ARC Journal of Animal and Veterinary Sciences
Volume 5, Issue 2, 2019, PP 16-24
ISSN No. (Online) 2455-2518
DOI: http://dx.doi.org/10.20431/2455-2518.0502003
www.arcjournals.org

Trials for Improvement of the Immune Response of Foot and Mouth Disease Vaccine Using Oil/Gel Mixture as an Adjuvant

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Abstract

Background: *Adjuvants play an important role in the efficacy of vaccines, the protective immune response produced by vaccines could vary according to the kinds of adjuvants.*

Objective: *Comprehensive sero-immunological study was conducted to reveal the adjuvant's effect of on the immune response of polyvalent Foot and mouth disease (FMD) vaccine in cattle.*

Methods: *This study was conducted on twenty-seven cattle in Kaliobia governorate, Egypt. Twenty animals was divided into four groups, each of five animals, one group vaccinated intramuscularly (I/M) with trivalent FMD ISA 206 oil vaccine, second group vaccinated intramuscularly (I/M) with trivalent FMD ISA 206-aluminum hydroxide gel vaccine, third group vaccinated intramuscularly (I/M) with trivalent ISA 201 oil vaccine, fourth group vaccinated intramuscularly (I/M) with trivalent ISA 201- aluminum hydroxide gel vaccine, three cattle were used as negative control (non-vaccinated) and four cattle were used for safety test. Serum samples were collected from vaccinated animals for 10 months. The cellular and humeral immune responses were monitored.*

Results: *Our results showed that the groups immunized with the vaccines prepared with ISA 201 and ISA 201 +aluminum hydroxide gel induced better cellular immunity than that induced by vaccines prepared with ISA 206 and ISA 2016 +aluminum hydroxide gel, also found that vaccines prepared with ISA 206 and ISA 206 + aluminum hydroxide gel induce better humeral immunity than that induced by vaccines prepared with ISA 201 and ISA 201 +aluminum hydroxide gel. Immune response with aluminium hydroxides gel overcomes that with ISA oils alone.*

Conclusion: *Finally, we concluded that aluminum hydroxide gel improves the effects of ISA adjuvants.*

Keywords: *FMD vaccine, ISA, aluminum hydroxide gel.*

1. INTRODUCTION

Foot-and-mouth disease (FMD) is an acute infectious disease that infects cloven-hoofed mammals, such as pigs, cattle, cattle and goats **Dara, et al., (2013)**. The causative agent is a single stranded positive- sense RNA virus that belongs to the genus Aphthovirus in the family Picornaviridae. The virus has seven serological types, identified as; O, A, C, SAT1, SAT2, SAT3 and Asia1 **Dara, et al., (2013)**.

FMD is characterized by fever, lameness and vesicular lesions on the feet, tongue, snout, and teats, with high morbidity and low mortality **Rodriguez and Grubman (2009)**.

In Egypt, the disease is enzootic and outbreaks have been reported since 1950 and Mousa et al., 1974). Type O was the most prevalent since 1960 **Zahran (1960), Farag et al., (2005) and Satya (2009)**. FMDV serotype A was isolated during 2006 in Egypt through live animals importation where sever clinical signs were recorded among cattle and buffaloes **Abed El-Rahman (2006)**., Also FMDV serotype SAT2 was recorded in Egypt **Shawkey et al., (2013) and Nader et al., (2014)**.

Control of FMD in animals was considered to be important to effectively contain the disease in endemic areas, so that vaccination of animals is effective in limiting the spread of FMD **Longjam et al., (2011)** and **Depa et al., (2012)**.

Adjuvants, also can prolong the immune response and stimulate specific components of the immune response either humeral or cell mediated immunity **(Barnett (2003) and Lombard et al. (2007)**. An ideal adjuvant is one which can stimulate the humeral immune response early (onset), and promote production

of high antibody titers that would long duration. It should also stimulate the cellular immune response **Park (2013)**.

