# STUDY OF HUMORAL AND CELLULAR IMMUNE RESPONSE IN FMD VACCINATED SHEEP WITH DIFFERENT INACTIVATED FMD VACCINES

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***Abstract***

This study has been designed as a trial for comparing of ISA206 adjuvanted Foot and Mouth Disease (FMD) vaccine (commercial vaccine), commercial FMD vaccine containing vitamin (A), commercial FMD vaccine containing vitamin (E-Selenium) and commercial FMD vaccine containing both vitamin (A) and vitamin (E-Selenium) together in eliciting immunity. The obtained results revealed that the duration of humoral immunity elicited by commercial vaccine only was 30 weeks, while, it was 32 weeks in commercial vaccine containing vitamin (A), 36 weeks in commercial vaccine containing vitamin (E-Selenium) and 38 weeks in commercial vaccine containing both vitamin (A) and vitamin (E-Selenium) together in vaccinated sheep. Results of cellular immunity evaluation indicated that mean optical density (mean OD) correspond to non-specific mitogens using MTT kit reached its highest values by 21$^{st}$ day post vaccination in all vaccinated sheep and mean OD values correspond to FMDV as specific mitogen were higher than that of non-specific mitogen. Also, the highest mean OD values were obtained in vaccinated sheep with commercial vaccine containing both vitamin (A) and vitamin (E-Selenium) together. It could be concluded that usage of inactivated oil FMD vaccine using Montanide ISA 206 containing both vitamin (A) and vitamin (E-selenium) resulted in longer lasting immunity than that with Montanide ISA 206 only, besides the stimulating effect of both vitamin (A) and vitamin (E-selenium) in both humoral and cellular immunity.

## INTRODUCTION

Foot and Mouth Disease (FMD) is a highly contagious disease and one of the most devastating diseases of cloven-hoofed animals, including domestic animals such as cattle, buffaloes, sheep, goats and pigs, as well as antelope, hison and other wild bovines and deer (OIE, 2012).

This disease has a significant economic impact on livestock industry worldwide. The majority of the economic losses results either from mortality of young animals, loss of milk and meat and drastic fall in production performance or indirect losses due to the imposition of trade restriction (Verma, 2008).

FMD virus is aphathovirus of Family Picornaviridae, There are seven recognized serotypes of FMD (O, A, C, Asia, SAT1, SAT2 and SAT3) which differ in

distribution across the world (Pereira, 1977) and comprise more than 65 subtypes (Sharma and Kakkar, 2005).

In Egypt, the disease is enzootic and outbreaks have been reported since 1950. FMDV serotypes (SAT2, A) and (O) were last reported in the years 1950, 1972 and 2000, respectively (Aidaros, 2002). Type (O) was the most prevalent since 1960 and onwards (Farag *et al.*, 2005).

Since 1950, 1953 and 1956 serotype (A) was not recorded in Egypt (Zahran, 1960). Recently, serotype (A) of FMD virus was introduced to Egypt through live animals importation and severe clinical signs occurred among cattle and buffaloes (Abd El-Rahman *et al.*, 2006). Also, FMDV serotype (SAT2) outbreaks in Egypt were officially reported by OIE (2012). In addition to this, endemic serotypes A and O continue to circulate in country (Lockart *et al.*, 2012 and Heba Attia, 2017).

Most of Foot and Mouth Disease vaccines are made of binary ethyleneimine (BEI) inactivated virus. Oil adjuvants are generally preferred due to introducing longer lasting immunity (Hunter, 1998). The commercial FMD vaccine is adjuvanted with Montanide ISA206 which is double water-in-oil-in-water (W/O/W) adjuvant eliciting protective humoral immune response and cellular immunity in vaccinated animals (Clo *et al.*, 2008).

Kutukculer *et al.* (2000) reported that both vitamin A and vitamin E-selenium can be used as adjuvant inducing high serum neutralization antibody titers and stimulate cellular immunity. Also, Rajeesh *et al.* (2008) found that using vitamin E-selenium as an adjuvant elicits high and prolonged immune response in vaccinated animals.

This study is carried out as an attempt to compare between (ISA206), (ISA206 and vitamin A), (ISA206 and vitamin E-Selenium) and (ISA206 and both vitamin A and vitamin E-Selenium together) vaccine in eliciting both humoral and cellular immunity.

## MATERIAL AND METHODS

**1. Animals:**

Twenty five sheep of 6 months (local breed) were clinically healthy and free from antibodies against FMD as proved by using SNT and ELISA.

**2. FMD virus strains:**

Vaccinal strains serotypes O Pan Asia 2012, A/Iran 05, and SAT2/Egy/2012 of FMDV were used for production of trivalent FMD vaccines and neutralization test.

**3. FMD vaccines:**

**a. Commercial FMD vaccine:**

Inactivated FMD oil trivalent vaccine.

**b. Commercial FMD vaccine containing vitamin (A):**

Inactivated FMD oil trivalent vaccine containing vitamin (A) 200,000 IU/dose (Semba *et al*., 1998).

**c. Commercial FMD vaccine containing vitamin (E-selenium):**

Inactivated FMD oil trivalent vaccine containing vitamin (E-Selenium) 500mg/dose (Milad *et al*., 2001).

**d. Commercial FMD vaccine containing both vitamin (A) and vitamin (E-selenium:**

Inactivated FMD oil trivalent vaccine containing both vitamin (A) 200,000IU/dose and vitamin (E- selenium) 500 mg/dose.

**4. Experimental Design:**

Four groups of sheep (5 animals/group) were vaccinated with the tested vaccines. Five animals were kept without vaccination as a control negative group. Serum samples were collected weekly post vaccination for one month and then every 2 weeks until the end of the experiment (38 weeks). Heparinized blood samples for MTT assay of cell mediated immunity were also collected.

**5. Estimation of the immune response:**

**5.1. Humoral immunity:**

It was estimated using SNT according to OIE Manual (2012) and ELISA according to Hamblin *et al.* (1986).

**5.2. Cellular immunity:**

It was estimated using MTT kit according to Verma (2010).

**6. Statistical analyses**

Results were analyzed using ANOVA test (Sendecor, 1971).

