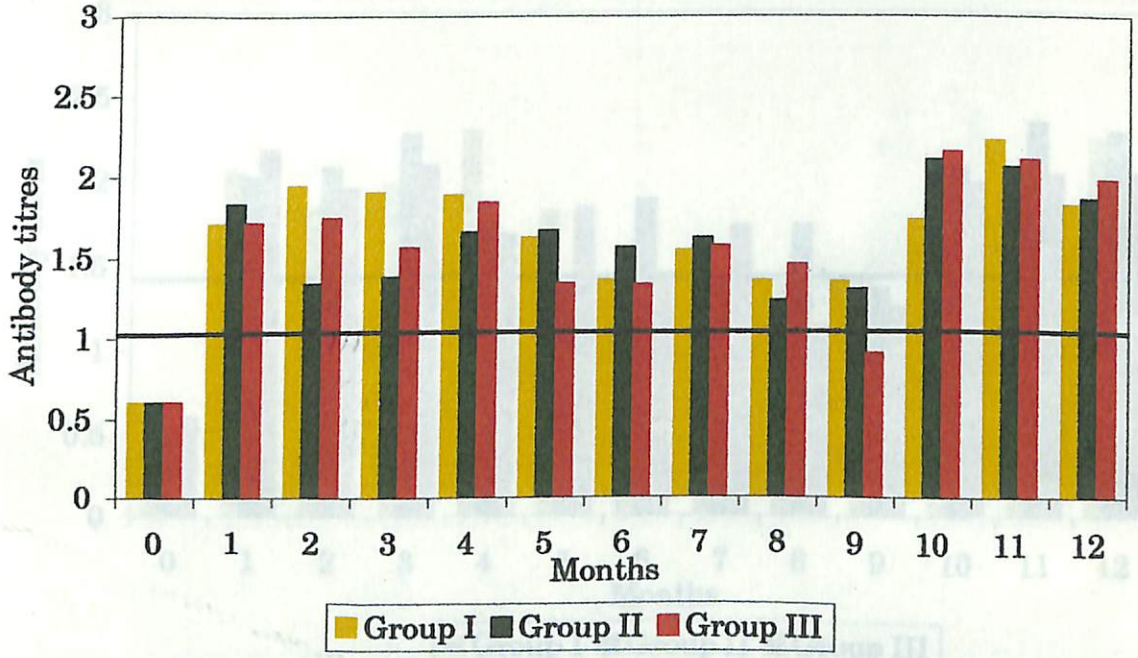

Fig. 2a Comparison of the mean FMDV type A antibody titres of three groups

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

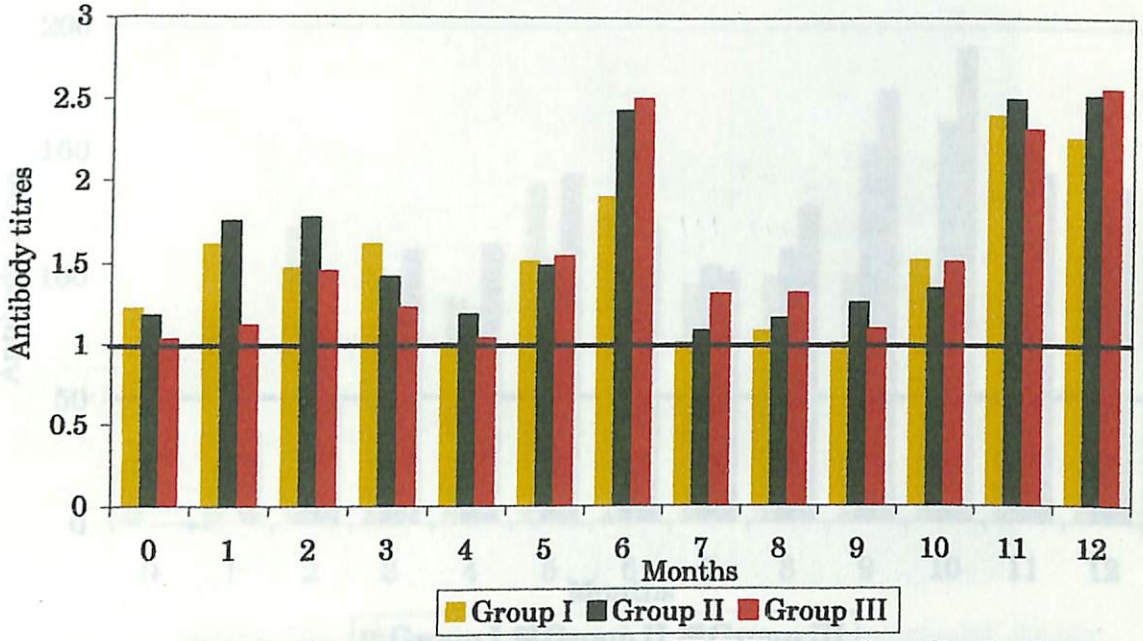

Fig. 3a Comparison of the mean FMDV type C antibody titres of three group

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