The oil adjuvant has the capability for generating a rapid, high and long-lasting immune response. Generally, the Montanide series of oil adjuvants (SEPPIC, France) has a clear immunological effect for inactivated vaccine in different susceptible animals **Dara, et al., (2013), Ehab et al., (2015) and Fakhry et al., (2012)**.

Vaccines containing aluminum hydroxide and saponin as adjuvants have several deficiencies such as the induction of short-lived antibody responses which require relatively frequent revaccinations at intervals of 6 or even 4 months. In contrast, oil-based adjuvant FMD vaccines appear to have several advantages such as the induction of high titers and long-lived antibody responses,resulting in more effective protection **Aucouturier et al., (2001), Cloete al., (2008)**. Unlike alum-based adjuvant vaccines, oil-based adjuvant vaccines can overcome interference by maternal antibodies in neonates and can consequently be applied earlier in life **Iyer et al., (2000) and (2003)**.

The objective of this study was to reveal the adjuvant's effect of different adjuvants on the immune response of trivalent Foot and mouth disease (FMD) vaccine in cattle.

2. Materials and Methods

2.1. Animals (Cattle)

Twenty seven calves, native breed, between 6 to 9 months old and weighted between 300 and 400 kg, twenty calves were divided into four groups, each of five animals, one group vaccinated intramuscularly (I/M) with trivalent FMD ISA 206 oil vaccine, second group vaccinated intramuscularly (I/M) with trivalent FMD ISA 206- aluminum hydroxide gel vaccine, third group vaccinated intramuscularly (I/M) with trivalent ISA 201 oil vaccine,, fourth group vaccinated intramuscularly (I/M) with trivalent ISA 201- aluminum hydroxide gel vaccine, three cattle were used as negative control (non-vaccinated) and four cattle were used for safety test.

2.2. FMD Virus Strains

FMD virus local strains (O /pan Asia2, A/ Iran 05, SAT2/ Egypt 2012 and SAT2/ Egypt 2018) were locally isolated and were identified by Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo. These viruses were used in vaccine preparation and serum neutralization test (SNT).

2.3. Adjuvants

A-ISA206: Montanide ISA 206 was obtained from Seppic, Paris, France.

B-ISA201: Montanide ISA 201 was obtained from Seppic, Paris, France.

C-Aluminium hydroxide gel: 2.5% aluminum hydroxide gel was prepared as an alum-based adjuvant.

2.4. FMD Polyvalent Vaccine

Local strains of FMD were propagated in baby hamster kidney cells- clone 21 (BHK-21) cell line and inactivated by Binary Ethylenemine (BEI).

A. Preparation of the ISA 206 adjuvant vaccine

Inactivated ISA 206 oil adjuvanated FMD Vaccines were formulated according to **Barnett.et.al. (1996).** The ratio of the aqueous antigen to the oil adjuvant was 50:50 (w/w) according to **OIE (2000).**

B. Preparation of the ISA206 - Aluminium Hydroxide Gel Adjuvant Vaccine

Inactivated ISA 206 oil adjuvanated FMD Vaccines were formulated according to **Barnett.et.al. (1996).** The ratio of the aqueous antigen to the oil adjuvant was 50:50 (w/w) according to **OIE (2000)**. Aluminium hydroxide gel was added as 30% of the aqueous phase of the vaccine bulk.

C. Preparation of the ISA201 Adjuvant Vaccines

Inactivated ISA 201 oil adjuvanated FMD Vaccines were formulated according to **Barnett.et.al. (1996).** The ratio of the aqueous antigen to the oil adjuvant was 50:50 (w/w) according to **OIE (2000).**

D. Preparation of the ISA206 - Aluminium Hydroxide Gel Adjuvant Vaccines

Inactivated ISA 201 oil adjuvanated FMD Vaccines were formulated according to **Barnett.et.al. (1996).** The ratio of the aqueous antigen to the oil adjuvant was 50:50 (w/w) according to **OIE (2000)**. Aluminium hydroxide gel was added as 30% of the aqueous phase of the vaccine bulk.