## RESULTS

From Tables (1 to 6), the results of humoral immune response revealed that mean protective serum antibody titre evaluated using SNT and ELISA were as follows:

The first group reached the SNT protective level (1.5 $\log_{10}$) at 3$^{rd}$ week post vaccination (WPV) with mean antibody titers of (1.68 $\log_{10}$/ml for "O" serotype, 1.68 $\log_{10}$/ml for "A" serotype) and at the 4$^{th}$ WPV (1.59 $\log_{10}$/ml) in case of "SAT2" serotype and to ELISA protective level (1.65 $\log_{10}$) at the 3$^{rd}$ WPV (1.89 $\log_{10}$/ml for "O" serotype and 1.68 $\log_{10}$/ml for "SAT2" serotype) and 1.59 $\log_{10}$/ml for "A" serotype at the 2$^{nd}$ WPV.

The highest level of mean antibody titers were obtained at 10$^{th}$ week post vaccination (WPV) (2.64 $\log_{10}$/ml for "O" serotype, 2.67 $\log_{10}$/ml for "A" serotype and 2.46 $\log_{10}$/ml for "SAT2" serotype) by SNT and by ELISA (2.73 $\log_{10}$/ml for "O" serotype, 2.67 $\log_{10}$/ml for "A" serotype and 2.46 $\log_{10}$/ml for "SAT2" serotype).

The duration of protection lasted for 30, 36 and 28 WPV for "O", "A" and "SAT2" serotypes respectively in SNT and for 28, 32 and 26 WPV in ELISA respectively.

In the second group, FMD SNT antibody protective levels were obtained at the 3rd, 2nd and 4th WPV in "O", "A" and "SAT2" serotypes respectively while in case of ELISA they achieved at the 3rd WPV in "O" and "SAT2" serotypes, but at the 2nd WPV in "A" serotype. The highest level was recorded at 10th WPV (2.67, 2.73 and 2.43 $log_{10}$/ml for "O", "A" and "SAT2" serotypes respectively) by SNT and (2.76 and 2.73 $log_{10}$/ml for "O" and "A" serotypes respectively) and at the 12th WPV (2.55 $log_{10}$/ml for "SAT2" serotype) by ELISA with protection duration for 32, 36 and 30 WPV for "O", "A" and "SAT2" serotypes respectively in SNT and for 30, 36 and 28 WPV in ELISA respectively.

Regarding to the third group, SNT antibody protective levels were recorded at the 3rd, 2nd and 3rd WPV in "O", "A" and "SAT2" serotypes respectively while in case of ELISA they achieved at the 2nd WPV in "O" and "A" serotypes, but at the 3rd WPV in "SAT2" serotype. The highest level of mean SNT antibody titers were recorded at the 10th WPV (2.85 $log_{10}$/ml for "O" serotype, 2.94 $log_{10}$/ml for "A" serotype and 2.55 $log_{10}$/ml for "SAT2" serotype) by SNT and (2.94 $log_{10}$/ml for "O" serotype and 2.55 for "SAT2" serotype) and at the 12th WPV (3.00 $log_{10}$/ml for "A" serotype) by ELISA with protection duration lasted for 36, 38 and 32 WPV for "O", "A" and "SAT2" serotypes respectively in SNT and for 32, 36 and 30 WPV in ELISA respectively.

In the fourth group, the SNT protective levels reached at 3rd WPV with mean antibody titers of 1.77 $log_{10}$/ml for "O" serotype and 1.53 $log_{10}$/ml for "SAT2" serotype) and at the 2nd WPV (1.71 $log_{10}$/ml) in case of "A" serotype and to ELISA protective level at the 2nd, 1st and 3rd WPV (1.68, 1.68 and 1.74 $log_{10}$/ml for "O", "A" and "SAT2" serotypes respectively).

The highest level of mean SNT antibody titers were gained at the 10th to 12th WPV (2.94 $log_{10}$/ml for "O" serotype, 2.94 $log_{10}$/ml for "A" serotype and 2.61 $log_{10}$/ml for "SAT2" serotype) by SNT and (2.94 $log_{10}$/ml for "O" serotype, 2.97 $log_{10}$/ml for "A" serotype and 2.64 $log_{10}$/ml for "SAT2" serotype) by ELISA with protection duration lasted for 38, 38 and 32 WPV for "O", "A" and "SAT2" serotypes respectively in SNT and for 38, 38 and 26 WPV in ELISA respectively.

## DISCUSSION

From Tables (1 to 6), the results revealed that SNT and ELISA titers of FMD antibodies induced by oil Montanide ISA-206 trivalent FMD vaccines in group one went in hand with Barteling and Vreeswij, 1991 and Sonia, 2007) who reported that oil emulsion FMD vaccine (double oil emulsion) induced best results in comparison with alhydragel vaccine. Also agree with Patil *et al.* (2002) and Abeer *et al.* (2009) who

found that FMD vaccines adjuvanted with Montanide ISA-206 can promote long lasting immunity.

The obtained results in the 2nd group in which animals were vaccinated with oil Montanide ISA-206 trivalent FMD vaccine containing vitamin (A), the results agree with Semba *et al.* (1992) who reported that administration of high dose of vitamin (A) resulted in significantly higher titers of anti-tetanus toxoid after immunization compared with those in control group.

In addition, in the trial of Rahman *et al.* (1999), they reported that vitamin (A) had a positive effect on the antibody response to diphtheria toxoid antigen in diphtheria vaccine. Also, Semba (1999) and Bahl *et al.* (2002) resulted that when vitamin (A) administration with Polivirus type 1 vaccine, the protective titer against type 1 poliovirus was significantly higher in experimental group. Semba *et al.* (2005) reported that vitamin (A) has a great role in improving immunofunction by increasing antibody titer against Schwars Measles vaccine.

In the third group, in which animals were vaccinated with oil Montanide ISA-206 trivalent FMD vaccine containing vitamin (E-selenium), the results are supported by Martin Nemec *et al.* (1990) and Cherly (1996) who found that vitamin (E-selenium) injection increased Ig production and stimulated cellular immunity. Weber *et al.* (2008) suggested that antibody formation against Newcastle disease was improved by vitamin E-selenium administration.

Also, Kanchana and Jeyanthi (2010) reported that when useding vitamin (E-selenium) as adjuvant for BVD vaccine, it provided higher level of protection.

In the fourth group, in which animals were vaccinated with oil Montanide ISA-206 trivalent FMD vaccine containing both vitamin (A) and vitamin (E-selenium) together, the obtained results agreed with Kutukculer *et al.* (2000) who made Turkish trials for using vitamin (A), vitamin (E-selenium) and both vitamins (A) and (E-selenium) together as adjuvant and found that highest antibody response when used both vitamins (A) and (E-selenium) together as adjuvant.