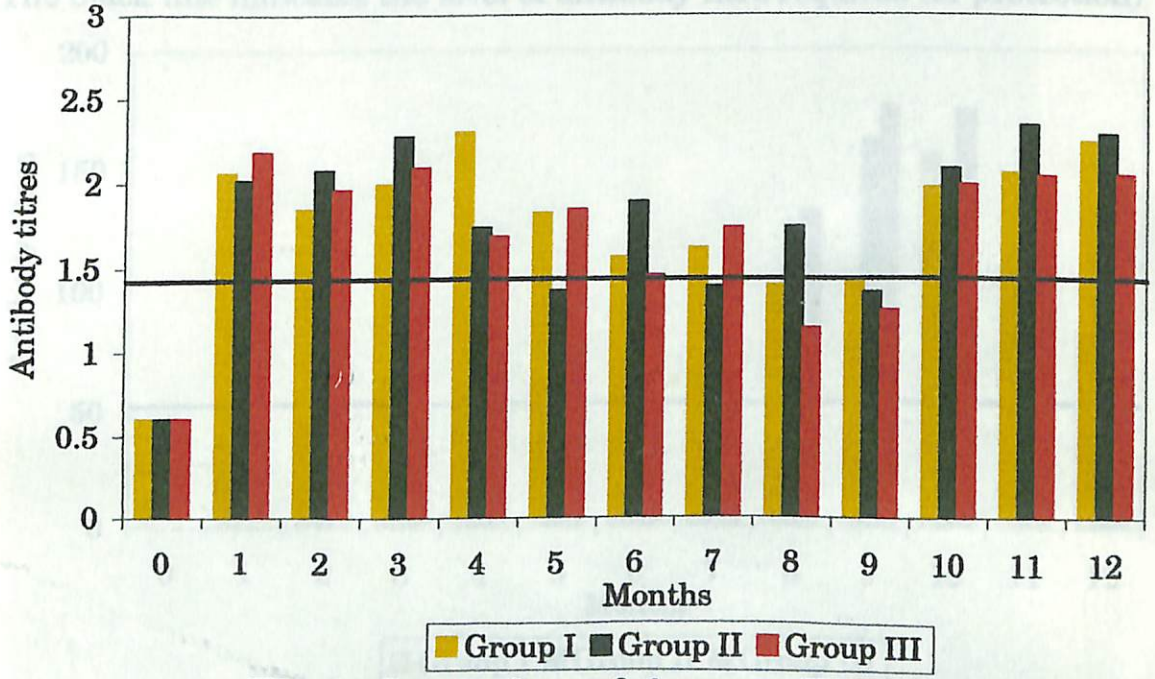

Fig. 4a Comparison of the mean FMDV type Asia-1 antibody titres of three group

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

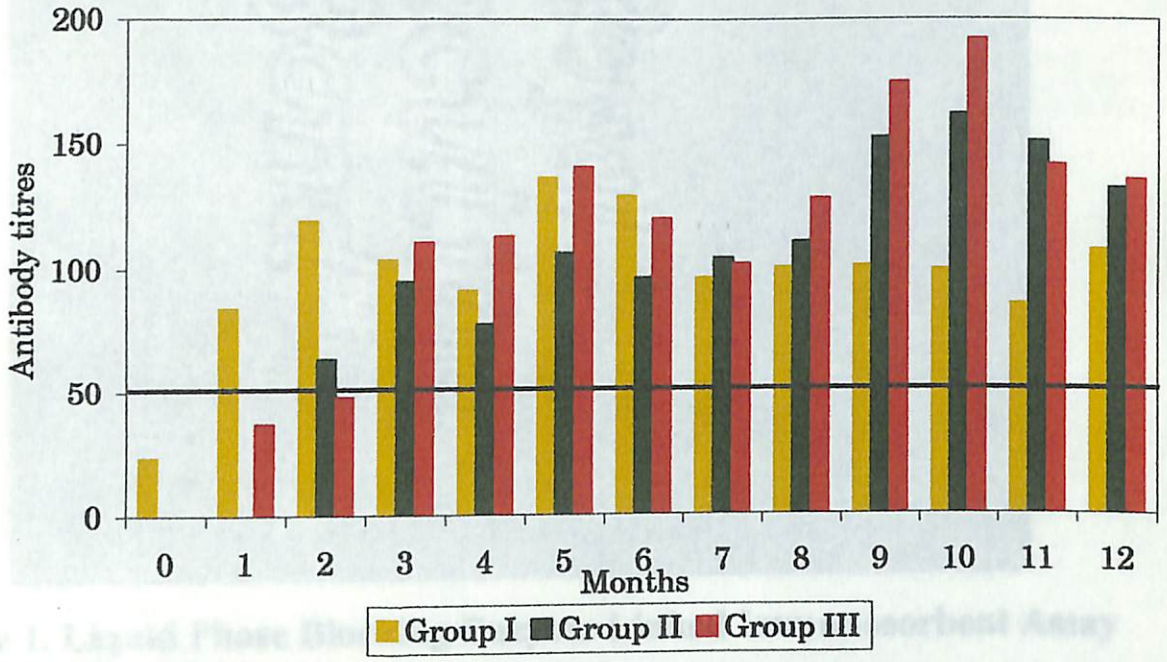

Fig.5a Comparison of the mean *Pasteurella multocida* antibody titres of three groups