Immune response of vaccinated cattle were determine by using Lymphocyte blastogenesis using XTT assay according to **Scudiero et al. (1988)** and SNT test against FMDV strains (O /pan Asia2, A/ Iran 05, SAT2/ Egypt 2012 and SAT2/ Egypt 2018) in serum samples according to **(Voller et al., 1976 and OIE 2012)**.

2.5. Safety of the Prepared Vaccines

Safety test for the formulated FMD vaccines: The inactivated FMD virus was tested for safety in vitro on BHK-21 cell line and the whole prepared vaccines in vivo in susceptible cattle **OIE (2000)**.

Safety of the prepared vaccines were done according to ***Code of Federal regulation of USA.* (1986), *Henderson* (1970) *and* OIE (2000)**.

3. Experimental Design

Twenty seven calves, native breed, between 6 to 9 months old and weighted between 300 and 400 kg, twenty calves were divided into four groups, each of five animals, one group vaccinated intramuscularly (I/M) with trivalent FMD ISA 206 oil vaccine, second group vaccinated intramuscularly (I/M) with trivalent FMD ISA 206- aluminum hydroxide gel vaccine, third group vaccinated intramuscularly (I/M) with trivalent ISA 201 oil vaccine,, fourth group vaccinated intramuscularly (I/M) with trivalent ISA 201- aluminum hydroxide gel vaccine, three cattle were used as negative control (non-vaccinated) and four cattle were used for safety test. Serum samples were collected from vaccinated animals for 10 months.

Heparinized blood samples were obtained from vaccinated and control non vaccinated animals at 0, 3, 7, 14, 21, 28,35 and 42 days post vaccination. Stimulation of the cellular immune response by the different prepared FMD vaccine was evaluated by Lymphocyte blastogenesis using cell proliferation kit (XTT kit).

Serum samples were collected weekly post vaccination for one month then every month post-vaccination till the end of experiment (ten months). The immune response was evaluated through SNT test.

4. Results and Discussion

Foot and Mouth Disease (FMD) is an acute disease caused by Foot and Mouth Disease Virus (FMDV) which causes important economy losses **(Orsel et al., 2007)**. The control of FMD in animals was considered to be important to effectively contain the disease in endemic areas, so that vaccination of animals is effective in limiting the spread of FMD.

FMD vaccines can be defined as a fixed formulation of that of specific amount of chemically inactivated virus strains and mixing with suitable adjuvant. Selecting the suitable vaccine formulation is dependent on several factors as the onset of protection and the duration of protection against FMD.

The effective formulation of inactivated FMD vaccines requires adjuvant aluminium hydroxide gel and mineral oils-based formulations have been widely employed in experimental studies to obtain a vaccine that stimulates a rapid and long-lasting protective immune response, the formulated vaccines are safe for animal use.

Results in table 1 showed that the five prepared vaccines were safe for use during the whole experiment time. These results were in agreement with **(OIE 2000)**.

Table1. *Safety of Polyvalent FMD Vaccines Tested*

Months post vaccination	Polyvalent FMD Vaccines			
	ISA201	ISA201＋AL*	ISA206	ISA206＋AL*
1	safe	safe	safe	safe
2	safe	safe	safe	safe
3	safe	safe	safe	safe
4	safe	safe	safe	safe
5	safe	safe	safe	safe
6	safe	safe	safe	safe
7	safe	safe	safe	safe
8	safe	safe	safe	safe
9	safe	safe	safe	safe
10	safe	safe	safe	safe

*AL * aluminium hydroxide gel*

The obtained results of cell mediated immune response using lymphocyte proliferation test for all animal groups expressed by ΔOD (Delta Optical Density) were as follow:

Group 1 (polyvalent FMD ISA 206 oil vaccine): Delta Optical Density was (0.583) by using FMD viruses at 3rd day post vaccination and still rise reached its highest level (1.557) at

3rd week post vaccination and continue high within examination time 35 days post vaccination.

