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# THE EFFICACY OF TWO FOOT AND MOUTH DISEASE VACCINATION REGIMENS FOR CALVES OF IMMUNIZED DAMS IN SAUDI DAIRY FARMS

(With 4 Tables and 4 Figures)

By

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## كفاءة نظامى تحصين العجول المولودة لامّات محصنة ضد مرض الحمى القلاعية فى مزارع الألبان بالمملكة العربية السعودية

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يعتبر نظام تحصين العجول المولودة لامّات محصنة مرات عديدة من المشكلات المعقدة فى مزارع الألبان التى تعتمد فى مكافحة مرض الحمى القلاعية على التحصين. وعلى هذا ، واعتمادا على انحدار المناعة الامية المكتسبة MDA اجريت هذه الدراسة لتحديد العمر والنظام الذى يتحتم به تحصين العجول ضد مرض الحمى القلاعية لكى تعطى اعلى استجابة مناعية. جرب نظامى تحصين على مجموعتين من العجول المغذاة باللبأ ( السرسوب) colostrum شملت كل منهما ١٦ عجلا رضيعا. فى النظام الاول حصنت العجول عند الشهر الرابع والخامس اما الثانى فعند الشهر الرابع والخامس والسادس من العمر بلقاح تجارى مخمد ضد مرض الحمى القلاعية. وقد قدرت الاجسام المناعية الامية المكتسبة وكذلك الناتجة عن التحصين بطريقة التعادل المصلى. بينت حيوانات المجموعتين عن وجود اجسام مناعية امية مكتسبة حتى نهاية الشهر الرابع من العمر، بينما كان فىبعض هذة العجول اجسام مناعية امية مكتسبة اثرت على تكوين الاجسام المناعية التعادلية للقاح حتى اليوم ٢١٠ من عمرها. كان الارتفاع فى متوسطات القوة العيارية للاجسام المناعية الناتجة عن التحصين عند الشهر الخامس (شهر واحد بعد جرعة اللقاح الابتدائية) قليلا عند مقارنتة بذلك الارتفاع المقدر بعد شهر من اعطاء الجرعة المنشطة الاولى. ومع ذلك فان القوة العيارية التى احدثتها الجرعة المنشطة الاولى فى ٧٥٪ و ٨١٪ من عجول نظام التحصين الاول والثانى على التوالى تعد واقية. ووجد ايضا ان الاجسام المناعية الامية المكتسبة اثرت على الاستجابة المناعية الخلطية Humoral antibodies الناتجة عن

اعادة التحصين بالجرعة المنشطة الاولى حيث ان ٢٥٪ و ١٩٪ من عجول نظام التحصين الاول والثانى على التوالى عند الشهر السادس من عمرها بينت انخفاضا فى القوة العيارية للاجسام المناعية التعادلية. كذلك فى نهاية الشهر السابع ( شهر واحد بعد الجرعة المنشطة الثانية) لوحظ زيادة اضافية للقوة العيارية فى جميع حيوانات نظام التحصين الثانى، بينما ٢٥٪ من حيوانات النظام الاول مازالت تبين انخفاضا فى قوتها العيارية. واستنادا الى النتائج المتحصل عليها ، فاننا نوصى بتحصين العجول عند الشهر الرابع من العمر متبوعة بجرعتين تنشيطيتين عند الشهر الخامس والسادس من العمر ( النظام الثانى).

## SUMMARY

The programme of immunization of calves born to repeatedly vaccinated dams is considered a complex problem in dairy farms in which control of FMD relies predominantly on vaccination. Consequently, depending on the waning of maternally derived antibodies (MDA), a study was undertaken to find out the age and the regimen at which calves could be vaccinated against Foot and Mouth disease (FMD) to elicit maximum immune response. Two vaccination regimens on two groups of colostrum-fed calves each of 16 animals were implemented . The animals were vaccinated with a commercial inactivated FMD vaccine at 4,5 months of ages (regimen I) and 4,5 and 6 months of ages (regimen II). The MDA level and the neutralising antibodies against FMD vaccine were assessed by microneutralization test . MDA could be detected in all animals of the two groups up to the age of 4 months, some animals had effective MDA up to the age of 210 days. It was found that calves with MDA did not merely fail to respond to primo-vaccination, but also serum titres were depressed: this depression was reverse proportional to the level of pre-existing MDA at the time of primo-vaccination. However, the increase in the mean of the vaccine induced antibody titres at 5 months of age ( one month after the primo-vaccination ) were lower as compared to the increase one month later after the booster dose, as the titres provoked by revaccination in 75% and 81% of calves of regimens I and II would be regarded as protective. MDA were also found to influence secondary humoral immune response as 25% and 19% of calves in regimens I and II at 6 months of age showed decrease in neutralizing antibodies titres. Further seroconversion was observed among all animals of regimen II at 7 months of age (one month after the second booster), while 25% of the animals of regimen I still showed considerable decrease in antibody titres. Based on the above findings, it is recommended that calves could be primo-vaccinated at 4