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

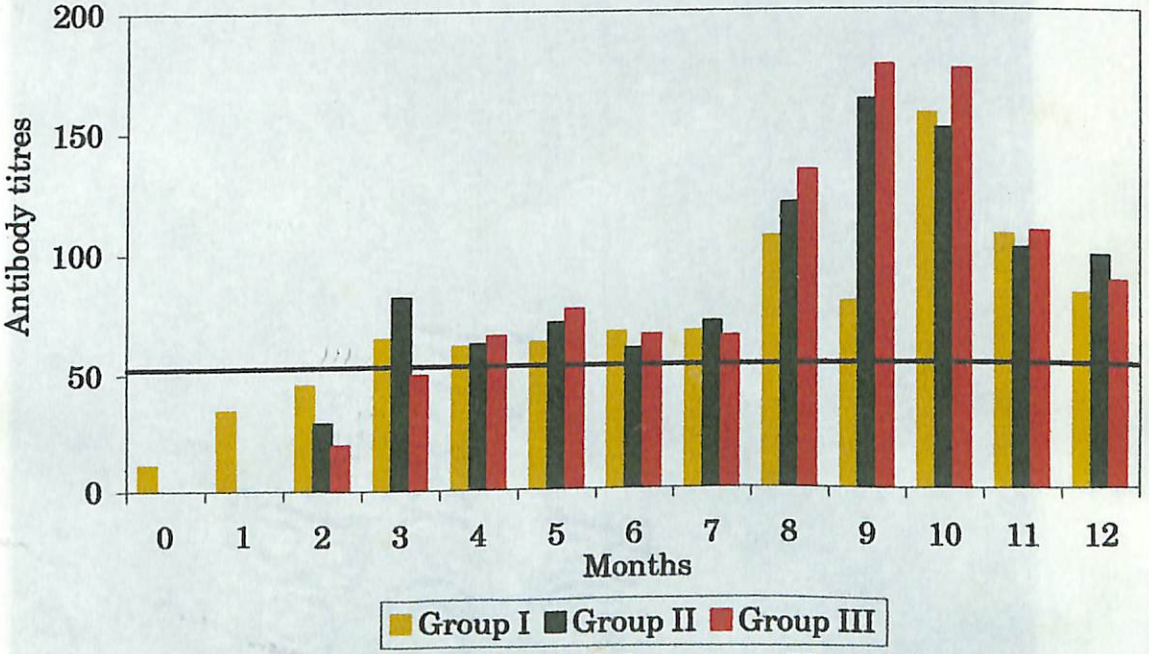

Fig. 6a Comparison of the mean *Clostridium chauvoei* antibody titres of three groups

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

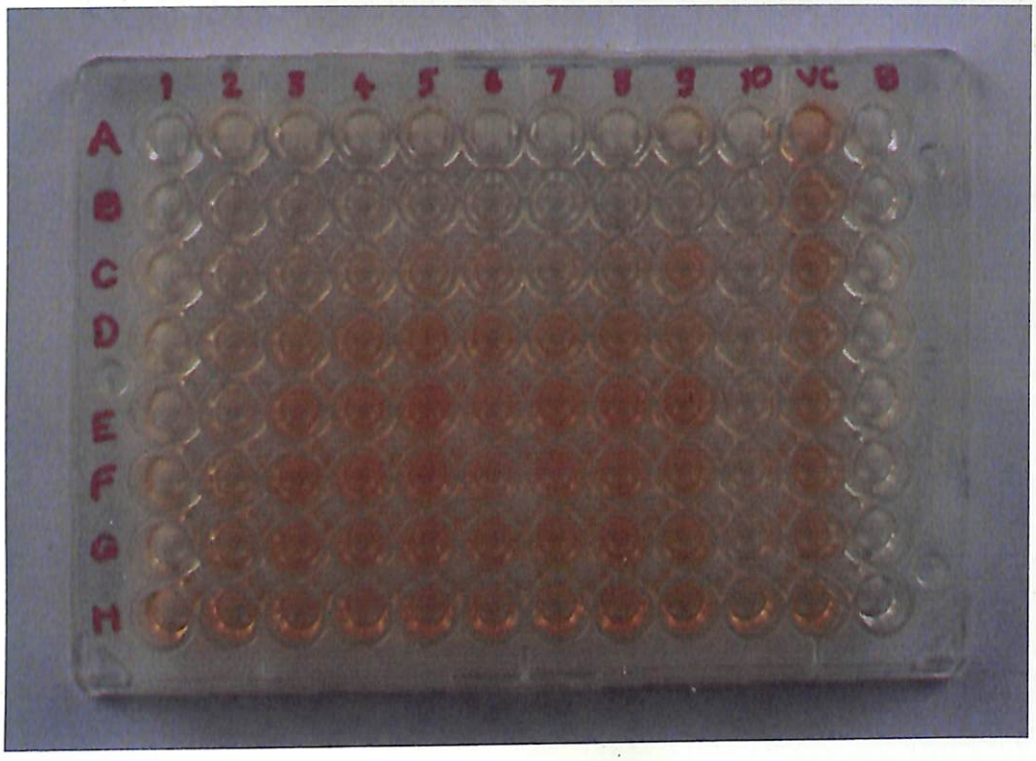

Plate 1. Liquid Phase Blocking Enzyme Linked Immunosorbent Assay (Test Plate)