Group 2 (polyvalent FMD ISA 206- aluminum hydroxide gel vaccine): Delta Optical Density was (0.536) by using FMD viruses at 3rd day post vaccination and still rise reached its highest level (1.166) at 3rd week post vaccination then declined gradually.

Group 3 (polyvalent ISA 201 oil vaccine): Delta Optical Density was (0.588) by using FMD viruses at 3rd day post vaccination and still rise reached its highest level (1.6628) at 3rd week post vaccination and continue high within examination time 35 days post vaccination.

Group 4 (polyvalent ISA 201- aluminum hydroxide gel vaccine): Delta Optical Density was (0.5956) by using FMD viruses at 3rd day post vaccination and still rise reached its highest level (1.7628) at 3rd week post vaccination then declined gradually as shown in tables (2, 3, 4 and 5).

Table2. *Delta optical density of the cell-mediated immune response of cattle vaccinated with polyvalent FMD ISA 206 oil vaccine using lymphocyte Proliferation (XTT) Assay*

Time post vaccination	ΔOD in buffy coat in Vaccinated cattle					Mean	Control non vaccinated animal
	1*	2	3	4	5		
Pre vaccination	0.051	0.050	0.049	0.048	0.046	0.0488	0.064
3rd day	0.590	0.578	0.578	0.592	0.572	0.583	0.065
1 week	0.851	0.854	0.852	0.850	0.847	0.8508	0.056
2 week	1.498	1.492	1.496	1.456	1.398	1.468	0.069
3 week	1.558	1.562	1.560	1.544	1.562	1.5572	0.067
4 week	1.258	1.262	1.260	1.255	1.250	1.257	0.065
5 week	0.830	0.832	0.830	0.845	0.798	1.257	0.064
6 week	0.640	0.644	0.641	0.651	0.611	0.827	0.065

Table 3: *Delta optical density of the cell-mediated immune response of cattle, vaccinated with polyvalent FMD ISA 206- aluminum hydroxide gel vaccine using lymphocyte Proliferation (XTT) Assay*

Time post vaccination	ΔOD in buffy coat in Vaccinated cattle					Mean	Control non vaccinated animal
	1*	2	3	4	5		
Pre vaccination	0.048	0.050	0.048	0.041	0.046	0.0466	0.064
3rd day	0.550	0.548	0.528	0.532	0.522	0.536	0.065
1 week	0.951	0.854	0.852	0.840	0.832	0.866	0.056
2 week	1.698	1.692	1.696	1.580	1.611	1.655	0.069
3 week	1.658	1.662	1.660	1.61	1.712	1.660	0.067
4 week	1.458	1.462	1.460	1.423	1.491	1. 459	0.065
5 week	0.940	0.944	0.941	0.924	0.919	0.934	0.064
6 week	0.827	0.828	0.827	0.862	0.896	0.848	0.065

Table4. *Delta optical density of the cell-mediated immune response of cattle, vaccinated with polyvalent ISA 201 oil vaccine using lymphocyte Proliferation (XTT) Assay*

Time post vaccination	ΔOD in buffy coat in Vaccinated cattle					Mean	Control non vaccinated animal
	1*	2	3	4	5		
Pre vaccination	0.048	0.050	0.048	0.036	0.041	0.044	0.064
3rd day	0.590	0.578	0.593	0.592	0.588	0.588	0.065
1 week	0.587	0.572	0.578	0.581	0.578	0.490	0.056
2 week	1.113	1.117	1.114	1.242	1.211	1.136	0.069
3 week	1.658	1.662	1.660	1.622	1.712	1.6628	0.067
4 week	0.782	0.780	0.782	0.783	0.758	0.777	0.065
5 week	0.683	0.683	0.684	0.662	0.668	0.676	0.064
6 week	0.636	0.641	0.638	0.611	0.609	0.627	0.065