months postpartum with two booster adiministered not later than 5 and 6 months of ages (regimen II) .

***Key words**: Foot & Mouth Disease, Vaccination, Calhood vaccination, Saudi Arabia.*

## INTRODUCTION

In order to achieve self-sufficiency in dairy production, several modern intensified dairy farms depending on the importation of cattle with high milking yield have been established in Saudi Arabia (AL-ODAN,1987). Despite vaccination, there were repeated outbreaks of FMD particularly in large dairy farms. During these outbreaks, it was observed that calves beyond four months old were less affected. Consequently, immunization of calves born to repeatedly vaccinated dams may represent a complex problem. The companies producing FMD vaccine recommended different primovaccination programmes for calves. The first programme proposed primo-vaccination at the age of 45 days followed by a boostering dose 15 days later and the calves were then repeatedly vaccinated with the main herd every 4 months.The second programme recommended only primovaccination at the age of 70 to 84 days, then repeated vaccination with the main herd every 6 months. The third one suggested primovaccination at 0 day (before fed colostrum) followed by a booster dose at 21 days of age. The aim of the present study was to implement a vaccination regimen and evaluate its efficacy for vaccinating calves against FMD.

## MATERIALS and METHODS

### Vaccination regimens

Two vaccination regimens on two groups of colostrum-fed one day old calves each of 16 animals were implemented. The calves were vaccinated at the ages of 4, 5 months (regimen I) and 4, 5 and 6 months (regimen II). The dose of the vaccine was 2 ml administrated subcutaneously.

### Vaccine

A quadrivalent inactivated commercial FMD vaccine was used. This vaccine contained the following virus strains: $O_1$ Lausanne, $O_1$ Manisa /68, A Iran 1987, $A_{22}$ Azerbaigan, A/SAU/41/91, $C_3$ Philippines 1976 and ASIA

1973. The vaccines were formulated according to the recommendations of the FMD World Reference Laboratory (WRL), Pirbright, UK.

**Serum neutralisation test:**

The micro-neutralisation test was applied as described by GOLDING et al., ( 1976)
using BHK-21 monolayers and $O_1$ Manisa /68 FMD virus strain.

**Serum samples**

Serum samples were collected from calves at the ages of 15,30,60,90,120,150,180 and 210 days. The sera were inactivated at 56 C° for 30 minutes.

## RESULTS

A general high level in MDA titres (740 to 96) in 78% (25 out of 32) of the experimental calves was observed by15 days of age. 53% of the tested calves had titres ranging from 48 to 96 at the age of 60 days. In all the calves tested, MDA titres persisted until the age of 4 months and some animals had effective MDA up to the age of 210 days during which the rate of fall-off was rapid in first 60 to 90 days of ages, but was gradual thereafter (Tables 1,2 and Figures 1,2).

In both regimens, at the time of primo-vaccination, MDA titres in some calves were 24-48, and in others the MDA titres had waned to low level (2-8). However, at one month post primo-vaccination some animals had marked decrease in the MDA titres ranging from 2 to 32 and others showed slight response to the primovaccination (Tables 1,2 and Figure 4 ).

By 6 months of age ( one month after the booster dose ) 75% and 81% of calves in regimens I and II revealed marked increased in vaccine induced antibody titres. On the other hand, MDA were also found to influnce secondary humoral immune response, as 25% and 19% of calves in regimens I and II showed decrease in neutralizing antibodies titres (Tables 1,2 and Figures 1,2).

Further seroconversion were observed among all animals of regimen II at 7 months of age (one month after the second booster), while 25% of the animals of regimen I still showed considerable decrese in antibody titres (Tables 1,2 and Figures 1,2).