The obtained results of cell mediated immune response using MTT kit for all animal groups expressed as mean Optical Density (mean OD) in table (7) showed that:

In the first group, mean ODs were (0.121 – 0.231) by using non-specific mitogen and FMD viruses (as specific mitogen) at the 3rd day post vaccination (DPV) and reached its highest level (0.510-0.600) at 21st day post vaccination for non-specific mitogen and FMD viruses as (specific mitogen) respectively.

The results of first group were supported by those of Soos and Tuboly (1983) and Sharma *et al.* (1985) who reported that cell mediated immune response was a constituent of immune response against FMD virus and were in agreement in some points with Abeer *et al.* (2009) who stated that FMD vaccine stimulated the cellular

immune response and lymphocyte stimulation by FMD as specific mitogen was greater than that by non-specific mitogen.

In the 2nd group, the mean OD was (0.197-0.289) at the 3rd DPV and (0.600-0.710) at 21st DPV for non-specific mitogen and FMDV as specific mitogen respectively.

These previously mentioned results were supported by Jason *et al.* (2002) who reported that vitamin "A" stimulate cellular response via stimulate T-cell proliferation.

Coutsoudis *et al.* (1992) reported that the beneficial effect of vitamin (A) supplementation with severe measles enhancing specific IgG antibody level and total lymphocyte proliferation. Stephensen (2001) reported that vitamin "A" stimulate the cell mediated activity by stimulating T-helper cell. Also, Ribeiro *et al.* (2003) found that vitamin "A" increase T-cell and B-cell count. Villamor and Fawzi (2005) found that vitamin "A" stimulate lymphocyte activation for cytokines production.

While, in the third group, mean OD was (0.245-0.345) at the 3rd DPV reached its highest value at 21st DPV (0.711-0.803) by using non-specific mitogen and FMDV (as specific mitogen) respectively.

These results agreed with Hogan *et al.* (1990) who reported that the E-selenium plays an important role in phagocytosis.

Howard *et al.* (1989) explained the mechanism of E-slenium by which enhances cellular immunity as it inhibits the generation of suppressor cell activity. Reffett Stabel *et al.* (1989) found that E-selenium administration increased T-lymphocyte blastogenesis in-vitro stimulation with mitogen in some studies. Also, Wuryastuti *et al.* (1993) reported that E-selenium increased the phagocytic activity and mitogenic response.

But in the 4th group, mean OD was (0.271-0.400) at 3rd DPV and (0.820-910) at 21st DPV by using non-specific mitogen and FMD (as specific mitogen) respectively.

The last mentioned results come in parallel with those found by Tengerdy (1990) who used both vitamin (A) and (E-selenium) together as adjuvant in vaccine preparation resulting in maximizing of both the cellular and humoral immune response than dietary supplementation.

Also, Semba *et al.* (2005) and Kanchana and Jeyanthi (2010) reported that both vitamin (A) and vitamin (E-selenium) stimulate virus function of T-lymphocyte enhancing the immune response of the host and affecting macrophage activity and provided higher level of protection against natural challenges.

Finally, it could be concluded that administration of inactivated oil vaccine with Montanide ISA 206, both vitamin (A) and vitamin (E-selenium) together resulted in longer lasting immunity than that with Montanide ISA 206 only, in addition to the stimulating effect of both vitamin (A) and vitamin (E-selenium) in both humoral and cellular immunity.

Table 1. Mean serum neutralization antibody titer against FMD serotype (O) in different sheep groups

| Weeks Post Vaccination | Sheep Groups | | | | |
|---|---|---|---|---|---|
| | Group (1) | Group (2) | Group (3)* | Group (4)** | Control –ve sheep group |
| 0 | 0.17 | 0.15 | 0.14 | 0.14 | 0.15 |
| 1 | 0.87 | 0.87 | 1.10 | 0.96 | 0.20 |
| 2 | 1.29 | 1.35 | 1.41 | 1.44 | 0.10 |
| 3 | 1.68 | 1.71 | 1.74 | 1.77 | 0.15 |
| 4 | 1.89 | 2.01 | 2.04 | 2.10 | 0.25 |
| 6 | 2.40 | 2.43 | 2.46 | 2.49 | 0.20 |
| 8 | 2.46 | 2.61 | 2.67 | 2.70 | 0.15 |
| 10 | 2.64 | 2.67 | 2.85 | 2.94 | 0.10 |
| 12 | 2.52 | 2.52 | 2.79 | 2.91 | 0.15 |
| 14 | 2.37 | 2.43 | 2.52 | 2.79 | 0.20 |
| 16 | 2.22 | 2.31 | 2.49 | 2.67 | 0.30 |
| 18 | 2.04 | 2.19 | 2.49 | 2.64 | 0.15 |
| 20 | 2.07 | 2.10 | 2.34 | 2.46 | 0.20 |
| 22 | 1.89 | 2.01 | 2.19 | 2.34 | 0.20 |
| 24 | 1.81 | 1.89 | 2.10 | 2.22 | 0.25 |
| 26 | 1.74 | 1.80 | 2.01 | 2.07 | 0.10 |
| 28 | 1.68 | 1.74 | 1.86 | 1.92 | 0.10 |
| 30 | 1.59 | 1.68 | 1.74 | 1.80 | 0.15 |
| 32 | 1.47 | 1.59 | 1.65 | 1.74 | 0.10 |
| 34 | 1.38 | 1.47 | 1.56 | 1.65 | 0.15 |
| 36 | 1.29 | 1.41 | 1.53 | 1.59 | 0.10 |
| 38 | 1.17 | 1.29 | 1.41 | 1.53 | 0.10 |

Group (1): Sheep vaccinated with inactivated oil trivalent FMD vaccine, (Control +ve)
Group (2): Sheep vaccinated with inactivated oil trivalent FMD vaccine containing (Vit. A)
Group (3): Sheep vaccinated with inactivated oil trivalent FMD vaccine containing (Vit. E-selenium)
Group (4): Sheep vaccinated with inactivated oil trivalent FMD vaccine containing (Vit. A & E-selenium)

- Serum neutralization antibody titer was expressed as $\log_{10}$
- Protective serum antibody titer = 1.5 $\log_{10}$ according to **OIE (2012)**

* There is a significant increase between Group (4) and Group (3), also between Group (3) and Group (2) (at $P < 0.05$)
** There is a significant increase between Group (4) and Group (1), also between Group (4) and Group (2) (at $P < 0.01$)