Table5. *Delta optical density of the cell-mediated immune response of cattle, vaccinated with polyvalent ISA 201- aluminum hydroxide gel vaccine using lymphocyte Proliferation (XTT) Assay.*

Time post vaccination	ΔOD in buffy coat in Vaccinated cattle					Mean	Control non vaccinated animal
	1*	2	3	4	5		
Pre vaccination	0.048	0.050	0.048	0.041	0.046	0.0466	0.064
3rd day	0.597	0.598	0.593	0.592	0.598	0.5956	0.065
1 week	0.951	0.854	0.852	0.840	0.832	0.8658	0.056
2 week	1.698	1.692	1.696	1.580	1.611	1.655	0.069
3 week	1.758	1.862	1.760	1.672	1.762	1.7628	0.067

4 week	**1.458**	**1.462**	**1.460**	**1.423**	**1.491**	**1. 459**	**0.065**
5 week	**0.940**	**0.944**	**0.941**	**0.924**	**0.919**	**0.934**	**0.064**
6 week	**0.827**	**0.828**	**0.827**	**0.862**	**0.896**	**0.848**	**0.065**

Tables 2,3,4 and 5 showed that vaccines prepared with ISA 201 and ISA 201＋aluminum hydroxide gel induced better cellular immunity than that induced by vaccines prepared with ISA 206 and ISA 2016＋aluminum hydroxide gel , these results appeared to be supported by **(Knudsen et al., (1979), Sharma et al., (1984)** who reported that cell mediated immune response was a constitute of immune response against FMD virus, and in agreement in some points with **(Knudsen et al., (1979), Mercedes et al.,(1996), Elwatany et al.,(1999), Sonia et al., (2010), Hiam et al., 2012** and **El-Din, W et al.,(2014)** who found that FMD vaccine stimulated the cellular immune response and lymphocyte stimulation by FMDV was greater than by mitogens (PHA) and appeared the highest increase in 1st and 2nd weeks post vaccination.

The SNT data in tables 6,7,8 and 9 showed the differences in the onset, intensity and duration of the antibodies elicited by the different vaccine formulations against FMD virus serotypes O, A, SAT2/2012 and SAT2/2018. Results showed that vaccines prepared with ISA 206 and ISA 206 ＋ aluminum hydroxide gel induce better humeral immunity than that induced by vaccines prepared with ISA 201 and ISA 201＋aluminum hydroxide gel, the results obtained are consistent with the statement of **Wisniewski et al., (1972)** they explained that the SNT measures those antibodies which neutralize the infectivity of FMD virion**.** The peak of antibody titre in all groups at 10-12 weeks post vaccination and continues with protective level till 32th week post vaccination. Our results also were consistent with the statement of **(Hamblin et al., (1986)** who explained that the SNT measures those antibodies which neutralize the infectivity of FMD virion, while ELISA probably measure all classes of antibodies even those produced against incomplete and non-infectious virus.

Table6. *Mean of serum antibody titers in cattle vaccinated with polyvalent FMD ISA 206 oil vaccine using SNT expressed* log_{10}