## DISCUSSION

Foot and Mouth Disease (FMD) is endemic in Saudi Arabia (Al-Mezaini et al.,1985) and causes severe economic losses (HAFEZ et al.,1994). In dairy farms in which control of FMD relies predominantly on vaccination, the programme of immunization of calves born to repeatedly vaccinated dams is considered a complex problem, due to the present of high titres of maternally derived antibody (MDA).

The companies producing FMD vaccine used in Saudi Arabia recommended different primo-vaccination programmes for protection of calves. The programme proposing a primo-vaccination at the age of 45 days followed by a booster dose 15 days later was previously evaluated by Massirio et al., (1988). In this programme there was no significant difference in the serum titres between the vaccinated and non vaccinated control groups. The second programme involving only vaccinated calves at the age of 70 to 84 days revealed no distinct serological response 21 days post vaccination. However, when the calves were exposed to a challenge test only 50% were protected (Faver et al.,1982). The third programme which involved a primo-vaccination at 0 day of age (before feeding colostrum) followed by a booster dose at 21 days of age was evaluated before the activation of the virology laboratory in the National Agriculture and Water Research Center in Riyadh by a big Saudi dairy farm in AL-Kharj region (unpublished data); the results of serological test carried out in the World Reference Laboratory (WRL) against $O_1$ BFS 1860 of FMD virus strain using ELISA on two groups of calves in a Saudi dairy farm in AL-Kharj region shown in Tables 3,4 and Fig.3 indicate that the mean serotitres of the vaccinated group of calves were lower than of the non-vaccinated group, and this depression of MDA titres indicated that vaccination of the young calves was not only ineffective in promoting any improvement in the antibody titres but also led to a strong depressive effect on the MDA titres which existed at the time of vaccination.

In the present study on MDA, a general high level in MDA titres in 78% (25 out of 32) of the experimental calves observed by the 15 days of age is an indicator that the consumed colostrum by the calves was of good quality and quantity. 53% of the tested calves had titres ranging from 48 to 96 by 60 days of age. The WRL for FMD, in Pirbright, UK ( Kitching, personal communication) considers a neutralizing titre of 45 as protective

against FMD in calves. On the other hand, Faver et al. (1982) in France found that calves with a titre of 80 were protected against challenge with FMD virus. The protection period in calves due to MDA against FMD virus were found to last until the ages between 56 and 90 days ( Hehler, 1922 ., Stryszak, 1970). However, in another study, the neutralizing MDA against FMD was found to remain until the age of 150 days (Nicholls et al.,1984). In the present study, By the age of 4 months MDA was detected in all calves, while some animals had effective MDA up to the age of 210 days (Tables 1,2). This long- lasting MDA could be due to the quantity and the quality of colostrum consumed by the calves and the vaccination schedule used on pregnant dams.

Our own observation and reports from different studies showed that :A) Effective MDA was present in all tested calves up to the age of 4 months., B) Calves beyond 4 months of age during FMD outbreaks in Saudi dairy farms were less affected., C) Early vaccination programmes under 4 months of age were ineffective., D) Less than 10 percent protection of calves which had MDA at the age above 4 months (Ahl and Wittman, 1987), and E) Calves with waning MDA at 5 to 6 months of age could become clinically infected with FMD virus prior to being able to respond to FMD vaccination (Kitching and Salt, 1995). Therefore, the calfhood primo-vaccination in the revised field schedule was delayed untill the animals were 4 months old.

Consequently, the present study was particularly involved in the evaluation of two other vaccination regimens in two groups of calves each of 16 animals following the waning of their MDA.The calves were vaccinated at 4,5 months of age in regimen I and 4,5 and 6 months of age in regimen II. However, in both regimens, the increase in the mean of the antibody titres at 5 months of age (one month after the primo-vaccination) was low, and not only the level of maternal antibodies came down completely but there was slight detected primary immune response also (Tables 1,2 and Figure 4). In contrast, calves which had pre-existing maternal antibodies, responded poorly, the degree of suppression of post-vaccination response of these calves appeared to be related to MDA titres at the time of primo-vaccination. We believe that the observed depression of MDA titres could be due to depletion of MDA by the antigen of the used vaccine. However, several explanations for this phenomenon were given by other investigators such as : 1) due to the rapid capture of the vaccine antigen by MDA (Solomon, 1971., Uhr and Moller, 1968)., 2) as a result of possible mediation by iso- and

idiotope specific supressor T lymphocytes (Flood et al., 1986., Okumura and Tada, 1986 and Solomon, 1971), 3) MDA act directly on B lymphocytes to down-regulate the proliferation and differentiation required for antibody production (Mossirio et al., 1988) and 4) the MDA complexed with vaccinal antigen may act through a regulatory network to either suppress antigen specific $TH_1$ cells or actively up-regulate the production of an antigen specific T suppressor cell population (Harte and Playfair, 1983).