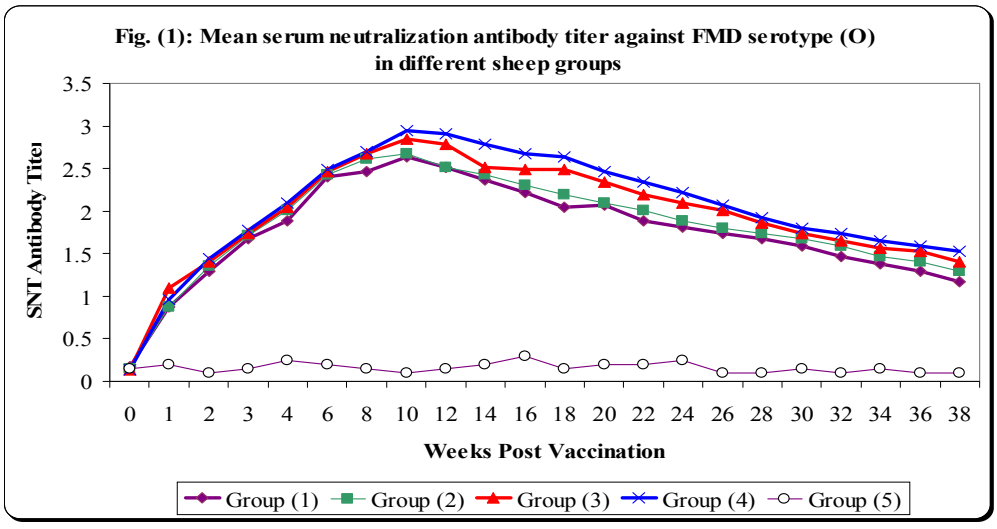

Table 2. Mean ELISA antibody titer against FMD serotype (O) in different sheep groups

| Weeks Post Vaccination | Sheep Groups | | | | |
|---|---|---|---|---|---|
| | Group (1) | Group (2) | Group (3)* | Group (4)** | Control –ve sheep group |
| 0 | 0.17 | 0.23 | 0.42 | 0.15 | 0.15 |
| 1 | 1.17 | 1.17 | 1.26 | 1.38 | 0.25 |
| 2 | 1.53 | 1.59 | 1.71 | 1.68 | 0.25 |
| 3 | 1.89 | 1.98 | 2.04 | 2.07 | 0.20 |
| 4 | 2.13 | 2.19 | 2.19 | 2.31 | 0.25 |
| 6 | 2.34 | 2.40 | 2.25 | 2.49 | 0.30 |
| 8 | 2.49 | 2.70 | 2.76 | 2.79 | 0.25 |
| 10 | 2.73 | 2.76 | 2.94 | 2.94 | 0.15 |
| 12 | 2.58 | 2.64 | 2.91 | 2.94 | 0.25 |
| 14 | 2.34 | 2.43 | 2.76 | 2.82 | 0.25 |
| 16 | 2.25 | 2.40 | 2.61 | 2.70 | 0.30 |
| 18 | 2.04 | 2.22 | 2.52 | 2.67 | 0.30 |
| 20 | 1.98 | 2.16 | 2.40 | 2.49 | 0.20 |
| 22 | 1.80 | 2.10 | 2.25 | 2.37 | 0.25 |
| 24 | 1.80 | 1.98 | 2.16 | 2.25 | 0.20 |
| 26 | 1.65 | 1.86 | 2.10 | 2.19 | 0.20 |
| 28 | 1.65 | 1.80 | 1.92 | 2.11 | 0.20 |
| 30 | 1.47 | 1.68 | 1.86 | 2.07 | 0.25 |
| 32 | 1.41 | 1.56 | 1.77 | 1.98 | 0.25 |
| 34 | 1.26 | 1.50 | 1.62 | 1.92 | 0.20 |
| 36 | 1.26 | 1.44 | 1.56 | 1.83 | 0.20 |
| 38 | 1.05 | 1.32 | 1.50 | 1.71 | 0.20 |

Group (1): Sheep vaccinated with inactivated oil trivalent FMD vaccine, (Control +ve)
Group (2): Sheep vaccinated with inactivated oil trivalent FMD vaccine containing (Vit. A)
Group (3): Sheep vaccinated with inactivated oil trivalent FMD vaccine containing (Vit. E-selenium)
Group (4): Sheep vaccinated with inactivated oil trivalent FMD vaccine containing (Vit. A & E-selenium)

- ELISA antibody titer was expressed as $\log_{10}$
- Protective serum antibody titer = 1.65 $\log_{10}$ according to OIE (2012)

* There is a significant increase between Group (4) and Group (3), also between Group (3) and Group (2) (at $P < 0.05$)
** There is a significant increase between Group (4) and Group (1), also between Group (4) and Group (2) (at $P < 0.01$)

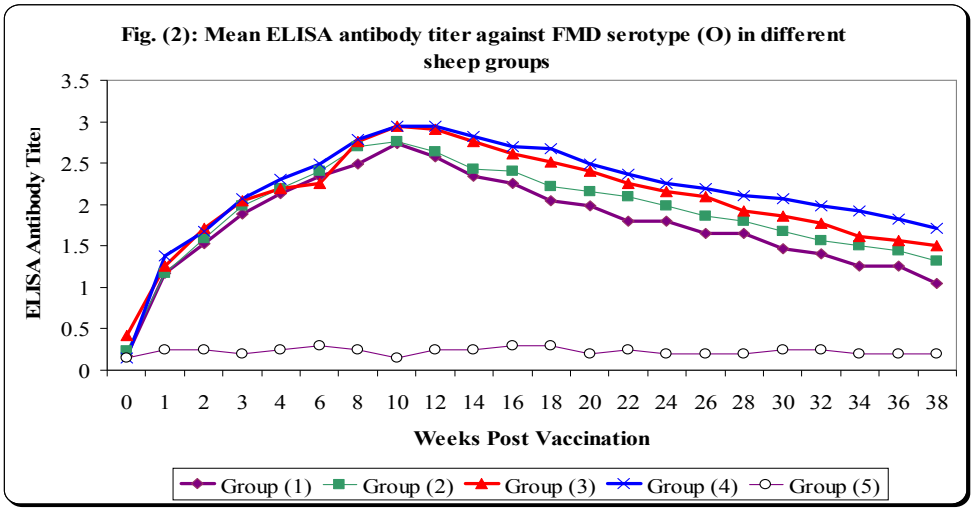

Table 3. Mean serum neutralization antibody titer against FMD serotype (A) in different sheep groups