Weeks post vaccination	**Mean antibody titer against FMD virus strains**				**Non vaccinated Group**
	FMD (O)	**FMD (A)**	**FMD (SAT/2012)**	**FMD (SAT/2018)**	
Pre-vaccination	**0.27**	**0.27**	**0.15**	**0.3**	**0.3**
1	**1.1**	**1.05**	**1.2**	**1.2**	**0.3**
2	**1.65**	**1.8**	**1.8**	**1.8**	**0.3**
3	**2.1**	**2.1**	**1.95**	**1.95**	**0.3**
4	**2.4**	**2.4**	**2.4**	**2.1**	**0.3**
6	**2.7**	**2.7**	**2.7**	**2.7**	**0.3**
8	**2.85**	**2.85**	**2.85**	**2.85**	**0.3**
10	**2.85**	**3.15**	**3.15**	**3.0**	**0.3**
12	**2.55**	**2.85**	**3.0**	**2.85**	**0.3**
14	**2.55**	**2.7**	**2.85**	**2.85**	**0.3**
16	**2.4**	**2.4**	**2.7**	**2.55**	**0.3**
20	**2.1**	**2.1**	**2.4**	**2.4**	**0.3**
24	**1.8**	**1.8**	**2.1**	**2.1**	**0.3**
28	**1.65**	**1.65**	**1.8**	**1.65**	**0.3**
32	**1. 5**	**1. 5**	**1.65**	**1.65**	**0.3**
36	**1.05**	**1.05**	**1.2**	**1.2**	**0.3**
40	**0.75**	**0.6**	**0.75**	**0.6**	**0.3**

Table7. *Mean of serum antibody titers in cattle vaccinated with polyvalent FMD ISA 206- aluminum hydroxide gel vaccine using SNT expressed* log_{10}

Weeks post vaccination	**Mean antibody titer against FMD virus strains**				**Non vaccinated Group**
	FMD (O)	**FMD (A)**	**FMD (SAT/2012)**	**FMD (SAT/2018)**	
Pre-vaccination	**0.27**	**0.27**	**0.15**	**0.3**	**0.3**
1	**1.1**	**1.05**	**1.2**	**1.2**	**0.3**
2	**1.65**	**1.8**	**1.8**	**1.8**	**0.3**
3	**2.1**	**2.1**	**1.95**	**1.8**	**0.3**
4	**2.4**	**2.4**	**2.4**	**2.1**	**0.3**

6	2.7	2.7	2.7	2.7	0.3
8	2.85	2.85	2.85	2.85	0.3
10	3.15	3.15	3.1	3.0	0.3
12	315	3.0	3.0	3.0	0.3
14	2.55	2.7	2.85	2.7	0.3
16	2.4	2.4	2.7	2.55	0.3
20	2.1	2.1	2.4	2.1	0.3
24	1.8	1.8	2.1	2.1	0.3
28	1.65	1.65	1.8	1.65	0.3
32	1. 5	1. 5	1.65	1.5	0.3
36	1.05	1.05	1.2	1.2	0.3
40	0.75	0.6	0.75	0.6	0.3

Table8. *Mean of serum antibody titers in cattle vaccinated with polyvalent ISA 201 oil vaccine using SNT expressed* log_{10}

Weeks post vaccination	Mean antibody titer against FMD virus strains				Non vaccinated Group
	FMD (O)	FMD (A)	FMD (SAT/2012)	FMD (SAT/2018)	
Pre-vaccination	0.27	0.27	0.15	0.3	0.3
1	1.1	1.05	1.2	1.2	0.3
2	1.65	1.8	1.8	1.8	0.3
3	2.1	2.1	1.95	1.95	0.3
4	2.4	2.4	2.4	2.1	0.3
6	2.7	2.7	2.7	2.7	0.3
8	2.85	2.85	2.85	2.85	0.3
10	2.85	3.0	2.85	2.85	0.3
12	2.55	2.85	2.85	2.85	0.3
14	2.55	2.7	2.7	2.7	0.3
16	2.4	2.4	2.7	2.55	0.3
20	2.1	2.1	2.4	2.1	0.3
24	1.8	1.8	2.1	2.1	0.3
28	1.65	1.65	1.8	1.65	0.3
32	1. 5	1. 5	1.65	1.5	0.3
36	1.05	1.05	1.2	1.2	0.3
40	0.75	0.6	0.75	0.6	0.3