However, the responses to secondary vaccination (boostering ) were more variable than primary responses, as at the time of booster dose , MDA titres in some calves were still relatively moderate; in others the MDA titres had waned to low levels and yet others which had responded to primary vaccination were capable of true anamnestic response.

The titres provoked by revaccination (one month post boostering) would be regarded as protective in 75% and 81% of calves of regimens I and II respectively (Tables 1,2 and Figures 1,2).The obtained results agree with Dobourget et al., (1989). MDA were also influencing secondary humoral immune response, as 25% and 19% of calves of regimens I and II at 6 months of age failed to respond to the boostering vaccination because they had relatively high titres of MDA at the time of primo-vaccination (Tables 1,2) .

However, in regimen I, at the age of 7 months, of the remaining calves 25% did not reveal any marked increase in vaccine-induced antibody titres. In regimen II, although the majority of calves (81%) at the age of the 7 months (one month post second boostering vaccination) showed a slight increase in antibody titres, the remaining calves (19%) revealed also marked increase in vaccine induced antibody titres more than the protective level (Table 2 and Figure 2 ). This explaination could be that the first booster might have acted as a primary dose for the calves which had moderate MDA titres at the time of primo-vaccination (19%), and the second boostering dose acted as the first booster for them. Based on the above finding, it is recommended that calves born to repeatedly vaccinated dams should be primo-vaccinated at 4 months postpartum with two boosters adiministered not later than 5 and 6 months of ages (regimen II). Although, this regimen proved to be the most effective vaccination programme for calves of well vaccinated dams, yet there is an inevitable lag period (in calves at the age between 4 to 6 months) between vaccination and the development of active immunity. It is during this period that the prevalent virus can establish themselves in young calves.Consequently, to protect calves from FMD virus

during the lag period, it can be provided either by their isolation from FMD virus (Kitching and Salt, 1995), or to produce a FMD vaccine that does not interfere with the MDA .

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**Table 1 Maternally derived and vaccine induced antibody titres of calves of group I**

| Animal Number | Neutralizing antibody titres against $O_1$ Manisa / 68 FMD virus strain | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Age in days | | | | | | | |
| | 15 | 30 | 60 | 90 | 120 | 150 | 180 | 210 |
| 1 | 320* | 64 | 48 | 32 | 8 | 16 | 512 | 320 |
| 2 | 256 | 160 | 32 | 12 | 8 | 8 | 160 | 256 |
| 3 | 256 | 16 | 64 | 8 | 4 | 16 | 1024 | 512 |
| 4 | 256 | 160 | 96 | 48 | 32 | 16 | 24 | 16 |
| 5 | 256 | 64 | 48 | 16 | 4 | 24 | 320 | 256 |
| 6 | 512 | 128 | 96 | 48 | 48 | 32 | 16 | 16 |
| 7 | 32 | 16 | 12 | 8 | 4 | 32 | 512 | 740 |
| 8 | 128 | 64 | 48 | 16 | 8 | 12 | 256 | 204 |
| 9 | 512 | 48 | 48 | 32 | 48 | 32 | 16 | 12 |
| 10 | 128 | 96 | 48 | 16 | 4 | 12 | 512 | 740 |
| 11 | 256 | 48 | 32 | 12 | 8 | 4 | 256 | 320 |
| 12 | 32 | 24 | 16 | 8 | 4 | 12 | 256 | 512 |
| 13 | 64 | 12 | 16 | 8 | 4 | 16 | 320 | 512 |
| 14 | 128 | 64 | 48 | 32 | 24 | 16 | 12 | 8 |
| 15 | 256 | 48 | 32 | 8 | 4 | 4 | 128 | 256 |
| 16 | 512 | 48 | 12 | 8 | 4 | 8 | 256 | 512 |
| Mean | 244 | 66 | 44 | 20 | 14 | 16 | 286 | 317 |
| ST.D. | ±160 | ±47 | ±26 | ±14 | ±16 | ±9 | ±263 | ±250 |

