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RESEARCH

Cytokines profile and antibody response of calves vaccinated with conventional inactivated Trivalent FMD vaccine

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ABSTRACT

In the present study, eight (sero-negative for FMDV) native breed calves (6-12 months of age) were used to study the cytokine profile including IL-2, IL-4, IFN-γ and antibody response of calves vaccinated with conventional inactivated trivalent FMD vaccine. Expression of Th1 and Th2 was determined using quantitative ELISA. Results reveled peaks of IL-4 at 7th day, IFN-γ at 14th day and IL-2 at 21st day post vaccination. Lymphocytic proliferation assays demonstrated the continuous response of lymphocytes started at the 3rd day till reaches peak at 28th day post vaccination then start to decline. Whereas the peak titers for antibodies were reported at the 28th day post vaccination. Indeed, the study reports the role of ail adjuvant in stimulating the cellular response for inactivated vaccine.

Keywords: FMD, Cytokines, MTT, VNT and cross-protection.

BACKGROUND

Foot and mouth disease (FMD) is a highly contagious and infectious disease affecting biungulate species both wild and domestic animals. Infected cases by Foot and mouth disease virus (FMDV) are characterized by vesicles in the buccal cavity, on the teats, udders, on the coronary bands and in the interdigital cleft then erosions develop and finally ulcers. Deaths of young animals under six months old age (OIE, 2017) with potentially disastrous consequences (Tajdini F. et al. 2014).

In endemic areas with FMD virus, vaccination is the most successful control strategy for the virus (Rodriguez and Grubman, 2009). The vaccination decision is complex to take and varies according to the economic, political and scientific state of the country (Karin Orsel and Annemarie Bouma, 2009) Even that it considered best method of control during outbreaks in developing countries as the cost of eradication is high to afford and the animal population distributed randomly all over the country.

Vaccination with inactivated FMD vaccine with one or multiple doses parenterally are being applied in routine field vaccination campaigns (Kahn et al., 2002).

Using oil as an adjuvant in FMD vaccines formulation resulted in trapping of 140-400 times more virus particles than the vaccines produced using aluminum hydroxide (Maes and Fernandes, 1969). Oil adjuvanted vaccines provide a long lasting immunity compared with aluminum hydroxide saponine vaccine (Auge de mello p. et. al. 1977).

The use of adjuvants may lessen the antigenic load or the multiple immunizations to urge protective immune response (Coffman et al., 2010). In this study, FMD vaccine formulated using Montanide ISA 206 as an adjuvant was injected subcutaneously. Cytokines profile and antibody response in vaccinated calves were determined in 42 days duration.

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MATERIALS AND METHODS

Animals

Eight Native breed (baladi) calves with 6-12 months of age (serologically negative against FMDV tested by Virus neutralization test were used in the study according to "European Communities Council Directive 1986 (86/609/EEC)" guidelines and recommendations. All experimental animals were isolated in veterinary serum and vaccine research institute isolators.

FMD viruses

FMD virus strain O Pan-Asia/2012, A/Iran/05, SAT 2/EGY/2012, O/ East Africa 3 (EA-3) were obtained from Veterinary Serum and Vaccine Research Institute and Central Laboratory for Evaluation of Veterinary Biologics (CLEVB) which was used in both vaccine formulation, virus neutralization test and challenge test.

Vaccine formulation

The three virus strains were propagated on BHK-21 clone 13 cell line at 37 ^{o}C for 18-21 hours, cytopathologic effect was observed. After harvestation, three cycles of freezing and thawing were done followed by clarification at 2500 rpm for 30 minutes for the removal of any cell debris. Then inactivation of the viruses was carried out using binary ethylene -imine (BEI) (Bahnemann, 1975). Montanide ISA 206 (Incomplete Seppic Adjuvant) VG (Vegetative origin) was used as an adjuvant. The inactivated antigen with concentration of 3-4 μg was used in formulation of the trivalent vaccine administrated subcutaneously according to Pan et al., 2014. The antigen was quantified by sucrose density gradient centrifugation (SDG) technique described by (Barteling and Meloen, 1974). According to manufacturer's guidelines for Montanide ISA 206 formula, the antigen-adjuvant ratio was determined. Sterility and safety tests were performed according to OIE (2008).