| Weeks Post Vaccination | Sheep Groups | | | | |
|---|---|---|---|---|---|
| | Group (1) | Group (2) | Group (3)* | Group (4)** | Control –ve sheep group |
| 0 | 0.20 | 0.14 | 0.14 | 0.23 | 0.30 |
| 1 | 1.08 | 1.26 | 1.32 | 1.38 | 0.30 |
| 2 | 1.38 | 1.53 | 1.62 | 1.71 | 0.15 |
| 3 | 1.68 | 1.77 | 1.83 | 1.86 | 0.10 |
| 4 | 1.92 | 2.01 | 2.10 | 2.13 | 0.10 |
| 6 | 2.22 | 2.31 | 2.34 | 2.40 | 0.25 |
| 8 | 2.52 | 2.58 | 2.64 | 2.67 | 0.30 |
| 10 | 2.67 | 2.73 | 2.94 | 2.87 | 0.30 |
| 12 | 2.64 | 2.67 | 2.94 | 2.94 | 0.10 |
| 14 | 2.52 | 2.58 | 2.82 | 2.82 | 0.10 |
| 16 | 2.43 | 2.46 | 2.70 | 2.76 | 0.30 |
| 18 | 2.34 | 2.40 | 2.58 | 2.64 | 0.15 |
| 20 | 2.16 | 2.16 | 2.46 | 2.55 | 0.15 |
| 22 | 1.95 | 2.16 | 2.22 | 2.46 | 0.30 |
| 24 | 1.98 | 2.07 | 2.19 | 2.43 | 0.30 |
| 26 | 1.92 | 1.98 | 2.01 | 2.28 | 0.10 |
| 28 | 1.83 | 1.86 | 1.92 | 2.04 | 0.10 |
| 30 | 1.74 | 1.77 | 1.83 | 2.04 | 0.15 |
| 32 | 1.65 | 1.71 | 1.71 | 1.89 | 0.25 |
| 34 | 1.56 | 1.59 | 1.65 | 1.89 | 0.25 |
| 36 | 1.50 | 1.53 | 1.56 | 1.74 | 0.10 |
| 38 | 1.41 | 1.44 | 1.50 | 1.59 | 0.10 |

Group (1): Sheep vaccinated with inactivated oil trivalent FMD vaccine, (Control +ve)
Group (2): Sheep vaccinated with inactivated oil trivalent FMD vaccine containing (Vit. A)
Group (3): Sheep vaccinated with inactivated oil trivalent FMD vaccine containing (Vit. E-selenium)
Group (4): Sheep vaccinated with inactivated oil trivalent FMD vaccine containing (Vit. A & E-selenium)

- Serum neutralization antibody titer was expressed as $\log_{10}$
- Protective serum antibody titer = 1.5 $\log_{10}$ according to OIE (2012)

* There is a significant increase between Group (4) and Group (3), also between Group (3) and Group (2) (at $P < 0.05$)
** There is a significant increase between Group (4) and Group (1), also between Group (4) and Group (2) (at $P < 0.01$)

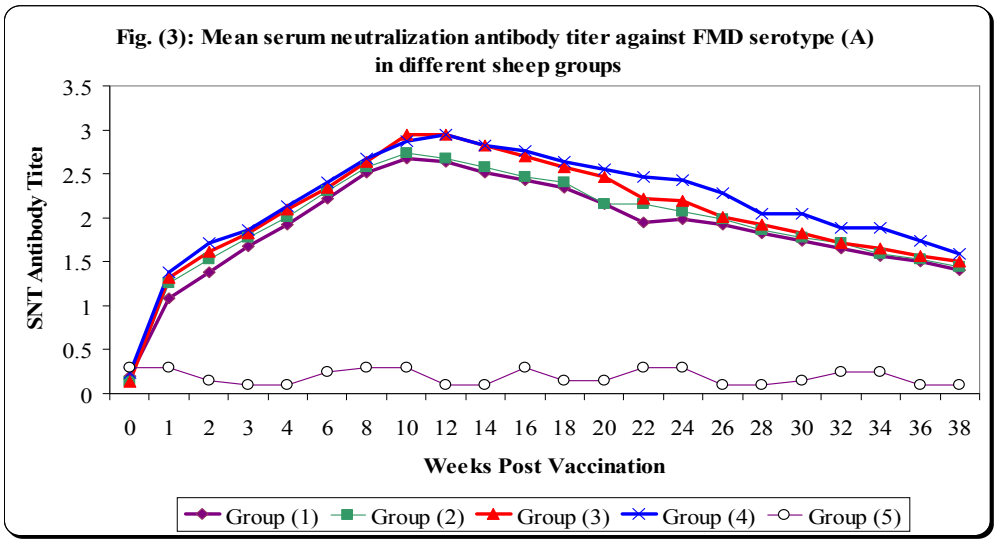

Table 4. Mean ELISA antibody titer against FMD serotype (A) in different sheep groups

| Weeks Post Vaccination | Sheep Groups | | | | |
|---|---|---|---|---|---|
| | Group (1) | Group (2) | Group (3)* | Group (4)** | Control –ve sheep group |
| 0 | 0.19 | 0.20 | 0.20 | 0.19 | 0.15 |
| 1 | 1.41 | 1.56 | 1.62 | 1.68 | 0.10 |
| 2 | 1.59 | 1.83 | 1.92 | 2.01 | 0.30 |
| 3 | 2.16 | 2.16 | 2.19 | 2.25 | 0.15 |
| 4 | 2.37 | 2.34 | 2.43 | 2.46 | 0.30 |
| 6 | 2.52 | 2.40 | 2.55 | 2.64 | 0.25 |
| 8 | 2.67 | 2.73 | 2.82 | 2.82 | 0.30 |
| 10 | 2.67 | 2.73 | 2.94 | 2.97 | 0.25 |
| 12 | 2.40 | 2.70 | 3.00 | 2.97 | 0.20 |
| 14 | 2.52 | 2.61 | 2.88 | 2.91 | 0.20 |
| 16 | 2.46 | 2.52 | 2.73 | 2.79 | 0.25 |
| 18 | 2.43 | 2.46 | 2.67 | 2.76 | 0.30 |
| 20 | 2.34 | 2.28 | 2.52 | 2.64 | 0.25 |
| 22 | 2.07 | 2.22 | 2.37 | 2.52 | 0.30 |
| 24 | 2.07 | 2.16 | 2.34 | 2.40 | 0.15 |
| 26 | 1.95 | 2.04 | 2.19 | 2.34 | 0.25 |
| 28 | 1.92 | 1.98 | 2.07 | 2.19 | 0.30 |
| 30 | 1.74 | 1.86 | 1.92 | 2.13 | 0.30 |
| 32 | 1.71 | 1.80 | 1.89 | 2.01 | 0.30 |
| 34 | 1.62 | 1.71 | 1.77 | 1.92 | 0.25 |
| 36 | 1.62 | 1.68 | 1.74 | 1.83 | 0.20 |
| 38 | 1.56 | 1.56 | 1.62 | 1.71 | 0.20 |

