Comparative Study for Immune Efficacy of Two Different Adjuvants Bivalent FMD Vaccines in Sheep

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Abstract: This work was planed to study the immune response in sheep vaccinated with bivalent inactivated aluminum hydroxide gel ($AL(OH)_3$) and oil adjuvant (Montanide ISA206) FMD vaccine by ELISA and SNT. Thirty sheep were used and were classified into three groups: 1st group (10 animals) vaccinated with bivalent $AL(OH)_3$ gel inactivated FMD vaccines; 2nd group (10 animals) vaccinated with bivalent inactivated oil-adjuvant FMD vaccine and 3rd group (10 animals) non-vaccinated control group. The immune response in the 1st group revealed that specific FMD antibodies titers were detected after 2 weeks post vaccination (WPV) by ELISA (one or more $\log_{10}$) and after 3 WPV by SNT (1.2 or more $\log_{10}$); FMD serum antibodies were peaked at 8 WPV then gradually decreased until 18 WPV and 16 WPV for serotype O and A respectively and all vaccinated sheep became seronegative at 24 WPV. The duration of protective immunity with aluminum hydroxide gel bivalent (O1 and A/Egypt 2006) FMD vaccine was 12 -13 weeks by SNT and ELISA. Whereas the immune response in the 2nd group revealed that specific FMD antibodies were detected after 2 WPV by ELISA (one or more $\log_{10}$) and after 3 WPV by SNT (1.2 or more $\log_{10}$); FMD serum antibodies were peaked at 12 WPV then gradually decreased until 32WPV and all vaccinated sheep became seronegative at 40 WPV. The duration of protective immunity with oil adjuvant (Montanide ISA206) (O1 and A/Egypt 2006) FMD vaccine was 29-30 weeks by SNT and ELISA. All control animals were negative by SNT and ELISA a along time of the experiment. It could be concluded that vaccination of sheep with bivalent inactivated oil adjuvant Montanide ISA 206 vaccine gave higher long lasting immunity than AL (OH)3, and could replace the commercial aluminum hydroxide FMD vaccine.
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1. Introduction:

Foot and mouth disease (FMD) is a highly contagious disease affecting cloven-hoofed animals. It causes production loss and constraint imposed on international trade in live animals and their products (KO et al., 2009). FMD is associated with an aphthovirus (family Picomaviridae) which occurs as seven major serotypes: A, 0, C, Southern African Territories (SAT) 1, SAT 2, SAT 3 and Asia 1. However, there are a number of immunologically and serologically distinct subtypes with different degrees of virulence, especially within the A and 0 types. As there is no cross-immunity between serotypes, immunity to one type does not confer protection against the others (Knowles & Samuel, 2003 and Radostits et al., 2010).

The typical severity of FMD and the level and duration of infectiousness vary widely among hosts, with sheep showing less clinical evidence of infection than cattle or pigs (Pay, 1988). All of the most recent outbreaks of FMD within and around the European Union member states have involved sheep (Donaldson &Doel, 1992; Kitching, 1996 and Ferguson, et al., 2001). In Egypt over the last forty – five years (1960-2005) only serotype O1 had been isolated yearly while FMD virus serotype A was not recorded in Egypt since 1956 and it was confirmed in Egypt since March 2006 through live animals importation (Abed El Rhaman et al., (2006).

Newer techniques for identifying subtypes involve enzyme-linked immunosorbent assay (ELISA), reverse transcriptasepolymerase chain reaction (RT-PCR) and nucleotide sequence analysis (Radostits et al., 2010). The virus neutralization test is the reference test to detect antibodies against FMDV (De Clercq, 2002). ELISA preferable in FMD diagnosis as it is serotype specific, sensitive, quantitative and quicker to perform (Mackay et al., 2001).

In the endemic countries, eradication does not seem possible within the foreseeable future and countries free of the disease may require regional vaccination during outbreaks. Because of the increasing occurrence of antigenically dissimilar substrains, the production of vaccines from locally

isolated virus is becoming a more common practice (Radostits et al., 2010).

The progress in FMD vaccine production was primarily directed towards safety of the vaccine, purity of the antigen, selection of proper adjuvant and endurance of immunity (Osama, 1992). An important consideration in the selection of an adjuvant system is that the vaccine should give minimal reaction in the vaccinated animal (Doel, 1999). Current research in Egypt is directed towards developing and evaluating adjuvants that result in a high and long lasting immunity (Deghaidy et al., 2002). In view of the above argument this work was planned to evaluate the immune response in sheep vaccinated either with bivalent inactivated aluminum hydroxide gel FMD or oil adjuvant (Montanide ISA206) FMD vaccine by SNT and ELISA.