* The reciprocal of the highest serum dilution which neutralizes 100 TCID50

**Table 2 Maternally derived and vaccine induced antibody titres of calves of group II**

| Animal Number | Neutralizing antibody titres against $O_1$ Manisa / 68 FMD virus strain | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Age in days | | | | | | | |
| | 15 | 30 | 60 | 90 | 120 | 150 | 180 | 210 |
| 1 | 512* | 128 | 64 | 32 | 24 | 16 | 12 | 512 |
| 2 | 256 | 96 | 48 | 16 | 8 | 16 | 256 | 512 |
| 3 | 256 | 128 | 48 | 24 | 12 | 8 | 160 | 256 |
| 4 | 32 | 16 | 8 | 4 | 2 | 8 | 128 | 256 |
| 5 | 64 | 8 | 8 | 4 | 4 | 12 | 256 | 320 |
| 6 | 740 | 128 | 48 | 24 | 32 | 16 | 12 | 320 |
| 7 | 48 | 8 | 8 | 4 | 4 | 8 | 128 | 256 |
| 8 | 64 | 16 | 12 | 4 | 2 | 8 | 256 | 512 |
| 9 | 128 | 48 | 12 | 8 | 4 | 16 | 256 | 512 |
| 10 | 512 | 128 | 64 | 32 | 24 | 16 | 12 | 320 |
| 11 | 96 | 32 | 24 | 8 | 2 | 8 | 128 | 320 |
| 12 | 256 | 128 | 48 | 24 | 12 | 8 | 256 | 320 |
| 13 | 96 | 32 | 16 | 8 | 2 | 4 | 320 | 512 |
| 14 | 256 | 48 | 12 | 8 | 12 | 4 | 128 | 320 |
| 15 | 320 | 96 | 48 | 32 | 8 | 2 | 96 | 512 |
| 16 | 256 | 96 | 64 | 24 | 12 | 8 | 128 | 320 |
| Mean | 243 | 71 | 33 | 16 | 10 | 10 | 158 | 380 |
| ST.D. | ±200 | ±49 | ±22 | ±11 | ±9 | ±5 | ±99 | ±108 |

* The reciprocal of the highest serum dilution which neutralizes 100 TCID50 of virus

**Table 3 Reciprocal ELISA titres against $O_1$ BFS 1860 FMD virus strain in sera of calves vaccinated at 0 and 21 days of age**

| Animal Number | Reciprocal ELISA titres against $O_1$ BFS 1860 FMD virus strain | | | | |
|---|---|---|---|---|---|
| | Age in days | | | | |
| | Days 21 | Days 42 | Days 63 | Days 84 | Days 105 |
| 1 | 128 | 45 | 45 | 45 | 22 |
| 2 | 45 | 22 | 11 | 90 | 32 |
| 3 | 724 | 362 | 256 | 128 | 90 |
| 4 | 90 | 64 | 45 | 45 | 22 |
| 5 | 256 | 128 | 90 | 90 | 32 |
| 6 | 64 | 32 | 22 | 16 | 11 |
| 7 | 362 | 126 | 64 | 64 | 22 |
| 8 | 362 | 181 | 90 | 45 | 64 |
| 9 | 90 | 45 | 22 | 45 | 11 |
| 10 | 512 | 90 | 45 | 64 | 90 |
| 11 | 181 | 90 | 45 | 22 | 16 |
| 12 | 90 | 32 | 16 | 11 | 8 |
| 13 | 90 | 45 | 45 | 22 | 11 |
| 14 | 181 | 90 | 90 | 32 | 22 |
| 15 | 362 | 128 | 90 | 90 | 22 |
| 16 | 181 | 90 | 45 | ND | 22 |
| 17 | 64 | 181 | 90 | 128 | 11 |
| 18 | 90 | 64 | 8 | 11 | 90 |
| 19 | 90 | 45 | 32 | ND | 8 |
| 20 | 126 | 181 | 90 | 32 | 22 |
| 21 | 256 | 128 | 90 | 32 | 22 |
| 22 | 181 | 90 | 128 | 90 | 22 |
| 23 | 181 | 64 | 45 | 64 | 11 |
| 24 | 128 | 90 | 45 | 22 | 8 |
| Mean | 202 | 101 | 66 | 54 | 29 |
| ST.D. | ±163 | ±163 | ±65 | ±54 | ±26 |