Immunization of Calves

The calves were divided into 2 groups; Vaccinated group (n=5) calves and control non-vaccinated group (n=3). Each calf was vaccinated by 3 ml of the prepared vaccine subcutaneously, Blood was collected from the jugular vein at day 0 (Pre-Vaccination) to confirm the sero-negativity of calves, day 3, 7, 14, 21, 28, 35 and 42 post vaccination. Part of the collected blood was received on Heparin for lymphocytic proliferation assay and the other part was left to be used in other assays.

Challenge test

Both vaccinated and non-vaccinated calves were challenged intradermaolingually at 3-5 different sites with 0.3 ml of 10^4 ID50 (OIE,2017) FMDV; O/East Africa 3 (EA-3) at 28 days post vaccination. Daily observation of calves for 7 days post challenge was carried out for detection of clinical symptoms as excessive salivation, rise in temperature and appearance of vesicles in oral cavity and on the interdigital cleft according to OIE,2008. Determination of interleukin-2, interleukin-4 and interferon gamma in serum of immunized calves. To evaluate the cytokines expression levels of the vaccinated calves. IL-2, IL-4 and IFN-γ were detected using Bovine IL-2, IL-4 and IFN-γ ELISA development kit from MABTECH® (Sweden). All samples were duplicated and the results were presented as a mean per group.

Lymphocytic proliferation assay

Lymphocyte proliferation of the vaccinated calves was done using MTT colorimetric assay according to Nagarajan et al., (2011). Lymphocyte cell suspension was seeded to the 96 well tissue culture plate 2000 cell/ well and 100 µl of growth media was added in each well. The plate was incubated at 37°c for 24-48 hours. Then 50 µl of MTT (1x) was added in each well and incubated at 37°c for 1-4 hours. one hundred and fifty µl of DMSO was added to dissolve the MTT formazan (stopping solution). The plate was read via ELISA reader at wavelength 570 nm. Triplicate cultures were set for each treatment.

Results were expressed as SI (Stimulation index)

$$\frac{O.D.570\ of\ sample\ -\ O.D.of\ negative\ control}{O.D.570 of\ negative\ control}$$

Virus neutralization Test:

The serum neutralizing antibodies titers were quantified using virus neutralization assay. The serum was collected and heat inactivated at 56 ^{O}C for 30 minutes The obtained sera were incubated with 100 $TCID_{50}$ FMDV for 1 hour and added on the BHK-21 clone 13 followed by incubation at 37 ^{O}C for 48 hours The titers were calculated as the Log10 of the reciprocal antibody dilution which able to neutralize 100 $TCID_{50}$ of the virus (OIE, 2017).

RESULTS

Protection percentage in vaccinated calves

The vaccinated group shows 80% protection while the control challenged group shows rise in temperature, erosion in the tongue dorsum and in the interdigital cleft.

IL-2 response

IL-2 levels were elevated 7dpv (1.163 ± 0.070) followed by decrease in 14dpv (0.772 ± 0.002) and finally reach it peaks at 21 dpv (1.783 ± 0.115) as shown in (fig.1).

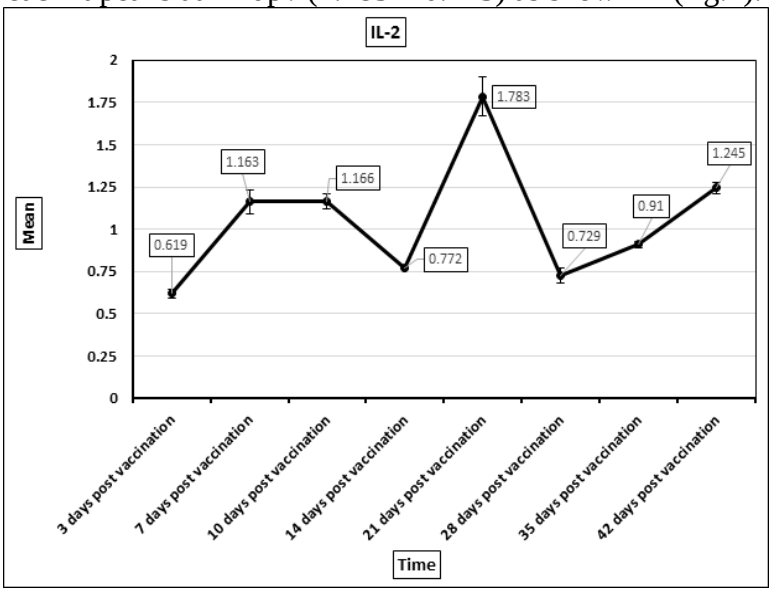

Fig. 1: Mean optical densities obtained for IL-2 in sera of vaccinated calves along the 42 days of the experiment.