2. Materials and Methods

2.1. Materials:

2.1.1. Animals:

Thirty apparently healthy local breed male sheep aged 10 -12 month and weighting about 25-30 kg body weight used in this study. They were proved to be free from FMDV type "A, O" antibodies titers using SNT and ELISA.

1.2. Serum Samples:

Ten ml of blood were collected in clean sterile dry screw capped bottle from sheep jugular vein before and after vaccination. The collected blood were left to clot at room temperature for one hour and centrifuged at 3000 rpm for 15 minutes. Sera were aspirated by Pasteur pipette in other clean dry crocked bottle which labeled in a serial number and stored at –20 ^{o}C until used.

2.1.3. Biological reagents:

2.1.3.1. FMDV:

Locally isolated FMD virus type O strain (O1/3/93 Egypt) first appeared in Aga 1993 and FMD virus type A strain (A/EGY/1/2006) first appeared in Ismailia 2006 stored at -70 ºC and used for determination of antibodies against FMD virus type O and A.

2.1.3.2. Cell cultures (BHK_{21}, $clone_{13}$):

It was supplied from Institute of animal health, Pirbright, UK. and propagated in FMD department, Veterinary Serum and Vaccine Research Institute, Cairo according to the technique described by (Macpherson & Stocher, 1962).

2. 1.3.3. New-born calf serum: (Sigma, Germany). Used as 8% in growth medium according to (Telling and Roblett, 1969).

2.1.3.4. Bivalent inactivated FMD vaccine with AL $(OH)_3$ gel and oil emulsion Montanide ISA206. Vaccines were kindly supplied by serum and vaccine research and production institute, Abassia, Cairo.

2.1.4. Chemical reagents and buffers

2.1.4.1. Reagents for SNT:

It was performed using the micro technique as described by Ferreira, (1976).

2.1.4.2. Reagents for ELISA:

It was carried out according to the method described by Voller et al., (1976).

2.2. Methods:

2.2.1. Experimental vaccination of bivalent inactivated aluminum hydroxide gel and oil adjuvant (Montanide ISA206) FMD vaccine in sheep.

The selected 30 sheep used in experimental vaccination were classified into three groups:

- 1st group (10 animals) administrated S/C with 1ml aluminium hydroxide gel inactivated FMD vaccines.
- 2nd group (10 animals) administrated I/M with 1ml of inactivated oil-adjuvanted FMD vaccine.
- 3rd group (10 animal) non-vaccinated sheep and used as control group.

Serum samples were collected from experimentally vaccinated and control sheep weekly interval for 8 weeks then every 2 weeks until disappearance of antibodies. The serum samples were tested serologically by ELISA and SNT according to Voller et al., (1976) and Ferreira, (1976) respectively.

2.2.2. Statistical analysis

Data were analyzed using Microsoft office excel 2007 program and statistical analysis system (SPSS) package ***(Littell et al., 1991)***.

3. Results

The mean antibody titer by SNT reached to 1.2 $\log_{10}$ or more in all vaccinated sheep with $AL(OH)_3$ gel and oil adjuvant bivalent FMD vaccine after 3 WPV in both serotypes A, O. Antibody titers reached to the maximum mean titer (1.755 $\log_{10}$ and 1.71 $\log_{10}$) at 8 WPV with in serotypes O and A respectively in $AL(OH)_3$ adjuvant vaccine while in oil adjuvant vaccine maximum mean titer (2.52$\log_{10}$ and 2.55$\log_{10}$) obtained at 12 WPV in both serotype A in serotype O. In $AL(OH)_3$ gel bivalent FMD vaccinated group antibodies showed gradually decreased until 16-18 WPV and all vaccinated sheep became seronegative at 24 WPV. While in oil adjuvant vaccinated group Antibodies showed gradually decreased until 32 WPV and all vaccinated sheep became seronegative at 40 WPV (Table 1).

Table (1) Comparative means of serum neutralizing antibody titers for sheep vaccinated by AL (OH)3 and Montanide ISA206 oil adjuvenated FMD bivalent (A,O) vaccines.

(Log10) antibodies titers means obtained by SNT					
Type of vaccine		AL(OH)3		Montanide ISA206 OIL	
FMD serotype		A	O	A	O
Weeks post vaccination	0	0.24	0.295	0.389	0.499
	1	0.465	0.505	0.645	0.735
	2	0.915	0.915	1.065	1.095
	3	1.23	1.335	1.47	1.515
	4	1.38	1.41	1.65	1.74
	5	1.485	1.545	1.77	1.905
	6	1.56	1.65	1.95	2.025
	7	1.635	1.71	2.175	2.16
	8	1.71	1.755	2.25	2.295
	10	1.59	1.695	2.43	2.475
	12	1.53	1.545	2.52	2.55
	14	1.38	1.455	2.385	2.49
	16	1.32	1.35	2.325	2.46
	18	1.17	1.275	2.295	2.385
	20	1.11	1.185	2.16	2.34
	22	0.9	1.065	2.055	2.19
	24	0.73	0.855	1.905	2.04
	26	0.525	0.66	1.77	1.905
	28	N.D*	N.D	1.65	1.74
	30	N.D	N.D	1.515	1.605
	32	N.D	N.D	1.35	1.395
	34	N.D	N.D	1.155	1.17
	36	N.D	N.D	1.005	1.05
	38	N.D	N.D	0.765	0.84
	40	N.D	N.D	0.615	0.585