**Table 4 Reciprocal ELISA titres against $O_1$ BFS 1860 FMD virus strain in sera of calves of non vaccinated group**

| Animal Number | Reciprocal ELISA titres against $O_1$ BFS 1860 FMD virus | | | | |
|---|---|---|---|---|---|
| | Age in days | | | | |
| | Days 21 | Days 42 | Days 63 | Days 84 | Days 105 |
| 1 | 512 | 181 | 128 | 181 | 64 |
| 2 | 362 | 90 | 90 | 8 | 90 |
| 3 | 181 | 64 | 22 | 32 | 32 |
| 4 | 181 | 90 | 90 | 64 | 32 |
| 5 | 512 | 181 | 128 | 90 | 64 |
| 6 | 362 | 181 | 128 | 90 | 90 |
| 7 | 362 | 181 | 181 | 181 | 181 |
| 8 | 128 | 256 | 181 | 128 | 128 |
| 9 | 362 | 181 | 90 | 90 | 181 |
| 10 | 90 | 181 | 181 | 128 | 181 |
| 11 | 90 | 90 | 90 | 64 | 32 |
| 12 | ND | 128 | 181 | 64 | 45 |
| 13 | 362 | 32 | 45 | 45 | 45 |
| 14 | 256 | 181 | 128 | 128 | 90 |
| 15 | 181 | 64 | 90 | 64 | 90 |
| 16 | 181 | 90 | 90 | 64 | 90 |
| 17 | 90 | 128 | 128 | 45 | 90 |
| 18 | 181 | 90 | 45 | 32 | 64 |
| 19 | 128 | 90 | 181 | 32 | 64 |
| 20 | 90 | 64 | 181 | 22 | 90 |
| 21 | 181 | ND | 181 | 22 | 64 |
| 22 | 181 | 90 | 256 | 22 | 90 |
| 23 | 90 | 90 | 181 | 22 | 90 |
| 24 | 362 | 128 | 181 | 16 | 32 |
| 25 | 181 | 90 | 90 | ND | 22 |
| Mean | 236 | 123 | 130 | 68 | 81 |
| ST.D. | ±132 | ±55 | ±56 | ±50 | ±46 |

**Figure 1 Maternally derived and vaccine induced antibody titres against strain O1 Manisa/68 of FMD virus of calves vaccinated at 4,5 months of age (regimen I)**

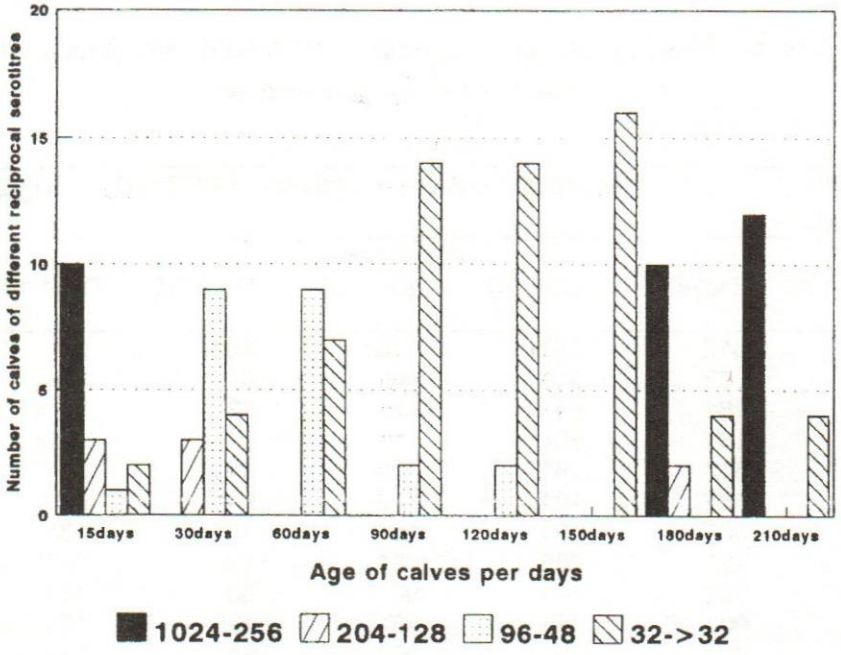
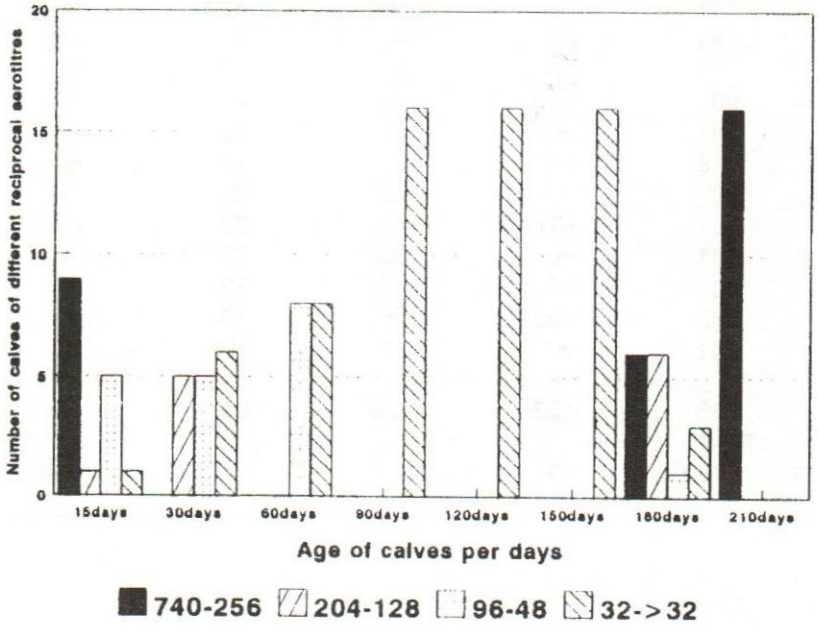



