EVALUATION OF THE IMMUNE RESPONSE OF TURKISH FMD VACCINE IN IRAQI AWASSI SHEEP EXPERIMENTALLY

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ABSTRACT

This work was designed to determine the protective immune response of widely used FMD vaccines in Iraqi Awassi sheep experimentally. Sixteen Awassi sheep were selected as seronegative for FMD (8 male and 8 female) 9-12 months aged divided into vaccinated group and control group (8 animals for each group), sheep in vaccinated group inoculated by 1 ml of trivalent FMD vaccine subcutaneously in right axilla and the control groups gave 1 ml of sterile PBS at same site, serums were collected from all experiment at 0, 14, 21, 28, 42, 60 days post vaccination (DPV), so booster dose was inoculated at day 42 post vaccination to four (4) animals in vaccinated group.

LPB-ELISA exhibited peak of antibodies against A FMDV serotype (0.695± 0.06) at 28 DPV, that higher significantly than Abs against O serotype (0.562 ± 0.2), and interestingly, the booster dose at 42 DPV increased Abs titer against O strain (0.829 ± 0.153) significantly higher than against A strain (0.783 ± 0.12).

This vaccine, enhance cellular immune response by increase the concentrations of IL4 and INF-Ŷ that measuring by commercial kits, IL4 concentration elevated and the peak reach at 28 days of experiment (14.62 ± 0.48), but the significant increase of INF production at 28 days of experiment when compare vaccinated group (162.2 ± 12.) with control group (145.7 ± 5.07) and the higher peak of INF concentration recorded in 42 days post vaccination (175.6 ±20)

We concluded, the vaccination against FMD in Awassi sheep by commercial vaccine (Turkish) was enhancement humeral immune response especially against A serotype, so it effective to stimulate cellular immune response and produce protective immune response against FMD, and the booster dose of vaccine is recommended.

I. INTRODUCTION

Foot and mouth disease (FMD) is acute and highly contagious disease affecting all ruminants and cloven-footed animals (Alexandersen and Mowat, 2005). The FMD virus (FMDV), belongs to the Picornaviridae family, the main member of the genus Aphthovirus (King et al., 2011). The FMD disease has been a major cause of economic losses to livestock industries worldwide due to fatal cases that restricted to young animals (Alexandersen et al., 2003), and the infected animals significantly reduce productive performance and international trade of animals and animal-derived products is severely restricted between countries with different sanitary status of the disease (Perry and Rich, 2007).

FMD has been dramatically demonstrated in the last two decades by outbreaks occurred in different countries irrespectively of their economic development (Muroga et al., 2012; Perez et al., 2004; Thompson et al., 2002). Vaccination is almost the only weapon in the fight against viral animal diseases because wide-spectrum antivirals are not available and sanitary measures are generally ineffective the field (Diaz-San et al., 2017)

The FMD vaccine is one of the earliest animal vaccines. inactivated whole virus vaccines are being used throughout the world today (Parida, 2009)
Vaccination has been successfully applied as the main control measure for FMD in different countries, in most cases by means of polyvalent oil vaccines formulated using inactivated virus from strains previously detected in the region (Saraiva and Darsie, 2004).

Good quality vaccines may prevent the development and transmission of the disease and decrease the incidence of persistently infected animals (Cox et al., 2006; Orsel et al., 2005). FMDV structural proteins are highly variable and seven antigenically distinct serotypes have been described for this virus (Knowles and Samuel, 2003). Consequently, protection provided by FMD vaccines, which is closely related to the induction of specific antibody responses (Maradei et al., 2008). Serological assays, such as virus neutralization tests (VNT) and liquid-phase blocking ELISA (LPB-ELISA), were designed to detect levels of antibodies against viral capsid proteins and represent a major tool to predict the immunological and protective status of the animals and susceptible populations against FMDV (Barnett et al., 2003; Goris et al., 2008; Maradei et al., 2008).

Cellular responses against FMDV have also been studied by detection of IFN-γ, showing correlation with protection against homologous virus challenge in sheep (Barnett et al., 2004).

Type I and type III interferons (IFN) are important parts of the early innate immune response to viral infection and are often crucial in controlling or eliminating infection Zhang et al., 2002).

This work was planned to solve a problem that in spite of FMD vaccination of local animals by commercial FMD vaccines would be re-infected with FMD by detect the immune efficacy of selective widely use FMD vaccine in Awassi sheep experimentally and to evaluate the immune response of Turkish FMD vaccine in Iraqi Awassi sheep and determine their longevity.