Group (1): Sheep vaccinated with inactivated oil trivalent FMD vaccine, (Control +ve)
Group (2): Sheep vaccinated with inactivated oil trivalent FMD vaccine containing (Vit. A)
Group (3): Sheep vaccinated with inactivated oil trivalent FMD vaccine containing (Vit. E-selenium)
Group (4): Sheep vaccinated with inactivated oil trivalent FMD vaccine containing (Vit. A & E-selenium)

- ELISA antibody titer was expressed as $\log_{10}$
- Protective serum antibody titer = 1.65 $\log_{10}$ according to OIE (2012)

* There is a significant increase between Group (4) and Group (3), also between Group (3) and Group (2) (at $P < 0.05$)
** There is a significant increase between Group (4) and Group (1), also between Group (4) and Group (2) (at $P < 0.01$)

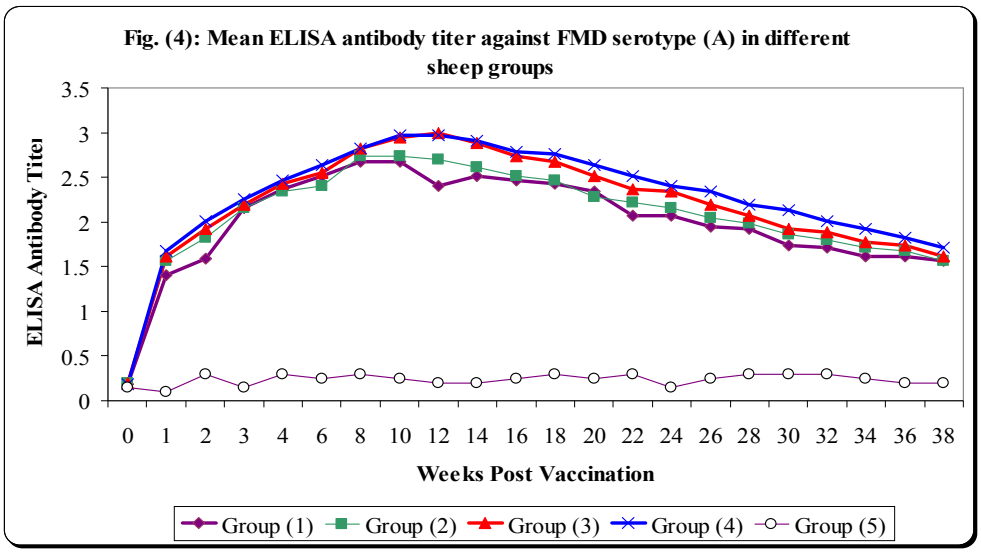

Table 5. Mean serum neutralization antibody titer against FMD serotype (SAT2) in different sheep groups

| Weeks Post Vaccination | Sheep Groups | | | | |
|---|---|---|---|---|---|
| | Group (1) | Group (2) | Group (3)* | Group (4)** | Control –ve sheep group |
| 0 | 0.27 | 0.14 | 0.15 | 0.14 | 0.15 |
| 1 | 0.66 | 0.66 | 0.78 | 0.78 | 0.10 |
| 2 | 0.96 | 1.23 | 1.29 | 1.29 | 0.15 |
| 3 | 1.26 | 1.41 | 1.56 | 1.53 | 0.10 |
| 4 | 1.59 | 1.71 | 1.74 | 1.83 | 0.10 |
| 6 | 1.86 | 1.95 | 2.01 | 2.07 | 0.25 |
| 8 | 2.22 | 2.16 | 2.25 | 2.34 | 0.20 |
| 10 | 2.46 | 2.43 | 2.55 | 2.61 | 0.15 |
| 12 | 2.31 | 2.43 | 2.49 | 2.58 | 0.20 |
| 14 | 2.25 | 2.28 | 2.34 | 2.46 | 0.15 |
| 16 | 2.10 | 2.22 | 2.19 | 2.31 | 0.30 |
| 18 | 2.04 | 2.07 | 2.07 | 2.16 | 0.20 |
| 20 | 1.92 | 2.01 | 2.01 | 2.07 | 0.15 |
| 22 | 1.80 | 1.86 | 1.89 | 1.92 | 0.10 |
| 24 | 1.71 | 1.83 | 1.86 | 1.89 | 0.15 |
| 26 | 1.56 | 1.68 | 1.71 | 1.74 | 0.20 |
| 28 | 1.56 | 1.62 | 1.71 | 1.74 | 0.25 |
| 30 | 1.41 | 1.53 | 1.56 | 1.59 | 0.15 |
| 32 | 1.26 | 1.47 | 1.53 | 1.59 | 0.10 |
| 34 | 1.26 | 1.32 | 1.41 | 1.44 | 0.15 |
| 36 | 1.05 | 1.23 | 1.26 | 1.26 | 0.20 |
| 38 | 0.96 | 1.14 | 1.20 | 1.26 | 0.20 |

Group (1): Sheep vaccinated with inactivated oil trivalent FMD vaccine, (Control +ve)
Group (2): Sheep vaccinated with inactivated oil trivalent FMD vaccine containing (Vit. A)
Group (3): Sheep vaccinated with inactivated oil trivalent FMD vaccine containing (Vit. E-selenium)
Group (4): Sheep vaccinated with inactivated oil trivalent FMD vaccine containing (Vit. A & E-selenium)

- Serum neutralization antibody titer was expressed as $\log_{10}$
- Protective serum antibody titer = 1.5 $\log_{10}$ according to OIE (2012)

* There is a significant increase between Group (4) and Group (3), also between Group (3) and Group (2) (at $P < 0.05$)
** There is a significant increase between Group (4) and Group (1), also between Group (4) and Group (2) (at $P < 0.01$)