IL-4 response

At 7 dpv, IL-4 reach its maximum level (0.123 ± 0.001) and start to decline at 10 dpv (0.087 ± 0.001) then increase at 14 dpv (0.108 ± 0.028) as in (fig. 2).

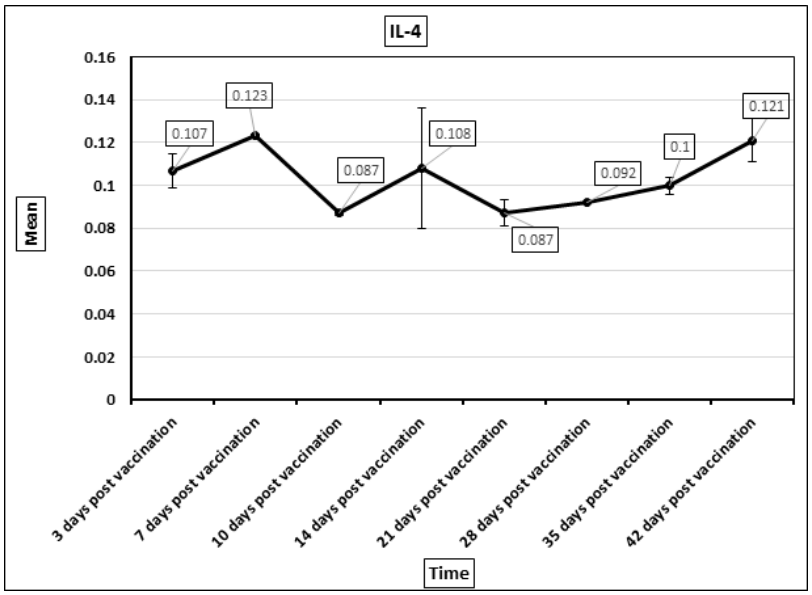

Fig. 2: Mean optical densities obtained for IL-4 in sera collected from vaccinated calves along the 42 days of the experiment.

IFN-Gamma response

IFN-Gamma begin to increase on a weekly basis till it reached its higher level at 14dpv (0.291 ± 0.033) followed by decrease until the fourth week of observation (fig. 3).

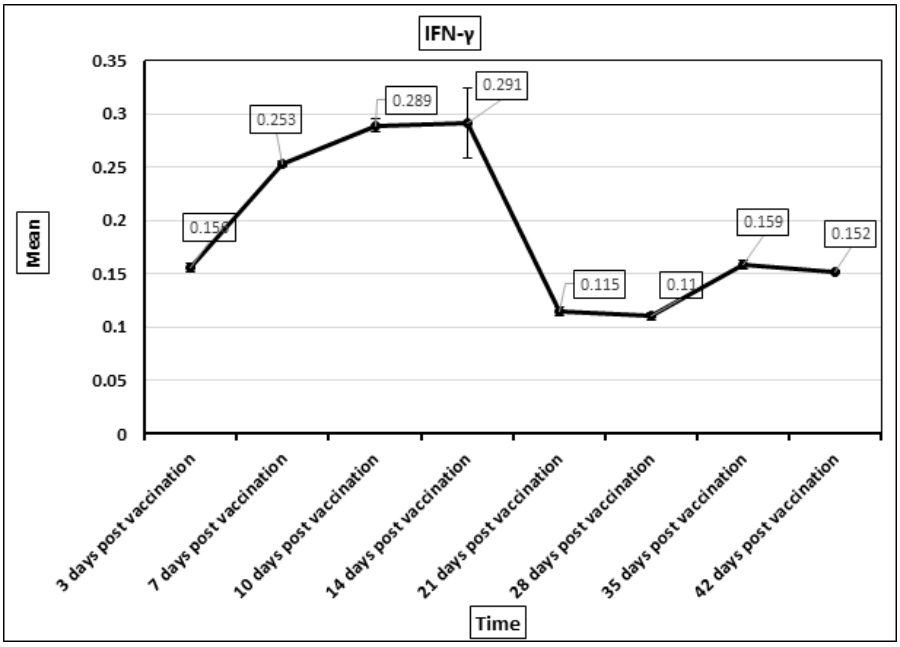

Fig. 3: Mean optical densities obtained for IFN-γ in vaccinated calves sera along the 42 days of the experiment.

Lymphocytic proliferation assay

At 7dpv, the vaccinated group reveals an increase in lymphocytes proliferation and reach its peak stimulation index level at 28 dpv (0.844 ± 0.009) as in (fig.4).