**Keywords:** FMD, LPB ELISA, Abs, IL4, INF, Awassi sheep.

### II. MATERIAL AND METHODS

**Vaccine:**

Turkish inactivated trivalent FMD virus vaccine for serotype (O1), (A/T1) and Asia with BEL duvanted with Aluminum Hydroxide and Saponine was supplied by Teaching Veterinary hospital in Aldiwaniyh city \ Iraq.

**Animals:**

A total of 16 local Awassi sheep (8 males (rams) and 8 females (ewes) 9-12 months old and about 25-30 kilo grams body weight, were used for experimental studies. The experimental animals were reared under similar supervision and nutrition during experiment.

Sheep were clinically healthy and free from antibodies against FMDV (type O and A). These animals were divided into two groups randomly, each group had been 8/4 males and 4 females) sheep, the animals in vaccinated (T) group inoculated by 1 ml of Turkey vaccine subcutaneously in right site of axilla, and sheep in the control (C) group were inoculated with 1 ml of sterile PBS subcutaneously in right site of axilla, and four (4) sheep of vaccinated group were inoculated with booster dose of Turkey vaccine at 42 day post vaccination vital signs (respiration and heart rates, temperatures) of all animals in experiment were undergoing for monitoring for 3 days post vaccination.

**Serum Samples:**

Serum samples were collected before vaccination (day zero) and at 14,28, 42,60 days post-vaccination (DPV) from experimental sheep, part of each serum was inactivated at 56°C for 30 minutes for LPB ELISA and other serums parts were stored at -20°C until used in IL4, INF concentration measurement.

**ELISA test:** Liquid phase blocking ELISA was carried out for the detection of FMDV antibodies in serum using FMD Antibody Detection Kit (BDSL, UK), according to Hamblin et al. (1986).

**Determination of Serum Cytokine Levels:** Serum gamma interferon (IFN-γ) and interleukin 4 (IL-4) levels were measured using ELISA kits (RayBiotech, Inc, GA, USA). Cytokine levels were evaluated by interpolation of cytokine standard curves, according to the manufacturer’s instructions.
Statistical analysis
Data were analyzed using analysis of variance (ANOVA) in the SPSS statistical software package. Multiple comparisons of means were made using Least Significant Differences tests at $P<0.05$. The results represent as the mean ± standard error.

Ethical guidelines: For the animal experimentation, international as well as institutional guidelines were followed.

III. RESULTS AND DISCUSSION

Post vaccination clinical physical observations

No severe clinical reaction due to vaccination were observed, the means of temperature, respiration and heart rates were slightly elevated gradually after 12 hrs post vaccination in Awassi sheep in vaccinated group and these means return to normal values after 48 hrs, but these elevations are not significant ($P>0.05$) in comparison with control group, (table 4-1).

Table (4-1): Physical examination (temperature, respiratory and heart rates) in animals of experiment

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Times\hours Post vaccination</th>
<th>Groups of animals M±SE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>$T$</td>
</tr>
<tr>
<td>Temperature\C$^\circ$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>39.2±0.30 Aa</td>
<td>39.2±0.27 Aa</td>
</tr>
<tr>
<td>12</td>
<td>39.2±0.30 Aa</td>
<td>39.8±0.15 Aa</td>
</tr>
<tr>
<td>24</td>
<td>39.3±0.36 Aa</td>
<td>39.6±0.35 Aa</td>
</tr>
<tr>
<td>36</td>
<td>39.3±0.28 Aa</td>
<td>39.4±0.24 Aa</td>
</tr>
<tr>
<td>48</td>
<td>39.2±0.56 Aa</td>
<td>39.2±0.12 Aa</td>
</tr>
<tr>
<td>Respiration rate \minute</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>29.6±1.18 Aa</td>
<td>29.4±0.39 Aa</td>
</tr>
<tr>
<td>12</td>
<td>29.5±1.65 Aa</td>
<td>30±1.34 Aa</td>
</tr>
<tr>
<td>24</td>
<td>29.6±1.54 Aa</td>
<td>29.3±1.01 Aa</td>
</tr>
<tr>
<td>36</td>
<td>29.6±1.22 Aa</td>
<td>29.4±1.34 Aa</td>
</tr>
<tr>
<td>48</td>
<td>29.5±1.04 Aa</td>
<td>29.2±1.34 Aa</td>
</tr>
<tr>
<td>Heart rate\minute</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>74.8±2.54 Aa</td>
<td>74.7±2.11 Aa</td>
</tr>
<tr>
<td>12</td>
<td>74.8±1.86 Aa</td>
<td>75.6±1.84 Aa</td>
</tr>
<tr>
<td>24</td>
<td>74.6±1.58 Aa</td>
<td>75.0±2.06 Aa</td>
</tr>
<tr>
<td>36</td>
<td>74.6±2.86 Aa</td>
<td>75.1±2.50 Aa</td>
</tr>
<tr>
<td>48</td>
<td>74.7±1.93 Aa</td>
<td>74.8±1.56 Aa</td>
</tr>
</tbody>
</table>