Columns 1 to 10 - Samples 1 to 10 in two - fold dilution from A to H

Column VC - Virus control **Column B** - Column blank

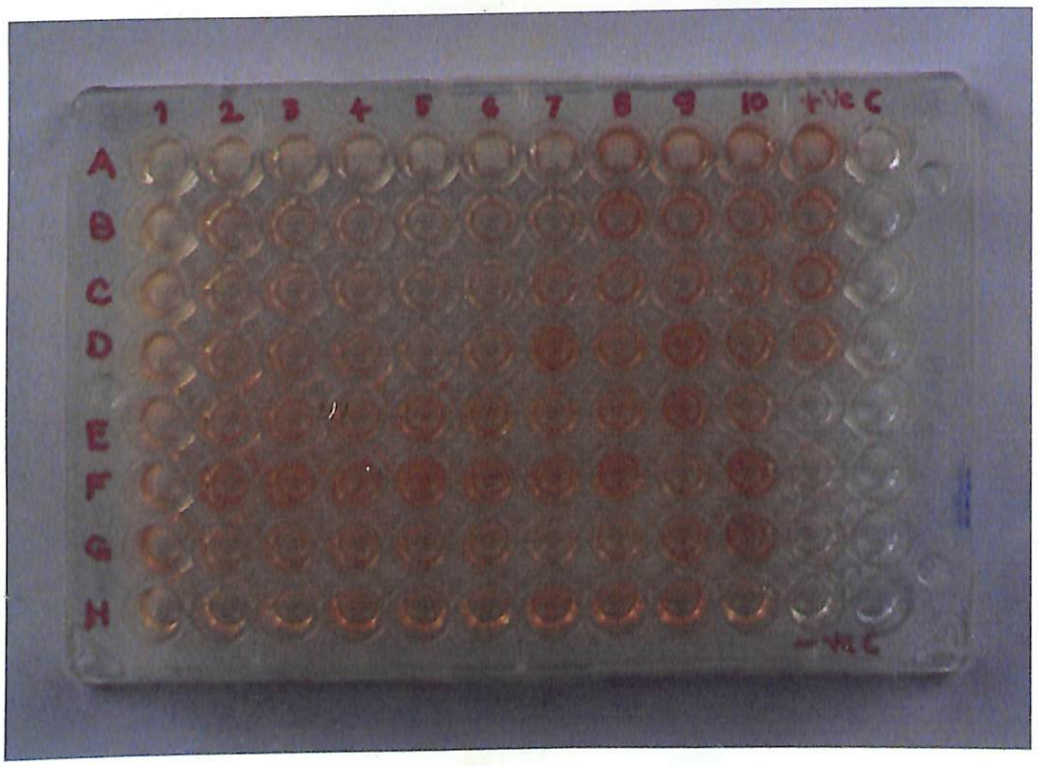

Plate 2. Indirect Enzyme Linked Immunosorbent Assay

Wells A1 to H10 - Samples 1 to 80

Eleventh Column - A11 to D11 - Positive control

E11 to H11 - Negative control

Column C - Column blank

Discussion

5. DISCUSSION

In the present study, immune response to combined Foot and Mouth Disease, Haemorrhagic Septicaema and Black Quarter vaccine was compared with that of their respective individual vaccine in cattle at monthly interval for a period of one year.

5.1 Seroconversion of FMDV type O antigen

For FMDV type 'O' antibody titre of 1.5 and above is taken as protective (Srinivasan, 2001).

5.1.1 Group I

In this study mean antibody titre of Group I animals reached the protective antibody titres within first thirty days. This result was in accordance with the observation made by Rao *et al.* (1993) who reported that after primary vaccination satisfactory antibody responses against type, O, A, C, Asia-1 antigen were recorded on 21st day itself.

Group I animals reached the peak titre four month after primary vaccination and there after it declined. These finding does not agrees with the findings of Srinivas *et al.* (1996) who reported that higher titres were recorded on 21st day after primary vaccination and the titres declined there after.

At 270 days after primary vaccination when the booster vaccination was done, group I animals showed protective titre. This finding agrees with the findings of Rao *et al.* (1993), who reported that serum neutralizing antibody titres in calves administered oil emulsion vaccine remained satisfactory level on 270 post vaccination.

Lower immune response during seventh and eighth month in group I might be due to animal to animal variation in the serum neutralizing antibody response, nutrition and stress, as it was reported by Pay (1991) who stated that the animal to animal 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 is quite large, and also nutrition and stress may play a role in influencing the magnitude of the antibody response to vaccination.

After booster vaccination at ninth month increase in mean antibody titre were recorded. This observation endorses the opinion of Tizard (1998) described that repeated injection of antigen produced immune response with shorter lag period and for a longer period of time than single inoculation.

5.1.2 Group II

The mean antibody titre of group II animals reached protective level within first thirty days. This result was in accordance with the observation made by Rao *et al.* (1993) who reported that after primary vaccination satisfactory antibody responses against type, O, A, C, Asia-1 antigen were recorded on 21st day itself.

Group II animals showed protective titre till 270 days after primary vaccination, these findings agrees with the findings of Rao *et al.* (1993), who reported that serum neutralizing antibody titres in calves administered oil emulsion vaccine remained satisfactory level on 270 post vaccination.

Lower immune response (1.4 ± 0.24) during sixth month in group II may be due to animal to animal variation in

the serum neutralizing antibody responses, nutrition and stress as described by Pay (1991).

Higher antibody titre in group II animal during 10th month is because of the anamnestic response produced by booster vaccination during ninth month as it was reported by Tizard (1998), that repeated injection of antigen produced immune response with shorter lag period and for a longer period of time than single inoculation.

5.1.3 Group III

Group III animals reached highest mean antibody titre within a month after primary vaccination there after it declined. These findings correlate with the findings of Srinivas *et al.* (1996), who reported that serum neutralizing antibody titres in calves administered oil emulsion vaccine remained satisfactory level on 270 days post vaccination.

Group III animals did not have the protective titres during eighth and ninth month after primary vaccination. This findings does not agree with the findings of Rao *et al.* (1993),

according to whom the titre was maintained till the end of ninth month.

Group III animal also showed increase in antibody titre during tenth month after booster vaccination at ninth month.

5.1.4 Comparison of type O FMDV antibody titres between groups

There was no significant difference between the three groups throughout the study period except during first month ($P \leq 0.05$) while there was a significant difference between group I and group III, though during that period all the three group animal showed protective titres. These finding correlates with the observation of Gugiu *et al.* (1989) who opined that the protection against FMD was not reduced by association with anti – Pasteurellosis vaccination. Also Afzal and Muneer (1990) reported that the combined water in oil adjuvanted HS and FMD vaccine induced antibody titres equivalent to oil adjuvant vaccine of HS and FMD when done separately in different group. Also these findings correlate with the findings of Reddy *et al.* (1997), who reported that serological response of cattle to all the three

antigens FMD, HS and BQ either alone or combined together were similar. These finding indicated that FMDV type 'O' antibody titres was same in animals vaccinated with combined vaccine and individual vaccine. Presence of other bacterial component in an FMD vaccine does not influence FMDV type 'O' antibody titres.

5.2 Seroconversion of FMDV type A antigen

For the protection of FMDV type 'A' the titre of one and above is taken as protective (Srinivasan, 2001).