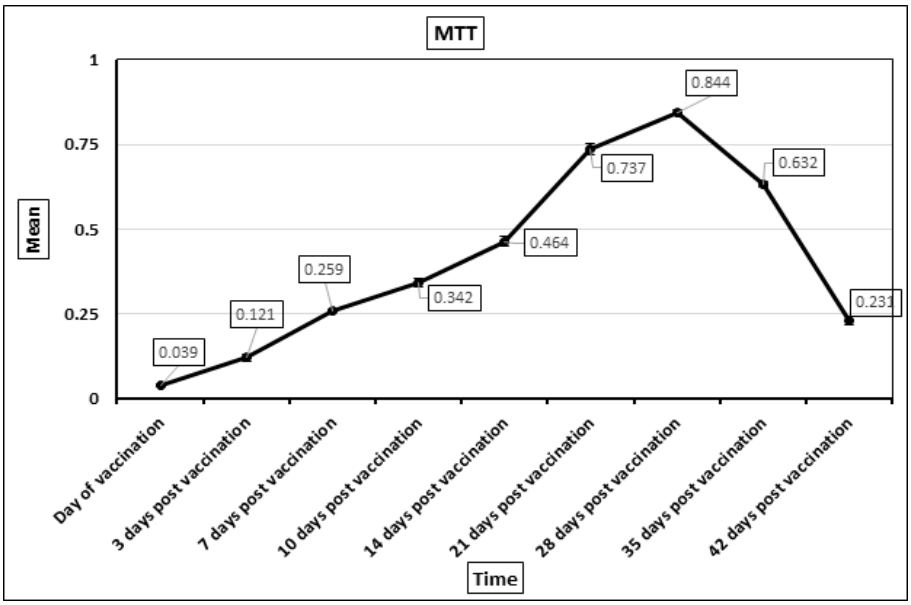

Fig. 4: Lymphocytic proliferation assay of PBMCs in experimental group following immunization with the prepared FMD vaccine (MTT assay) from 3 days to 42 days post vaccination. Data expressed as Mean (570nm).

Neutralizing antibodies response

Calves showed increasing levels of serum neutralizing antibodies shown in (fig. 5) week after week and peaked at 28 dpv (1.560 ± 0.624) which is considered protective as (fig. 5).

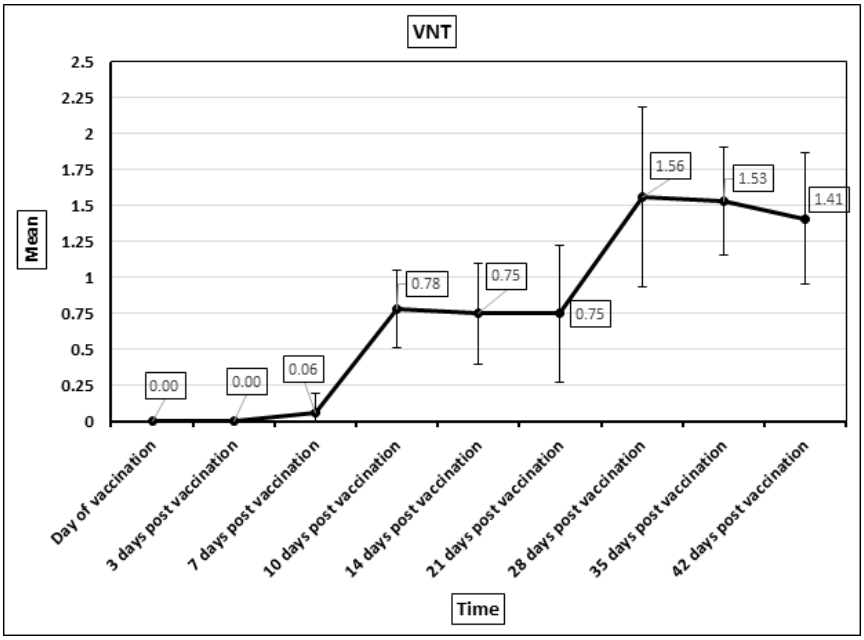

Fig. 5: Mean neutralizing antibody titer (log_{10}) induced by the FMD prepared vaccine in serum from 0 day to 42 days post vaccination. According to OIE,2017