Table9. *Mean of serum antibody titers in cattle vaccinated with polyvalent ISA 201- aluminum hydroxide gel vaccine using SNT expressed* log_{10}

Weeks post vaccination	Mean antibody titer against FMD virus strains				Non vaccinated Group
	FMD (O)	FMD (A)	FMD (SAT/2012)	FMD (SAT/2018)	
Pre-vaccination	0.27	0.27	0.15	0.3	0.3
1	1.1	1.05	1.2	1.2	0.3
2	1.65	1.8	1.8	1.8	0.3
3	2.1	2.1	1.95	1.8	0.3
4	2.4	2.4	2.4	2.1	0.3
6	2.7	2.7	2.7	2.7	0.3
8	2.85	2.85	2.85	2.85	0.3
10	2.85	3.15	3.1	3.0	0.3
12	2.55	2.85	3.0	2.85	0.3
14	2.55	2.7	2.85	2.85	0.3
16	2.4	2.4	2.7	2.55	0.3
20	2.1	2.1	2.4	2.1	0.3
24	1.8	1.8	2.1	2.1	0.3
28	1.65	1.65	1.8	1.65	0.3
32	1. 5	1. 5	1.65	1.5	0.3
36	1.05	1.05	1.2	1.05	0.3
40	0.75	0.6	0.75	0.6	0.3

The selection of adjuvants in FMD vaccine formulation is important for both early and long-lasting immunity and protection **Park et al., (2014)**. The aluminium hydroxide gel is the most commonly used adjuvant in commercial vaccines **Rimaniol and Gras (2004)**. A previous report showed that aluminium hydroxide gel induces Th2-type

responses in animal models, facilitating the dissemination of antibodies from the injected region **Gupta et al., (1995) and Brewer et al., (1996)**. In addition, the aluminium hydroxide gel was shown to play an important role in memory responses by inducing the differentiation of macro- phages. Gel-adjuvanated FMD vaccines are currently used only in cattle, because they offer only a short period of immunity, making them unsuitable for use in pigs **Park (2013)**.

Moreover, the immune responses in sheep and goats are poorer than those of oil-based vaccines **Patil et a., (2002 a,b)**. The combined components of oil and aluminium hydroxide gel have been used to protect against rabies in bovines **Reddy and Srinivasan (1996)**.

In this study, we tested a combination of oil with aluminium hydroxide gel to enhance the immune responses. We found that vaccines prepared with ISA 201 and ISA 201＋aluminum hydroxide gel induced better cellular immunity than that induced by vaccines prepared with ISA 206 and ISA 2016＋aluminum hydroxide gel, also found that vaccines prepared with ISA 206 and ISA 206＋aluminum hydroxide gel induce better humeral immunity than that induced by vaccines prepared with ISA 201 and ISA 201＋ aluminum hydroxide gel.

We concluded that aluminum hydroxide gel improves the effects of ISA adjuvants.

In the present study, we thus attempted to demonstrate that mixing of various oil-adjuvants and aluminium hydroxide gel would induces similar protection in cattle. We confirmed slight increases in the level of cellular and humeral immune response after using aluminium hydroxide gel.

Finally, Oil-adjuvanated vaccines can result in the formation of local lesions in the injected areas. Thus, to avoid granuloma, long-term studies with a reduced volume of vaccines should be carried out to identify whether such lesions increase or decrease when aluminum hydroxide gel is added to the vaccines. In addition, comparative studies on long-lasting immunity in experimental and target animals may be required for the development of new vaccines.

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Citation: *Wael Mosad, Ehab Elsayed, Amr I Hassanin, Assem A Mohamed, Walaa Shabana. Trials for Improvement of the Immune Response of Foot and Mouth Disease Vaccine Using Oil/Gel Mixture as an Adjuvant. ARC Journal of Animal and Veterinary Sciences. 2019; 5(2):16-24. doi: dx.doi.org/10.20431/2455-2518.0502003.*