5.2.1 Group I

Mean antibody titres of group I reached protective titres within first thirty days. This agrees with the findings of Rao *et al.* (1993) who reported that after primary vaccination satisfactory antibody responses against type, O, A, C, Asia-1 antigen were recorded on 21st day itself.

Group I animals reached the peak mean antibody titre during second month. This does not aggrees with the findings of

Rao *et al.* (1993) who reported that highest antibody titre was obtained 90 days post vaccination with oil adjuvant vaccine. Throughout the study period group I animal showed the protective titre.

At ninth month the titre was 1.343 ± 0.1, which rose to 1.729 ± 0.1 after one month and the antibody titre doubled (2.224 ± 0.2) to the required protection level after two month after booster vaccination.

5.2.2 Group II

Group II animals reached protective titre within first thirty days after primary vaccination. This agrees with the findings of Rao *et al.* (1993), according to whom the protective titre were reached within 21st day itself.

The peak antibody titre was reached within a month and this findings agrees with the findings of Srinivas *et al.* (1996), who reported that higher titres were recorded on 21st day after primary vaccination and the titres declined there after.

Group II animals showed the protective titre throughout the study period. After booster vaccination at ninth month the antibody titre doubled to required level for protective titre within a month. This observation is in accordance with the observation of Tizard (1998), who described that repeated injection of antigen produces immune response with shorter lag period and for a longer period of time than single inoculation.

5.2.3 Group III

Mean antibody titre of Group III animals reached the protective titre within first thirty days and this agrees with the findings of Rao *et al.* (1993), according to whom the protective titre were reached within 21st day itself.

Group III animals reached the peak mean antibody titre during fourth month and these findings does not agree with the findings of Srinivas *et al.* (1996) who recorded the highest titre in 21st days after primary vaccination.

After primary vaccination Group III animals showed protective titre till eighth month only. At ninth month when the

booster was given the titre was just below the protective titre level (0.889 ± 0.18). After booster vaccination at ninth month increase in mean antibody titre raised to double the protective level within a month. This observation endorses the opinion of Tizard (1998), that repeated injection of antigen produced immune response with shorter lag period and for a longer period of time than single inoculation.

5.2.4 Comparison of type A FMDV antibody titre between groups

There was no significant difference in antibody titres between three groups throughout the study period except during second and third month ($P \leq 0.05$) where there was a significant difference between group I and II, and group I animal showed higher antibody titres than group II animals. All the animals during that period showed protective antibody titres. These findings correlates with the findings of Darie *et al.* (1979) who opined that there was no interference with the immunogenic effect of Clostridial, Anthrax and FMD vaccine. Also Joseph and Hedger (1984) opined that simultaneous administration of FMD and HS inactivated vaccine produced no adverse effect and the serological response did not differ from the response to either

vaccine given separately. Same was of the opinion of Reddy *et al.* (1997) when compared combined FMD, HS and BQ vaccine with their individual vaccine. These findings indicated that FMDV type 'A' antibody titres was same in animals vaccinated with combined vaccine and individual vaccine.

5.3 Seroconversion of FMDV type C antigen

For the protection of FMDV type 'C' the titre of one and above is taken as protective (Srinivasan, 2001).

5.3.1 Group I

Group I animal showed protective FMDV type 'C' antibody titre at the time of primary vaccination. It shows the presence of maternal antibody to FMDV type 'C' even at fourth month of age.

Following primary vaccination in group I animal showed the rise in antibody titre during first month and there was no reduction in the antibody titre due to presence of colostral antibody. These result is in accordance with the observation

made by Sadir *et al.* (1988) who reported that FMDV oil adjuvant vaccine are highly efficient from 30 days on wards even in the presence of colostral antibody. These findings does not agree with the findings made by Reddy *et al.* (1997) and Nicholls *et al.* (1984) who reported low serum antibody titres to FMD virus antigens in calves may be because of persistence of maternally derived antibodies.

Group I animals reached the peak antibody titre six month after primary vaccination and there after it starts declining. This finding does not agree with the findings of Rao *et al.* (1993) who reported that highest antibody titrewas obtained 90 days post vaccination. Group I animal showed protective titre throughout the study period.

After booster vaccination at ninth month increase in antibody titre was observed within a month and the antibody titre doubled the protective level (2.395 ± 0.12) within two month. This observation was in accordance with the observation of Tizard (1998), repeated injection of antigen produced immune response with shorter lag period and for a longer period of time than single inoculation.

5.3.2 Group II

Group II animals also showed maternal antibody at the time of primary vaccination at fourth month of age. After primary vaccination Group II animals showed the rise in antibody titre during first month and there was no reduction in antibody titre due to presence of colostral antibody. This is in accordance with the observation made by Rao *et al.* (1993) who reported that presence of maternal antibody did not influence the serological response in oil adjuvant vaccine.

Group II animals reached the peak antibody titre six months after primary vaccination and there after it started declining. This finding does not agree with the findings of Rao *et al.* (1993), who reported that highest antibody titre was obtained 90 days post vaccination. Throughout the study period Group II animals showed protective antibody titre.

Group II animals also responded positively to booster vaccination as the antibody titre doubled the protective level (2.5 ± .12) within two months after booster vaccination.

5.3.3 Group III

Group III animals also showed maternal antibody at the time of primary vaccination at fourth month of age. After primary vaccination group III animal showed the rise in antibody titre during first month and there was no reduction in the immune response due to presence of colostral antibody. These result in accordance with the observation made by Sadir *et al.* (1988) who reported that FMDV oil adjuvant vaccine are highly efficient from 30 days on ward even in the presence of colostral antibody.

Group III animals reached the peak antibody titre six month after primary vaccination and there after it starts declining. This finding does not agree with the findings of Rao *et al.* (1993) who reported that highest antibody titre was obtained 90 days post vaccination. Throughout the study period group III animal showed protective antibody titre.