**Figure 2 Maternally derived and vaccine induced antibody titres against strain O1 Manisa/68 of FMD virus of calves vaccinated at 4,5 and 6 months (regimen II)**



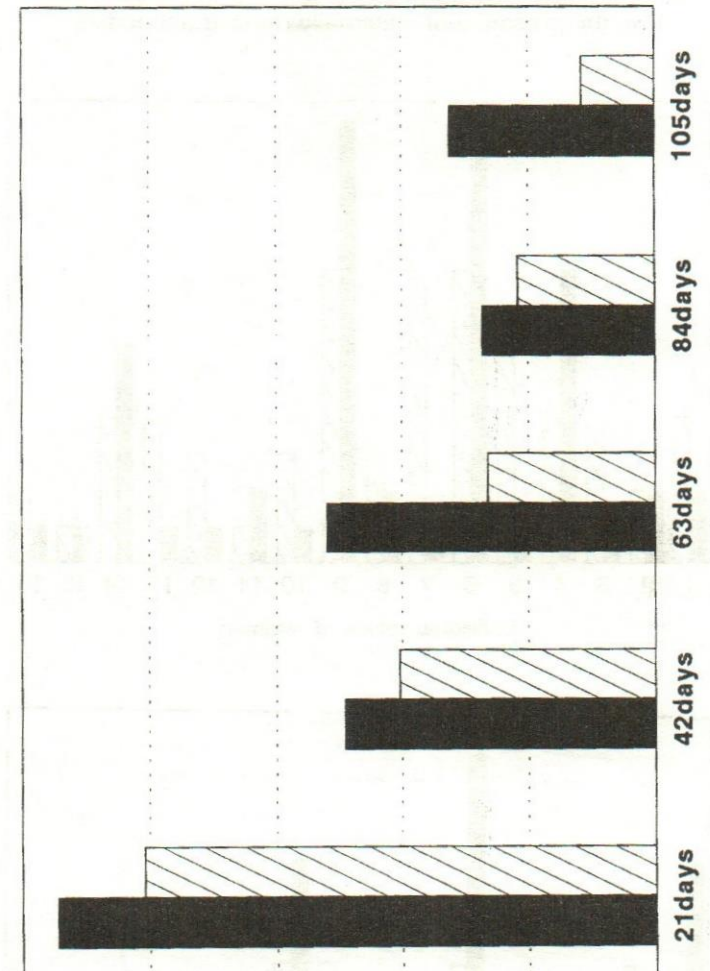



Figure 3 Assesment of antibody titres against O1 BFS 1860 strain of FMD virus in sera of a group of calves vaccinated at 0 and 21days of age and non vaccinated group.

Figure 4 Response of calves of regimens I and II to primovaccination with FMD in the presence of maternally derived antibodies