The different small letters horizontally refers to significant variations at ($p≤0.05$)

The different capital letters vertically refers to significant variations at ($p≤0.05$)

At Post vaccinations, only mini palpated swelling at site of Subcutaneous inoculation of vaccines and disappeared after 24hrs and any type of reaction was not observed at site of vaccine injection along time of experiment.

This results about slightly systemic reaction due to vaccination were in parallel to findings by Orsel et al. (2009) as an effective immune system and results of Lee et al. (2019) with variant degree of response depend on health status of animals, age and time of vaccination (summer, winter) and absence of any reaction at site of injection of vaccine and no clear and important gross physical reaction following vaccination (Sitt et al., 2019), so, the type of vaccine (mono,tri,quadri valents) and types of adjuvant involvement (Moosad, 2007).

Antibodies titration against A and O FMDV strains

The antibodies titration against A strain of FMD virus, in table (2) was appeared the antibodies titration as resultant of vaccination was in significant higher than control group at all times of experiment.
Table 2: Mean antibodies Abs titer against A strain of FMD virus in sera of animals in vaccinated and control groups

<table>
<thead>
<tr>
<th>Groups Days</th>
<th>0 M±SE</th>
<th>14 M±SE</th>
<th>28 M±SE</th>
<th>42 M±SE</th>
<th>60 M±SE</th>
</tr>
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<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T</td>
<td>0.079 ± 0.007 Ba</td>
<td>0.608 ± 0.04 Bb</td>
<td>0.695 ± 0.06 Bc</td>
<td>0.617 ± 0.11 Bb</td>
<td>0.783 ± 0.12 Bd</td>
</tr>
<tr>
<td></td>
<td>0.495 ± 0.171 Bc</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>C</td>
<td>0.078 ± 0.007 Ca</td>
<td>0.078 ± 0.007 Ca</td>
<td>0.078 ± 0.007 Ca</td>
<td>0.078 ± 0.007 Ca</td>
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</table>

The different small letters horizontally refers to significant variations at \((p \leq 0.05)\)

The different capital letters vertically refers to significant variations at \((p \leq 0.05)\)

The Abs titer against Turkey FMD – strain A vaccine was increased significantly at all times of experiment, and the higher Abs titer recorded at 28 day \((T/0.695 \pm 0.06)\) and these titration lowering at days \((42,60)\) as \((0.617 \pm 0.11, \ 0.495 \pm 0.171)\) respectively, table 2, figure 1.

<table>
<thead>
<tr>
<th>Groups Days</th>
<th>0 M±SE</th>
<th>14 M±SE</th>
<th>28 M±SE</th>
<th>42 M±SE</th>
<th>60 M±SE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td></td>
</tr>
<tr>
<td>T</td>
<td>0.078 ± 0.007 Aa</td>
<td>0.466 ± 0.07 Bb</td>
<td>0.562 ± 0.2 Ac</td>
<td>0.455 ± 0.05 Bb</td>
<td>0.829 ± 0.153 Bd</td>
</tr>
<tr>
<td></td>
<td>0.444 ± 0.109 Bb</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>0.065 ± 0.014 Ca</td>
<td>0.065 ± 0.014 Ca</td>
<td>0.065 ± 0.014 Ca</td>
<td>0.065 ± 0.014 Ca</td>
<td></td>
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</tbody>
</table>

The boostered dose of vaccine enhance Abs production against FMDV- A strain in high level at day 60 of experiment, and the mean Abs titration in vaccinated group was recorded at high significant level in comparison with their titration in different times post vaccination in this group \((T/0.783 \pm 0.12 boostered, T / 0.495 \pm 0.171 non boostered)\), (table 1, figure 1)
Figure 1: Antibodies titrations against A strain of FMDV in serum of animals of vaccinated (T) and control (C) groups.

In table (3) was showed the Turkey vaccine was evoked Abs production against FMDV –O strain that started in days 14 of experiment (T /0.466 ± 0.07) in compare with either day zero (T/0.078 ± 0.007) or with control group (0.065 ± 0.014), so the peak of Abs titration was showed at day 28 (T/ 0.562 ± 0.2), but the Abs against O FMD virus was lowering in 42,60 days (T/420.455 ± 0.05, T/600.444 ± 0.109), table 3, figure2.