After booster vaccination at ninth month increase in antibody titre was observed within a month and the antibody titre doubled the protective level within two month.

5.3.4 Comparison of type C FMDV antibody titre between groups

All the three group did not show any significant differences in mean type 'C' FMD antibody titres throughout the study period, except during sixth month ($P \leq 0.01$) when there was a significant difference between group I and II and group I and III. Group I animal showed lower antibody titre than group II and III though the group I animals showed protective titre well above the protective level.

These findings correlates with the findings of Afzal and Muneer (1990) who reported that the combined water in oil adjuvanted HS and FMD vaccine induced antibody titres equivalent to oil adjuvanted vaccine of HS and FMD when done separately in different group. Also Srinivas *et al.* (1996) reported that simultaneous or singular administration of FMD and RP vaccine induces satisfactory serological response without any adverse effect. Same was of the opinion of Reddy *et al.* (1997) when compared combined FMD, HS and BQ vaccine with their individual vaccine. These finding indicated that FMDV type 'C' antibody titres was same in animals vaccinated with combined vaccine and individual vaccine.

5.4 Seroconversion of FMDV type Asia-1 antigen

For the protection of FMDV type Asia-1 the titre of 1.4 and above is taken as protective (Srinivasan, 2001).

5.4.1 Group I

Mean antibody titres group I animals reached protective titres within first thirty days. In this group animals after primary vaccination peak antibody titre reaches during fourth month only and these does not agree with the findings of Rao *et al.* (1993), who reported that highest antibody titre was obtained 90 days post vaccination. Nair and Sen (1993b) who reported that higher antibody level within 21 and 28 days post vaccination. Till ninth month after the primary vaccination group I animals maintained the protective antibody titre.

After booster vaccination at ninth month increase in antibody titre was observed within a month. This observation was in accordance with the observation of Tizard (1998), who described that repeated injection of antigen produced immune

response with shorter lag period and for a longer period of time than single inoculation.

5.4.2 Group II

Mean antibody titres of groups II reached protective titres within first thirty day. Group II animals reached the peak antibody titre during third month after primary vaccination, these observation endorses Rao *et al.* (1993), who reported that highest antibody titrewas obtained 90 days post vaccination.

Group II animal maintained protective antibody titre till eighth month after primary vaccination. Lower antibody titre during seventh month in group II may be due to animal to animal variation in the serum neutralizing antibody response, nutrition and stress as it was reported by Pay (1991).

After booster vaccination at ninth month increase in antibody titre was observed within a month. And the antibody titre reached the peak after two months. This observation was in accordance with the observation of Tizard (1998).

5.4.3 Group III

Mean antibody titres of groups III reached protective titres within first thirty day. Group III animals reached the peak antibody titre within a month after primary vaccination, these findings agree with the findings of Nair and Sen (1993b), who reported that higher antibody level within 21and 28 days post vaccination. Group III animals maintain the protective antibody titre till seventh month after primary vaccination. The titre during eighth and ninth month was below protective level, and rose to above the protective titre within a month of booster vaccination.

5.4.4 Comparison of type Asia-1 FMDV antibody titres between groups

There were no significant difference in antibody titres between three group except during fourth month ($P \leq 0.05$) when there was a significant difference between group I and II and group I and III, where group I animal showed higher antibody titres than group II and III animals. After primary vaccination and booster vaccination increase in antibody titres was observed in all the three group.

This finding correlates with the observation of Guigu *et al.* (1989) who opined that the protection against FMD was not reduced by association with anti-Pasteurellosis vaccination. Also Joseph and Hedger (1984) opined that simultaneous administration of FMD and HS inactivated vaccine produced no adverse effect and the serological response did not differ from the response to either vaccine given separately. Same was of the opinion of Reddy *et al.* (1997) when compared combined FMD, HS and BQ vaccine with their individual vaccine. These findings indicated that FMDV type Asia-1 antibody titre was same in animals vaccinated with combined vaccine and individual vaccine.

5.5 Seroconversion of *Pasteurella multocida* antibodies

For the protection of *Pasteurella multocida* the titre of 50 and above is taken as protective (Srinivasan, 2001).

5.5.1 Group I

Group I animals attained a protective level of mean *Pasteurella multocida* antibody titres in the first month.

Group I animal showed the protective titre throughout the study period. Group I animal which were vaccinated with oil adjuvant vaccine maintained the protective titre till ninth month after primary vaccination. This result was in accordance with the findings of Zaher *et al.* (1976) who reported that oil adjuvant Pasteurella vaccine conferred a high level of immunity for up a year under field condition, Alwis *et al.* (1978) opined that oil adjuvant HS vaccine gave immunity for six to nine month, also Muneer and Afzal (1989) concluded that oil adjuvant vaccine induced sustained high antibody titres in buffalo calves beyond 230 days after vaccination.

After booster vaccination at ninth month the antibody titre rose to a maximum (107.3 ± 11) after three month.

5.5.2 Group II

Mean antibody titres groups II reached protective titres within first thirty day. Group II animals, which were vaccinated with Aluminium hydroxide gel vaccine maintained protective titre till six month after primary vaccination. This finding agrees with the findings of Cameron and Bester (1986)

who opined that in cattle both alum precipitated and aluminium hydroxide adsorbed vaccine produced good response and there was no advantage gained with oil emulsion vaccine.

Following booster vaccination at 12th month of age the antibody titre increased to a maximum after two month and thereafter it declined. This observation was in accordance with the observation of Tizard (1998), who described that repeated injection of antigen produced immune response with shorter lag period and for a longer period of time than single inoculation.