DISCUSSION

In the present study, we prepared trivalent FMD inactivated vaccine containing 3 serotypes O Pan-Asia/2012, A/Iran/05, SAT 2/EGY/2012. The formulation of the prepared vaccine was based on the use of oil adjuvant Montanide ISA 206 according to the manufacture's guidelines in the form of water-in-oil-in-water which is currently used in the vaccine production institute. The addition of ISA 206 in the formula is expected to enhance the immune response of the vaccinated calves to the utilized vaccine as such oil is a mineral oil that stimulate the natural immunity and thus, reflected on the magnitude of the specific humoral immune response (Patil et al. 2002; Fowler et al. 2011). In the current study, the vaccinated calves demonstrated protection of 80% confirming that the prepared vaccine was valid in the challenge test (OIE, 2017). We used the O/East Africa-3 serotype of the FMD which is circulated in Egypt Since 2016 and causes many outbreaks in different governorates (Muhammad et al. 2018)

We decided to use such strain to standpoint the cross protection induced by the currently used seed virus O Pan-Asia/2012 which used as a vaccine strain for the last 7 years against the recent circulating strain O/East Africa-3. Results obtained for cytokines profile in the vaccinated calves revealed stimulation of cellular immunity as IL-2, IL-4 and IFN-γ expression levels which were increased started 7 days and peaked 21 days post vaccination.

Th1 (IL-2 and IFN-γ) and Th2 (IL-4) are the two types of cytokines known that they counteract with each other. IL-4 boost Th2 cytokines production collectively while suppress Th1 cytokines expression and vice versa (Forshubor et al. 1996; Bembridge et al. 1998).

Dendritic cells (DCs) begins the interaction simultaneously when facing either pathogens or receiving signals via chemokines from the periphery,

followed by production of cytokines such as IL-1, IL-2 and IL-12, which are crucial activators of natural killer cells (NK). NK cells in counter secret more cytokines, especially tumour necrosis factor alpha (TNF-α) and IFN- γ which have a regulatory impact on DC and an activating action on gamma delta T cells (γ δ T) (Toka, Golde. 2013). The major activities of IL-2 promote clonal expansion of antigen activated CD8+ T cell (Kendall A.S. 2000), serves as Growth factor for CD4+ T cell (Gullberg and Smith, 1986) and Natural killer cells (NK) (Caligiuri et al., 1990). While, IL-4 secreted from T cells and differentiated Th2 cells (Achsah D. Keegan, 2000), its main function; regulation of the naive CD4+ T cell differentiation (Seder and Paul, 1994 & Swain, 1994) into helper phenotype Th2 cells. Beside, co-stimulation of T and B cells proliferation (Achsah D. Keegan, 2000). Hence, it will reflect on the antibodies production in the vaccinated animals as seen in our study.

IFN-γ does not entail a molecular relation between IFN-α and IFN-β but it reflects sharing in the biological activity against viral infection for cell protection (Alfons Billiau and Koen Vandenbroeck, 2000) and its action is antagonized by IL-4 (Gautam, 1992).In the present study, we determined the expression level of IFN- ɣ in vaccinated calves and proved its induction as in fig.(3). IFN-ɣ is essential in the initial phase of immune reaction; aspecific inflammatory and antigen presenting, in the middle phase; expression and differentiation of antigen reactive lymphocytes clones and finally in the end phase; sustained inflammation). Collectively, as seen in our results IL-2, IL-4 and IFN- ɣ are expressed as inflammatory process to vaccination as in figures (I,2 and 3).

Therefore, IL-2, IL-4, IFN-γ have an impact on the humoral immunity i.e antibody production against the vaccine strain used which even low antibody production against the heterologous strain but, it contributes to the protection in combined with cellular mechanisms and cytokines production (heterotypic protection) (Cox et al. 2003; Parida et al. 2006) and IFN-γ play an important role in protection as well (Oh et al. 2006)

The early expression of Th1 cytokine represented by IFN-gamma that appear in the first week post vaccination corresponds with the early defense by the immune system. On the other hand, lymphocytes start to proliferate from the third day following vaccination and reached its highest level during experiment in the day 28 post vaccination then begin to decline after 7 days post challenge and decrease furthermore after 14 days post challenge. The levels of antibody titers in vaccinated calves reached its crest at 28 days post vaccination then decline at day 42 post vaccination.

CONCLUSION

Indeed, the study reports the induction of cellular and humoral immune response in vaccinated calves proving the success of the vaccine uptake, presentation and stimulation as well as heterogeneous response that lead to protection against different strain of the O serotypes and the ability of O/Pan-Asia/2012 seed virus to cross react with broad spectrum of strains belongs to the same serotype.

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