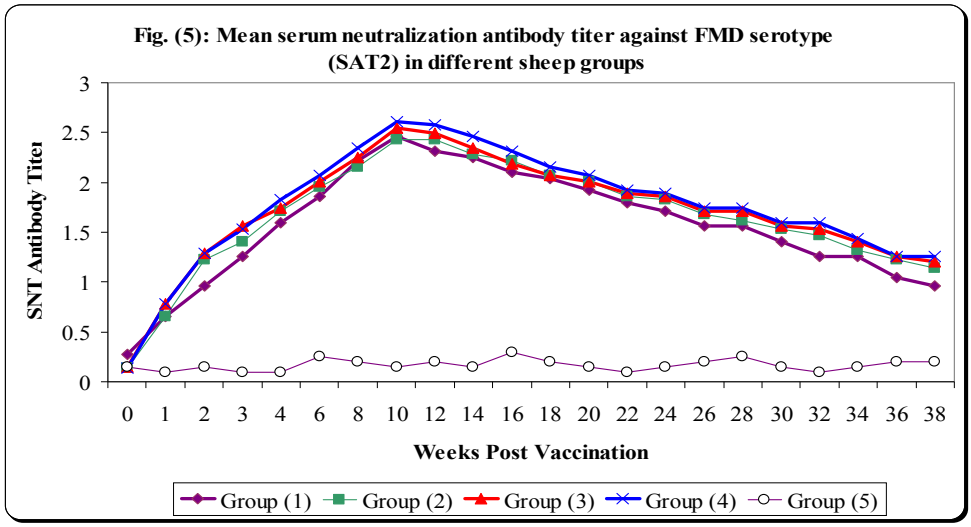


**Fig. (5): Mean serum neutralization antibody titer against FMD serotype (SAT2) in different sheep groups**

Table 6. Mean ELISA antibody titer against FMD serotype (SAT2) in different sheep groups

| Weeks Post Vaccination | Sheep Groups | | | | |
|---|---|---|---|---|---|
| | Group (1) | Group (2) | Group (3)* | Group (4)** | Control –ve sheep group |
| 0 | 0.20 | 0.14 | 0.15 | 0.15 | 0.30 |
| 1 | 0.96 | 0.93 | 0.93 | 0.96 | 0.25 |
| 2 | 1.26 | 1.50 | 1.59 | 1.53 | 0.30 |
| 3 | 1.68 | 1.80 | 1.80 | 1.74 | 0.30 |
| 4 | 1.89 | 2.01 | 1.95 | 1.98 | 0.25 |
| 6 | 1.95 | 2.04 | 2.10 | 2.07 | 0.20 |
| 8 | 2.25 | 2.34 | 2.37 | 2.49 | 0.20 |
| 10 | 2.46 | 2.52 | 2.55 | 2.64 | 0.20 |
| 12 | 2.43 | 2.55 | 2.55 | 2.64 | 0.25 |
| 14 | 2.25 | 2.49 | 2.40 | 2.49 | 0.30 |
| 16 | 2.10 | 2.31 | 2.34 | 2.40 | 0.30 |
| 18 | 2.04 | 2.13 | 2.28 | 2.25 | 0.30 |
| 20 | 1.92 | 1.98 | 2.16 | 2.07 | 0.30 |
| 22 | 1.86 | 1.89 | 2.04 | 1.92 | 0.25 |
| 24 | 1.77 | 1.83 | 2.01 | 1.89 | 0.20 |
| 26 | 1.65 | 1.74 | 1.86 | 1.74 | 0.20 |
| 28 | 1.59 | 1.68 | 1.89 | 1.59 | 0.20 |
| 30 | 1.50 | 1.56 | 1.71 | 1.59 | 0.20 |
| 32 | 1.35 | 1.41 | 1.53 | 1.59 | 0.25 |
| 34 | 1.23 | 1.26 | 1.56 | 1.47 | 0.20 |
| 36 | 1.26 | 1.20 | 1.35 | 1.41 | 0.20 |
| 38 | 1.08 | 1.02 | 1.35 | 1.32 | 0.20 |

Group (1): Sheep vaccinated with inactivated oil trivalent FMD vaccine, (Control +ve)
Group (2): Sheep vaccinated with inactivated oil trivalent FMD vaccine containing (Vit. A)
Group (3): Sheep vaccinated with inactivated oil trivalent FMD vaccine containing (Vit. E-selenium)
Group (4): Sheep vaccinated with inactivated oil trivalent FMD vaccine containing (Vit. A & E-selenium)

- ELISA antibody titer was expressed as $\log_{10}$
- Protective serum antibody titer = 1.65 $\log_{10}$ according to OIE (2012)

* There is a significant increase between Group (4) and Group (3), also between Group (3) and Group (2) (at $P < 0.05$)
** There is a significant increase between Group (4) and Group (1), also between Group (4) and Group (2) (at $P < 0.01$)

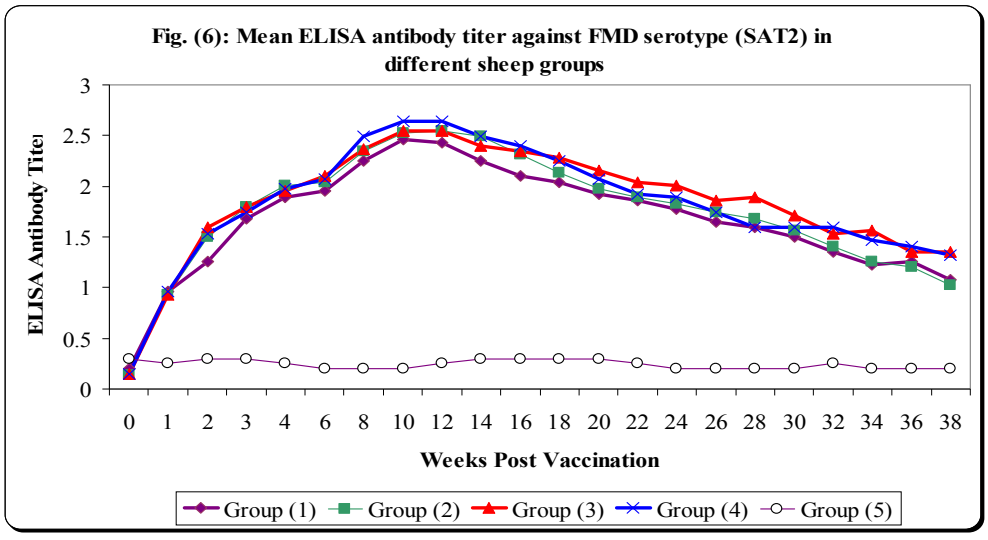

Table 7. Cell mediated immune response (expressed as Delta Optical Density) of vaccinated sheep were vaccinated with different inactivated trivalent FMD vaccines