5.5.3 Group III

Mean antibody titres of groups III reached protective titres within first thirty day. Group III animals, which were vaccinated with Aluminium hydroxide gel vaccine maintained protective titre till six month after primary vaccination. This finding agrees with the findings of Cameron and Bester (1986).

5.5.4 Comparison of antibody titres to Pasteurella multocida between groups

Significant difference in *Pasteurella multocida* antibody titre observed during first, second and ninth month was

due to difference in the vaccination schedule between three groups. There was no significant difference between three group throughout the study period except tenth ($P \leq 0.01$) and eleventh ($P \leq 0.05$) month where there was a significant difference between group I and II and group I and III.

These findings correlates with Joseph and Hedger (1984) opined that simultaneous administration of FMD and HS inactivated vaccine produced no adverse effect and the serological response did not differ form the response to either vaccine given separately. Also Srivastava *et al.* (1976) observed that combined *Pasteurella multocida* and *Clostridium chauvoei* oil adjuvant vaccine conferred a dependable grade of immunity which was comparable to that conferred by single vaccine against these diseases used alone and which was superior to the combined *Pasteurella multocida* and *Clostridium chauvoei* alum precipitated vaccine. Same was of the opinion of Reddy *et al.* (1997) when compared combined FMD, HS and BQ vaccine with their individual vaccine.

These findings indicated that *Pasteurella multocida* antibody titre was same in animals vaccinated with combined vaccine and individual vaccine.

Standard recommendation for HS aluminium hydroxide is at six month of age. In the present study oil adjuvanted combined FMD, HS and BQ vaccine were given at fourth month of age and all the animal showed HS antibody titre well above the protective level within a month after primary vaccination. From this it has been concluded that the combined vaccine provides early immunity as combined FMD, HS and BQ oil adjuvanted vaccination was carried out at four month of age itself.

5.6 Seroconversion of *Clostridium chauvoei* antigen

For the protection of *Clostridium chauvoei* the titre of 50 and above is taken as protective (Srinivasan, 2001).

5.6.1 Group I

Group I animals attained a protective level of mean antibody titres after three month and thereafter it showed protective antibody titres throughout the study period. This finding does agree with the findings of Awad *et al.* (1986) who opined that *Clostridium chauvoei* alum precipitated, aluminium

gel with saponin absorbed vaccine and oil adjuvant vaccine gave equal protection on 21st day after immunization in guinea pigs. In sheep, Cattle and Goat oil adjuvant BQ vaccine given strongest and rapid response. But in the study BQ combined oil vaccine gave protection three month after primary vaccination. Probably the vaccination at 4th month of age in this group might have delayed the antibody response. Also Awad *et al.* (1986) reported that two dose of oil adjuvant BQ vaccine provided protection in cattle and Buffalo upto nine month whereas in this study single injection at four month is given protective titre till ninth month where at ninth month booster vaccination was given.

After booster vaccination at ninth month peak antibody titre was observed within a month. This observation was in accordance with the observation of Tizard (1998), who described that repeated injection of antigen produced immune response with shorter lag period and for a longer period of time than single inoculation.

5.6.2 Group II

Group II animal attained a protective level of mean antibody titres within a month and there after it showed

protective antibody titres throughout the study period. This findings correlates with the findings of Awad *et al.* (1986), who opined that *Clostridium chauvoei* alum precipitated, aluminium gel with saponin absorbed vaccine and oil adjuvant vaccine gave equal protection on 21st day after immunization. But in the study BQ combined oil vaccine gave protection three month after primary vaccination.

After booster vaccination at 12th month of age peak antibody titre was observed within a month. This observation was in accordance with the observation of Tizard (1998).

5.6.3 Group III

Group III animals attained a protective level of mean antibody titres within a month and showed protective antibody titres throughout the study period. This finding does agree with the findings of Awad *et al.* (1986), who opined that *Clostridium chauvoei* alum precipitated, aluminium gel with saponin absorbed vaccine and oil adjuvant vaccine gave equal protection on 21st day after immunization. But in the study BQ combined oil vaccine gave protection three month after primary vaccination.

After booster vaccination at 12th month of age the highest mean antibody titre was obtained within a month. This observation was in accordance with the observation of Tizard (1998).

5.6.4 Comparison of antibody titre of Clostridium chauvoei between groups

BQ aluminium hydroxide gel vaccine provided protective titre within a month in group II and III, whereas it took three month in group I where oil adjuvant vaccine was used at fourth month. All the three groups did not showed any significant differences in mean *Clostridium chauvoei* antibody titres throughout the study period. Significant difference observed during second month and ninth month is due to the difference in the vaccination schedule between groups.

This findings correlates with the findings of Darie *et al.* (1979) who carried out simultaneous vaccination of Clostridial, Anthrax and FMD and concluded that there was no interference with the immunogenic effect of different vaccine. Same was of the opinion of Reddy *et al.* (1997) when compared combined FMD, HS and BQ vaccine with their individual vaccine.

This finding indicated that *Clostridium chauvoei* antibody titre was same in animals vaccinated with combined and individual vaccine.

5.7 Economic assessment of vaccine between three groups

5.7.1 Group I

The cost required for immunizing a calf for one year was Rs. 30.40 and only two times immunization were required for the first year.

5.7.2 Group II

For immunizing a calf for one year cost required is Rs.31.60 And four time immunization were required for the first year.

5.7.3 Group III

The cost required for immunizing a calf for one year was Rs. 31.60 and six times immunization were required for the first year.

5.7.4 Comparison of economic assessment of vaccination between three groups

Cost required for immunizing a calf for one year was Rs. 1.20 less in group I than group II and III. Group I animal require only two times injection, whereas group II animal require four times injection and group III animals require six times injection. So combined vaccine reduces labour cost for injection, number of visit and stress to the animals to a very great extent. Afzal and Muneer (1990) also concluded that combined HS and FMD vaccine could reduce the frequency and cost of vaccination. There fore combined vaccine can be preferred when compared to individual vaccine.