- All control animals were negative by serum neutralization test a along time of the experiment.
- N.D* = not done because the results of the last three successive samples were less than the protective level.

The mean antibody titer by ELISA reached to one $\log_{10}$ or more in all vaccinated sheep with AL $(OH)_3$ gel and oil adjuvant bivalent FMD vaccine after 2 WPV in both serotypes A, O. Antibody titers reached to the maximum mean titer ($2.054\log_{10}$ and $2.059\log_{10}$) obtained at 8 WPV in serotypes O and A respectively in $AL(OH)_3$ adjuvant vaccine, while in oil adjuvant vaccine maximum mean titer (2.844 $\log_{10}$ and 2.858 $\log_{10}$) obtained at 12 WPV in both serotype A in serotype O. In $AL(OH)_3$ gel bivalent FMD vaccinated group antibodies showed gradually decreased until 16-18 WPV and all vaccinated sheep became seronegative at 24 WPV. While in oil adjuvant vaccinated group Antibodies showed gradually decreased until 32 WPV and all vaccinated sheep became seronegative at 40 WPV (Table 2).

Table (2) Comparative means of ELISA antibody titers for sheep vaccinated by AL(OH)3 and Montanide ISA206 oil adjuvenated FMD bivalent (A,O) vaccines.

(Log10) antibodies titers means obtained by ELISA					
Type of vaccine		AL(OH)3		Montanide ISA206 OIL	
FMD serotype		A	O	A	O
Weeks post vaccination	0	0.209	0.376	0.47	0.543
	1	0.48	0.599	0.728	0.86
	2	1.006	1.002	1.172	1.263
	3	1.339	1.468	1.67	1.695
	4	1.632	1.638	1.845	1.924
	5	1.747	1.795	2.025	2.117
	6	1.868	1.898	2.29	2.257
	7	2.009	2.041	2.465	2.47
	8	2.059	2.054	2.632	2.581
	10	1.898	1.971	2.728	2.83
	12	1.801	1.842	2.844	2.858
	14	1.743	1.752	2.796	2.803
	16	1.669	1.651	2.739	2.705
	18	1.518	1.477	2.65	2.6
	20	1.34	1.269	2.496	2.415
	22	1.173	1.046	2.378	2.271
	24	0.928	0.814	2.155	2.135
	26	0.605	0.537	2.012	1.981
	28	N.D*	N.D	1.908	1.865
	30	N.D	N.D	1.797	1.754
	32	N.D	N.D	1.699	1.652
	34	N.D	N.D	1.58	1.48
	36	N.D	N.D	1.378	1.312
	38	N.D	N.D	1.002	1.013
	40	N.D	N.D	0.838	0.833

- All control animals were negative by ELISA test along time of the experiment.
- N.D* = not done because the results of the last three successive samples were less than the protective level.

4. Discussion

In order to adopt a good vaccination program to control of FMD disease, it is essential to know the specific serotype and the best time for revaccination. Essentially, there are three important factors for the production of FMD vaccines. First, the viral antigens should be propagated for large-scale production. Second, the virus must be treated in such a way that no residual infectivity remains. Third, a non-toxic adjuvant should be added to enhance the immune response to a satisfactory level (Deghaidy et al., 2002). Vaccination with good quality FMD vaccines helped in prevention of stock production losses and reduced the over all incidence of the disease (Hunter, 1997). Barnett et al., (1996) suggested that some of oil adjuvants of the Montanide series appeared to be promising candidates for the new generation FMD vaccines.