| Groups | | Days Post Vaccination | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 0 | 3 | 7 | 14 | 21 | 28 | 35 | 42 |
| 1 | Non-specific mitogen | 0.051 | 0.121 | 0.324 | 0.383 | 0.510 | 0.463 | 0.340 | 0.265 |
| | FMDV as specific mitogen | 0.060 | 0.231 | 0.420 | 0.490 | 0.600 | 0.550 | 0.490 | 0.350 |
| 2 | Non-specific mitogen | 0.067 | 0.197 | 0.401 | 0.464 | 0.600 | 0.565 | 0.480 | 0.390 |
| | FMDV as specific mitogen | 0.070 | 0.289 | 0.500 | 0.602 | 0.710 | 0.671 | 0.620 | 0.495 |
| 3* | Non-specific mitogen | 0.055 | 0.245 | 0.490 | 0.522 | 0.711 | 0.650 | 0.557 | 0.415 |
| | FMDV as specific mitogen | 0.076 | 0.345 | 0.589 | 0.700 | 0.803 | 0.755 | 0.700 | 0.600 |
| 4** | Non-specific mitogen | 0.059 | 0.271 | 0.574 | 0.646 | 0.820 | 0.768 | 0.691 | 0.470 |
| | FMDV as specific mitogen | 0.065 | 0.400 | 0.610 | 0.790 | 0.910 | 0.835 | 0.800 | 0.630 |
| 5 | Non-specific mitogen | 0.053 | 0.064 | 0.057 | 0.051 | 0.060 | 0.058 | 0.067 | 0.069 |
| | FMDV as specific mitogen | 0.061 | 0.059 | 0.053 | 0.060 | 0.054 | 0.052 | 0.061 | 0.060 |

Group (1): (Control positive group) Sheep vaccinated with inactivated trivalent FMD vaccine
Group (2): Sheep vaccinated with inactivated trivalent FMD vaccine containing Vit. A.
Group (3): Sheep vaccinated with inactivated trivalent FMD vaccine containing Vit. (E-selenium).
Group (4): Sheep vaccinated with inactivated trivalent FMD vaccine containing both Vit. A and Vit. (E-selenium).
Group (5): (Control negative group) sheep non-vaccinated

* There is a significant increase between Group (4) and Group (3), also between Group (3) and Group (2) (at $P < 0.05$)

** There is a significant increase between Group (4) and Group (1), also between Group (4) and Group (2) (at $P < 0.01$)

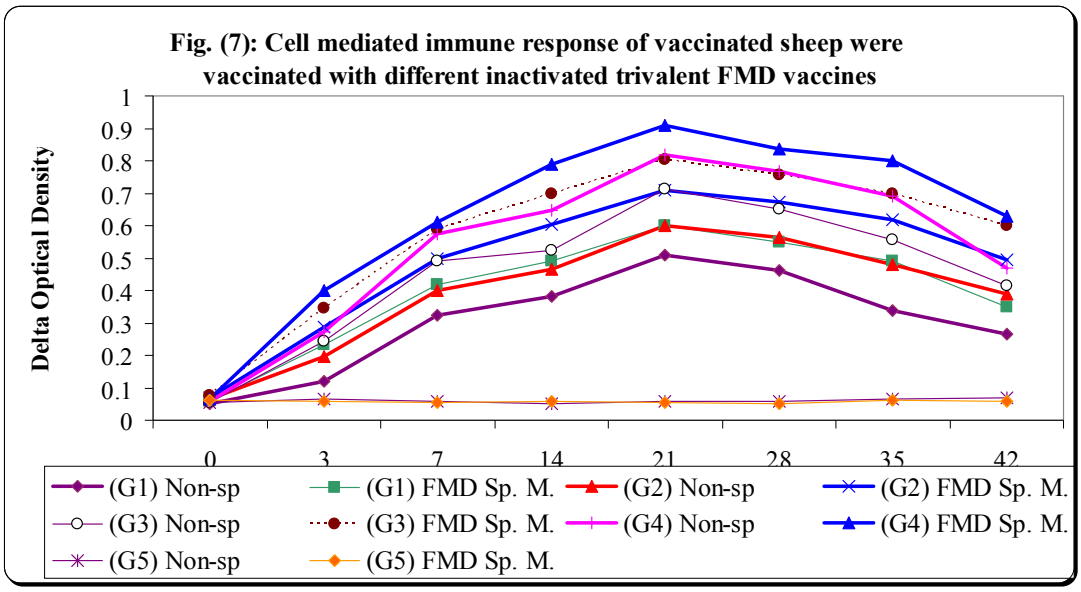


**Fig. (7): Cell mediated immune response of vaccinated sheep were vaccinated with different inactivated trivalent FMD vaccines**

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# دراسة المناعة المصلية والخلوية فى الأغنام المحصنة بأنواع مختلفة من لقاحات الحمى القلاعية المثبطة

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صممت هذه الدراسة كمحاولة لمقارنة تأثير كلاً من لقاح الحمى القلاعية المنتج حالياً ولقاح الحمى القلاعية مضاف اليه فيتامين "A" ولقاح الحمى القلاعية مضاف إليه فيتامين "E" سيلينيوم ونفس اللقاح مضاف إليه كلاً من فيتامين "A" – "E" سيلينيوم معاً. وقد أوضحت النتائج أن المناعة المصلية تمتد لمدة ٣٠ اسبوع فى أغنام المجموعة الأولى المحقونة باللقاح المنتج محلياً بينما المدة كانت ٣٢ اسبوع بالنسبة للمجموعة الثانية المحقونة بنفس اللقاح مضاف إليه فيتامين "A" ووصلت المدة الى ٣٦ اسبوع فى المجموعة الثالثة المحقونة باللقاح المنتج محلياً مضاف إليه فيتامين "E" سيلينيوم وكانت المدة ٣٨ اسبوع فى أمصال المجموعة الرابعة المحقونة باللقاح المنتج مضاف إليه كلاً من فيتامين "A" – "E" سيلينيوم معاً. أما المناعة الخلوية فقد أوضحت التجارب أن كل الأغنام المحقونة باللقاحات المختلفة للحمى القلاعية أعطت أعلى متوسط حسابى للكثافة الضوئية (Optical Density) بعد الحقن ب ٢١ يوم وذلك باستخدام تقنية (MTT-Kit) والتى تقيس الميتوجين غير المتخصصة للحمى القلاعية وعند قياس الكثافة الضوئية للميتوجين المتخصصة للحمى القلاعية وجد أنهما أعلى قيماً من تلك غير المتخصصة وذلك فى الأغنام المحصنة بلقاح الحمى القلاعية مضاف إليه فيتامين "A" – "E" سيلينيوم معاً.