Summary

6. SUMMARY

Immune response to combined Foot and Mouth Disease, Haemorrhagic Septicaemia and Black Quarter oil adjuvant vaccine and to their respective individual vaccine were studied in cattle of Kerala Agricultural University livestock farms at Mannuthy and Thumburmuzhi. Eighteen unvaccinated calves of four month of age were selected and were grouped into three of six each. Group I animals were vaccinated with combined FMD, HS, BQ oil adjuvant vaccine, Group II animals were vaccinated with FMD polyvalent oil adjuvant vaccine and with combined HS, BQ gel vaccine, Group III animals were vaccinated with FMD polyvalent oil adjuvant vaccine, monovalent HS gel vaccine and monovalent BQ gel vaccine as per the standard schedule. The antibody titres against Foot and Mouth Disease virus type O, A, C, and Asia-1 were assessed every month employing liquid phase blocking ELISA and antibody titres against *Pasteurella multocida* and *Clostridium chauvoei* were assessed Indirect ELISA.

Mean FMDV type 'O' antibody in all the three group did not differ significantly throughout the study period except

during first month ($P \leq 0.05$) where Group I animals showed lower antibody titres than Group II and III animals. All the three groups maintained protective mean type 'O' antibody titres throughout the study period, except for Group I during seventh and eighth month, Group II for sixth month and Group III for sixth, eighth, and ninth month.

Mean FMDV type 'A' antibody in all the three groups did not differ throughout the study period except during second ($P \leq 0.05$) and third ($P \leq 0.05$) month where Group I animals showed higher antibody titre than Group II and III. Group I and II animals showed protective titre throughout the study period. Group III animals showed the protective titre throughout the study period except ninth month.

There was no significant difference between type 'C' FMDV antibody titres between three groups throughout the study period except during sixth month where there was significant difference ($P \leq 0.01$) between Group I and II, and Group I and III, where Group I animals showed lower antibody titre during that month than other group animals. Group I, II and III animals showed protective antibody titres throughout the study period.

The mean FMDV type Asia-1 antibodies titres between three groups from zero to twelfth month did not differ significantly except during fourth month ($P \leq 0.05$) where there was a significant difference between Group I and II, and Group I and III, where Group I animals showed higher antibody level during this month than other two groups. All the three groups maintained protective mean type Asia-1 antibody titres throughout the study period, except eighth and ninth month of Group III.

Mean antibody titre to *Pasteurella multocida* between three groups did not differ significantly throughout the study period except tenth ($P \leq 0.01$) and eleventh ($P \leq 0.05$) month, where there was a significant difference between Group I and II, and Group I and III, where Group I animals showed lower antibody level than Group II and III animals. All the three groups maintained protective mean *Pasteurella multocida* antibody titre throughout the study period except for Group III during first month of study.

All the three groups did not show any significant difference in mean *Clostridium chauvoei* antibody titres

throughout the study period. Group I animals attained a protective level of mean antibody titres after three months and there after it showed protective antibody titres throughout the study period. Group II and III animals attained the protective level of mean antibody titres within a month and there after it showed protective antibody titres throughout the study period.

From this study it is concluded that the performance of combined vaccine is equally good as that of individual vaccine. There was no significant difference in cost of vaccine between three groups, but difference is there in number of times of immunization. Group I animals received only two times injection, whereas Group II animals four times injection and Group III animals six times injection. So combined vaccine reduces labour cost for injection, number of visit and stress to the animals to a very great extent. Therefore combined vaccine may be preferred when compared to individual vaccine.

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IMMUNE RESPONSE TO COMBINED FOOT AND MOUTH DISEASE, HAEMORRHAGIC SEPTICAEMIA AND BLACK QUARTER VACCINE AND THEIR RESPECTIVE MONOVALENT VACCINES IN CATTLE

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ABSTRACT OF A THESIS

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ABSTRACT

Immune response to combined Foot and Mouth Disease, Haemorrhagic Septicaemia and Black Quarter vaccine and to their respective individual vaccine were studied. Group I animals were vaccinated with combined FMD, HS and BQ oil adjuvant vaccine. Group II animals were vaccinated with FMD polyvalent oil adjuvant vaccine and combined HS, BQ gel vaccine. Group III animals were vaccinated with polyvalent FMD oil adjuvant vaccine, HS gel vaccine and BQ gel vaccine. Vaccinations were done in unvaccinated calves above four month of age. Booster vaccination were carried out after nine month after primary vaccination for oil adjuvant vaccine and six month after primary vaccination for gel vaccine in all group animals. Monthly assessment of antibody titres against O, A, C and Asia-1 antigens were made by liquid phase blocking ELISA and *Pasteurella multocida* and *Clostridium chauvoei* by Indirect ELISA.

All the three groups maintained protective antibody titre for FMD virus type 'O', 'A', 'C', 'Asia-1', *Pasteurella*

multocida and *Clostridium chauvoei* antibody titres throughout the study period.

All the three groups did not show any significant variation in antibody titres against FMDV type, 'O', 'A', 'C' and 'Asia-1'and *Pasteurella multocida* and *Clostridium chauvoei* though they are well above protective titres.

From this observation it is concluded that

1. All the three schedule of vaccination provides sufficient protective titre for FMDV type, 'O', 'A', 'C' and 'Asia-1'and Pasteurella multocida and Clostridium chauvoei though they are well above protective titres.

2. Combined vaccine performance is equally good as that of individual vaccine.

3. Combined vaccine reduces labour cost for injection, number of visit and stress to the animals to a very great extent. There fore combined vaccine may be preferred when compared to individual vaccine.