Table 3 : Mean antibodies Abs titer against O strain of FMD virus in serums of animals in vaccinated and control groups

The different small letters horizontally refers to significant variations at (p≤ 0.05)

The different capital letters vertically refers to significant variations at (p≤ 0.05)

The booster dose of turkey vaccine produced higher Abs titration at day 60 more than titration at any time of experiment (T/0.829 ± 0.153 boostered, 0.444 ± 0.109 non boostered), table 3, figure2.

Figure 2: Antibodies titrations against O strain of FMDV in serum of animals of vaccinated (T) and control (C) groups.
Our results showed the titration of Abs against A FMDV strain is higher than Abs against O FMDV strain, unlike results by El-Sayd et al.(2012) that Abs titration against O FMDV strain is higher than against AFMDV strain, while, findings by El-Bagoury et al .(2013) appeared no variations in Abs titration against A, O FMDV strains.

The higher protective serum of Abs titer produced by vaccinate sheep in experiment in comparing with protective Abs titer recorded by OIE(2009) is in agreement with many reports by (Patil et al.,2002, Barnard et al.,2005, Bayry et al., 1999). Many researchers pointed to vaccination against FMDV produced protective Abs titer in different level and longevity (Patil et al.,2002, Muthukrishnan et al.,2020), the peak of Abs titer against(A,O) FMDV strains showed at 28 days post vaccination, in similar of results obtained by Horsington et al.,2018 and Al-Saayed et al.(12), whereas, the Abs peak appeared around three months (Al-Bajory et al.,13)

Wang et al. (2013) was explain the importance of vaccination against FMD, antibodies against FMDV start to produce after 4-5 days post vaccination and their peak showed at first month post vaccination, and the Abs titration and their longevity were determined by many factors associated with vaccinated animals (age, sex, healthy status) vaccine containing strains, adjuvants, route of vaccination type.

The titrations of antibodies against FMDV begun to decline gradually at 60 days and the booster dose enhance to increase Abs production, and this our findings are very closely to Cui et al,(2020) about decline of Abs titer after 8 weeks post first vaccination and the booster dose of vaccine is very necessary to continue of the protective level of FMD Abs with longest time.

Interleukin 4 concentration
In general view to table (4) was showed that vaccine (Turkey) were enhancement the production of IL4 in compression with control group independent on time of experiment.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Days</th>
<th>0 M±SE</th>
<th>14 M±SE</th>
<th>28 M±SE</th>
<th>42 M±SE</th>
<th>60 M±SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>T</td>
<td></td>
<td>10.95 ± 0.46</td>
<td>13.75 ± 0.95</td>
<td>14.62 ± 0.48</td>
<td>14.13± 0.90</td>
<td>16.29 ± 0.87</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Aa</td>
<td>Ab</td>
<td>Ac</td>
<td>Abc</td>
<td>Bd</td>
</tr>
<tr>
<td>C</td>
<td></td>
<td>10.76 ± 0.59</td>
<td>10.96 ± 0.48</td>
<td>10.96 ± 0.52</td>
<td>10.98± 0.51</td>
<td>10.98 ± 0.51</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Aa</td>
<td>Ba</td>
<td>Ba</td>
<td>Ca</td>
<td>Ca</td>
</tr>
</tbody>
</table>

The different small letters horizontally refers to significant variations at (p≤ 0.05)

The different capital letters vertically refers to significant variations at (p≤ 0.05)y

In serum of Turkey vaccinated sheep, IL4 concentration elevated and the peak reach at 28 days of experiment (14.62 ± 0.48) and still highly significant (P≤0.05) to end of experiment(14.13± 0.90, 14.35 ± 0.29) in 42,60 days respectively in compare with their concentration in day zero(10.95 ± 0.46).

The increase of IL4 concentrations in serum of vaccinated animals were increasing significantly than it concentration in control group at all time post vaccination, (Table 4, Figure 3).

The effect of boostered dose was showed markedly at 60 day of experiment was revealed significant increase of IL-4 concentration in sheep serum of vaccinated group (T 60 / 16.29 ± 0.87 boostered, 13.85 ± 0.48 non boostered), (Table 4, Figure 3).
The current study about IL-4 concentration was in nearest with conclusions by Yassin et al.(2019) that IL-4 start to increase at 7 days and peaked at 21. Furthermore, McCullough et al.(2004) showed the significant increase of IL-4 concentration than in control group at ten days after immunization as indication of Th2 lymphocytes induction occurred.