The immune response in sheep vaccinated with $AL(OH)_3$ gel bivalent (O1 and A/Egypt 2006) FMD vaccine revealed that specific FMD antibodies titers were detected after 2 weeks post vaccination (WPV) by ELISA (one or more $\log_{10}$) and after 3 WPV by SNT (1.2 or more $\log_{10}$). These results were concordant with those reported by Rweyemamu et al., (1978) who observed that FMD vaccine type O induced neutralizing antibody titres of 2.35 ± 0.4 at 21 days post vaccination and Abeer, (1996) who detected that serum neutralizing antibody titre by SNT in sheep vaccinated group with FMD AL $(OH)_3$ monovalent O1 vaccine began at the first 2nd week post vaccination. Whereas Ehab, (2007) showed that immune response against FMDV in ewes vaccinated S/C with 1 ml inactivated monovalent $AL(OH)_3$ saponine FMDV/O1/93 started from the first WPV with mean antibody titer (0.48 and 0.78) log10 by SNT and ELISA respectively. This variation may be attributed to the difference of adjuvant use in vaccines. FMD serum antibodies were peaked at 8 WPV then gradually decreased until 18 WPV and 16

WPV for serotype O and A respectively and all vaccinated sheep became seronegative at 24 WPV. The duration of protective immunity with $AL(OH)_3$ gel bivalent (O1 and A/Egypt 2006) FMD vaccine was 12 -13 weeks by SNT and ELISA. Nearly similar results were reported by Abeer, (1996); Suhier et al ., (1998) ; Ismail et al., (2000); Daoud et al., (2002); Wafaa et al., (2002); Ahmed, (2007) and Ehab, (2007).

Regarding to the immune response in sheep vaccinated with oil adjuvant Montanide ISA 206 bivalent (O1 and A/Egypt 2006) FMD vaccine, the results revealed that specific FMD antibodies were detected after 2 WPV by ELISA (one or more log10) and after 3 WPV by SNT (1.2 log10). ELISA results were in parallel correlation with those obtained with SNT and this agreed with Fatthia, (2003) and Hamblin et al., (1986) who revealed that there was a linear correlation between SNT and ELISA test for detection of FMD antibody titer and ELISA test can detect antibodies earlier than the SNT. FMD serum antibodies were peaked at 12 WPV then gradually decreased until 32WPV and all vaccinated sheep became seronegative at 40 WPV. The duration of protective immunity with oil adjuvant (Montanide ISA206) (O1 and A/Egypt 2006) FMD vaccine was 29-30 weeks by SNT and ELISA (Table 1 &2). These results were similar to those reported by Cox et al., (2003) who found a rapid seroconversion in both sheep and pigs. The Montanide ISA 206 formulation gave longer lasting immunity than the conventional lower potency vaccines in ruminants. Emergency vaccination should be done with these high potency vaccines during an outbreak. The advantage of oil adjuvant was attributed to depot formation at the site of injection, a vehicle for transport of the antigen throughout the lymphatic system and slow antigen release with the stimulation of antibody producing cells. Moreover, being oil emulsion, Montanide ISA206 had various advantages, like viscosity, easy administration, greater stability and production of smaller nodules at the site of injection (Barnett et al., 1996), compared to other oil adjuvants, making as an ideal adjuvant candidate for FMD vaccines. The result of Barent and Cox (1999) has the superiority of the Montanide ISA206 preparation for longer term protection. It was also suggested that Montanide ISA206 could prevent the loss of potency was due to the proteolysis of VP1 or possibly the physical breakdown of the virus followed adsorption to the aluminum hydroxide gel (Doel and Pullen, 1990) and agree with the usage of Montanide ISA206 ready to formulate oil adjuvant can be sued in all target species is ideal for emergency vaccination (Barnett and Carabin, 2002). The usage of oil adjuvant (Montanide ISA206) improve, enhance cell mediated immunity and give higher level and long lasting immunity (Sonia, 2007). These results were in agreement with those reported by Fatthia, (2003) who observed that douple oil emulsion with Montanide ISA206 vaccine gave longer duration of protection than that obtained by alhydragel vaccine and both vaccines were safely used as they did not produce any local reaction at the site of inoculation; Mohamed, (1998) & Wafaa, (1999) detected that DOE vaccine containing Montanide ISA206 is highly efficient, fluidy with low viscosity which is easily dispersed from the place of injection and gave high Ab titers and longest duration of immunity than alhdyragel vaccine and Daoud et al., (2002) reported that the duration of immunity elicited by gel FMD vaccine was short lived and antibody concentrations rapidly fall after administration, while oils adjuventated FMD vaccines gave a longer duration of immunity and suggested that the oil adjuvenated vaccines had potential as an alternative to the conventional aluminum hydroxide FMD vaccine. Moreover Patil et al., (2002) reported that the oil adjuvant elicited a better immune response at any time than did aluminum hydroxide gel FMD quadrivalent vaccines in goats, and the response developed quicker. Local tissue reactions such as granulomas and cysts to oil-adjuvants have been not detected.

It could be concluded that the oil adjuvant vaccine elicited superior immune response at any given period of the study than $AL(OH)_3$ gel vaccine and immunity maintained for longer period. These observations suggest that the oil adjuvenated vaccine has potential to replace the commercial aluminum hydroxide FMD vaccine.

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