IL-4 secreted from T cells and differentiated Th2 cells (Achsah and Keegan, 2000), its main function; regulation of the naive CD4+ T cell differentiation (Cox et al. 2003) into helper phenotype Th2 cells. Beside, co-stimulation of T and B cells proliferation (Yassin et al., 2019).

Increasing of IL4 concentration after vaccination in our study also matching with report by Toellner et al.(2014) of the source of IL-4 during germinal center formation is still debated, it has been shown that natural killer T cells (NKT cells) and CD4+ T cells produce IL-4 after vaccination.

**Interferon gamma**

In table (5) that exhibit the relationship between vaccination and production of INF –γ was showed start to increase at 14 days post vaccination in vaccinated group, but the significant increase of INF production at 28 days of experiment when compare Turkey vaccinated group (162.2 ± 12.) with control group (145.7 ± 5.07) and the higher peak of INF concentration recorded in 42 days post vaccination (T / 175.6 ±20) and lowering at day 60 of experiment (172.13 ± 11.87), table 5, figure 4.

**Table 5: Interferon gamma (INF -γ) concentrations in animals of vaccinated and control groups .**

<table>
<thead>
<tr>
<th>Groups</th>
<th>0 M±SE</th>
<th>14 M±SE</th>
<th>28 M±SE</th>
<th>42 M±SE</th>
<th>60 M±SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>T</td>
<td>142.4 ± 13.09 Aa</td>
<td>148.7 ± 9.19 Aa</td>
<td>162.2±12.4 Ab</td>
<td>175.6 ± 20.1 Ac</td>
<td>195.06 ± 10.84 Bd</td>
</tr>
<tr>
<td>C</td>
<td>141.9 ± 3.6 Aa</td>
<td>143.27 ± 2.72 Aa</td>
<td>145.7±5.07 Ba</td>
<td>145.7 ± 7.96 Ba</td>
<td>145.7 ± 7.96 Ca</td>
</tr>
</tbody>
</table>

The different small letters horizontally refers to significant variations at (p< 0.05)
The different capital letters vertically refers to significant variations at (p≤ 0.05)y

The booster dose of Turkey vaccine enhance significant increase of INF –γ concentration at day 60 than other times in same vaccinated group (T / 195.06 ± 10.84), table 5, figure 4.

![Interferon Gamma](image)

**Figure 4:** Interferon gamma INF-concentrations in serum of vaccinated (T) and control (C) groups.

The present results is matching with Sharma et al.(2018) findings about start INF concentration increase at 14 days post vaccination and these increasing in INF as indication of role cellular immune response in protection from FMD infection (Wang et al.,2011) and agreement with reports by Parida et al.,2006 of the vaccination stimulate in INF production.

IN contrast, Eschbaumer et al.,2016 results in which no increase in INF production post vaccination because they were thought the increasing INF concentration is accompanied with viremia which occurred after infection but not post vaccination.

Our results, showed the humeral and cellular immune response were increased post FMD vaccination in similar of report by Cui etal.,(2019) of the increasing in INF and IL4 was in parallel with increasing in Abs titration due to that vaccination can activate Th1 and Th2 cells and antibodies producing immune cells.

The IFN-ϒ has been used for measurement of antigen-specific T cell activation and the proliferation and production of IFN-ϒ from lymphocytes derived from FMDV infected animals have been demonstrated after re-stimulating with vaccine antigen (Carr etal., 2013). A positive correlation has been found between IFN-ϒ and vaccine induced protection (Oh etal.,2012).

Moreover, Tewari and Jain (2019) was found that CD4+ T cells population has been detected in vaccinated animals as the major proliferating cells and produce interferon gamma in stimulated cells of vaccinated and subsequently infected animals.

**The current results are mimic findings by that, immunization that leads to a dominant IL-4 response from T cells and to dominant IFN-γ production are protective (Laudenbach et al.,2018).**

**IV. CONCLUSION**

It is concluded that Turkish inactivated trivalent FMD vaccine is capable to produce good immune response against FMD virus serotype-A and O in sheep. Also the vaccination was effective in induce cellular immune response to
contribute in FMD controlling and the boosting dose of vaccination is essential to give animal herds good immunity against FMD.

Author contributions
Dr. Qassim designed, coordinated, reviewed and corrected the manuscript, Dr. Heba and Dr. Assad performed the experiments, Dr. Mazin performed the serological tests, Dr. Qassim completed analysis and wrote the manuscript. All authors contributed to the article and approved the submitted version.

Conflict of Interest
The authors declare that they have no conflict of interest.

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REFERENCES:


