

## **ENHANCING EFFECT OF GINSENG STEM-LEAF SAPONINS (GSLs) ON THE IMMUNE RESPONSES IN VACCINATED CALVES WITH FMD BIVALENT VACCINE**

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### **ABSTRACT**

A comprehensive sero-immunological studies were conducted to reveal the adjuvant's effect of Ginseng Stem-leaf Saponins (GSLs) on the immune response of gel adjuvanted Bivalent Foot and Mouth Disease (FMD) vaccinated calves. These study conducted in two calve groups; group (A) vaccinated subcutaneously with bivalent Alhydrogel adjuvanted (30) % FMD vaccine, while group (B) vaccinated subcutaneously with bivalent FMD vaccine adjuvanted with both Alhydrogel and GSLs (10 mg/dose). The humeral and cellular immunoresponses were monitored in different tested groups that received the gel adjuvanted vaccine and the Alhydrogel-GSLs adjuvanted vaccine. Results indicated that the higher immune responses were found in calves vaccinated with Alhydrogel-GSLs adjuvanted vaccin up to 24 week while with Alhydrogel alone was only up to 18 week.

**Keywords:** Adjuvant, Calves, FMD vaccine, GSLs, Sero-immunological.

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## INTRODUCTION

Foot and mouth disease (FMD) is an acute contagious viral disease of cloven footed animals (Radostits *et al.*, 1995 and Orsel *et al.*, 2007). The causative agent is a single stranded positive-sense RNA virus that belongs to the genus Aphthovirus in the family Picornaviridae. There are seven immunologically distinct serotype of FMD virus, namely, O, A, C, Asia1, Sat1, Sat2 and Sat3 (Belsham, 1993). In Egypt, the disease is enzootic and outbreaks have been reported since 1950 (Mousa *et al.*, 1974).

Type O was the most prevalent since 1960 and onwards (Zahran, 1960, Daoud *et al.*, 1988 and Farag *et al.*, 2005). Since 1950, 1953 and 1956 serotype A didn't recorded in Egypt (Zahran, 1960), recently serotype A FMD virus introduced to Egypt through live animals importation, and the sever clinical signs occurred among cattle and buffaloes (Abd El-Rahman *et al.*, 2006). 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 (Nair and Sen,

1992). Most foot-and-mouth disease vaccines are made of BEI (binary Ethylenimine) inactivated virus that is adjuvanted with either aluminum hydroxide-saponin (AS) or oil adjuvant. Adjuvants, also can prolong the immune response and stimulate specific components of the immune response either humoral or cell mediated immunity (Dalsgaard *et al.*, 1990, Barnett *et al.*, 2003, .Pluimers, 2004 and Lombard *et al.*, 2007). Saponins extracted from ginseng stems and leaves (GSLs) has an adjuvant effects on the immune responses of buffalo to vaccination against foot-and-mouth disease virus (FMDV) (Xie *et al.*, 2004). This study was carried out as an attempt to detect the adjuvant effects of Saponin extracted from ginseng stems and leaves (GSLs) on the immune responses of calves to vaccination against foot-and-mouth disease virus (FMDV) to improve local inactivated FMD vaccine.

## MATERIAL & METHODS

### Animals:

Nine calves (local breed) were clinically healthy and free from antibodies against FMD virus as proved by using SNT and ELISA were used in this study.

**FMD viruses:**

FMD viruses O<sub>1</sub>/3/93-Egypt Strain and A<sub>1</sub>/Egypt/2006 are locally isolated strains of cattle origin. The viruses were typed at Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo and confirmed by Pirbright, International Reference Laboratories, United Kingdom.

**FMD vaccines:**

Inactivated bivalent FMD vaccines were prepared using the local strains O<sub>1</sub>/3/93 Egypt and A<sub>1</sub>/Egypt/2006, propagated in BHK-21 cell line. The viruses had a titer of 10<sup>8</sup> TCID<sub>50</sub> /ml for both and inactivated by Binary Ethylenimine (BEI), FMD vaccines with different adjuvant are formulized as follow:

**- Alhydrogel:**

The inactivated FMD viruses suspension was mixed with 30% Alhydrogel solution as adjuvant. *Mousa et al. (1976)*.

**- Ginseng Stems and leaves (GSLS) Saponine:**

The inactivated FMD viruses suspension was mixed with 30% Alhydrogel solution with adding 10 mg/ dose of Ginseng stems and leaves saponine. *Song and Hu (2009)*.

**Experimental Design:**

Two groups each group contain 3 calves, were vaccinated with the tested vaccines beside unvaccinated group (3 calves). Serum samples were collected weekly post vaccination for one month then every 2 weeks post-vaccination till the end of experiment. The immune response was evaluated through the estimation of cellular and humoral immune level using

Lymphocyte blastogenesis assay, SNT and ELISA.

**Serum neutralization test (SNT):**

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

**Enzyme linked immunosorbent assay (ELISA):**

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

Indirect solid phase ELISA was applied, patently prepared at department of FMD, Veterinary Serum and Vaccine Research Institute, Abbasia, Egypt.

**Evaluation of cell-mediated immunity in vitro using lymphocyte Proliferation (3-(4,5-Dimethylthiazol-2-yl)-2,5- (MTT) Assay:**

It was applied according to *Lucy, (1984)* following by modification adopted by *El-Watany et al. (1999)* and *Abeer (2001)*.

## RESULTS

### Humoral immune response of calves vaccinated with FMD vaccines:

Results of humoral immune response revealed that serum antibody protective titer evaluated by mean of SNT and ELISA were as follow:

**1<sup>st</sup> group** started at 2<sup>nd</sup> week post vaccination with the titers of 1.3 log<sub>10</sub> by SNT and 1.5 by ELISA for O<sub>1</sub> was with titer of 1.4 log<sub>10</sub> by SNT and 1.5 by ELISA for A<sub>1</sub>. The highest level of antibody titers were at the 6<sup>th</sup> week post vaccination as 2.1 log<sub>10</sub> by SNT and 2.4 by ELISA for O<sub>1</sub>, as 2.2 log<sub>10</sub> by SNT and 2.4 by ELISA for A<sub>1</sub>, and the immunity duration lasted for 18 weeks post vaccination as 1.2 log<sub>10</sub> by SNT and 1.5 by ELISA for O<sub>1</sub>, as 1.2 log<sub>10</sub> by SNT and 1.5 by ELISA for A<sub>1</sub>.

**2<sup>nd</sup> group** started at 2<sup>nd</sup> week post vaccination with the titers of 1.5 log<sub>10</sub> by SNT and 1.6 by ELISA for O<sub>1</sub> and was with titer of 1.6 log<sub>10</sub> by SNT and 1.6 by ELISA for A<sub>1</sub>. The highest level of antibody titers were at the 8<sup>th</sup> week post vaccination as 2.4 log<sub>10</sub> by SNT and 2.6 by ELISA for O<sub>1</sub>, as 2.4 log<sub>10</sub> by

SNT and 2.6 by ELISA for A<sub>1</sub>, and the immunity duration lasted for 24 weeks post vaccination as 1.2 log<sub>10</sub> by SNT and 1.5 by ELISA for O<sub>1</sub>, as 1.2 log<sub>10</sub> by SNT and 1.5 by ELISA for A<sub>1</sub>.

Tables (1, 2, 3 and 4).

### Evaluation of cell-mediated immunity in vitro using lymphocyte Proliferation (MTT) Assay:

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

**1<sup>st</sup> group-** Delta Optical Density was (0.152-0.11-0.128) by using phytohaemagglutinin (PHA), Pokeweed (pok) mitogens and FMD virus at 3<sup>rd</sup> day post vaccination and still rise reached its highest level (0.28-0.30-0.36) at 21<sup>st</sup> day post vaccination, then declined to( 6weeks ).

**2<sup>nd</sup> group-** Delta Optical Density was (0.232-0.191-0.309) by using (PHA), (pok) and FMD virus at 3<sup>rd</sup> day post vaccination and still rise reached its highest level (0.413-0.442-0.524) at 21<sup>st</sup> day post vaccination, then declined after (9 weeks).

Tables (1, 2, 3 and 4).

**Table 1.** Immune status (SNT titer) of calves vaccinated with Alhydrogel and modified Ginseng FMD vaccines against O<sub>1</sub>/3/93-Egypt virus.

Weeks post vaccination	Type of vaccines								Control group of calves*
	Alhydrogel FMD vaccine				Ginseng FMD vaccine				
	A1	A2	A3	Average*	A1	A2	A3	Average*	
0	0.3	0.0	0.6	0.3	0.3	0.0	0.0	0.2	0.0
1	0.9	1.0	0.9	0.9	1.2	0.9	1.2	1.1	0.0
2	1.5	1.2	1.2	1.3	1.8	1.2	1.5	1.5	0.3
3	1.8	1.5	1.5	1.6	2.1	1.5	1.8	1.8	0.3
4	1.8	2.1	1.5	1.8	2.4	1.8	2.1	2.1	0.3
6	2.1	2.4	1.8	2.1	2.4	2.1	2.4	2.3	0.6
8	1.8	2.1	2.1	2.0	2.4	2.1	2.7	2.4	0.6
10	1.5	1.8	1.8	1.7	2.1	2.4	2.4	2.3	0.6
12	1.5	1.5	1.8	1.6	2.1	2.1	2.4	2.2	0.3
14	1.5	1.5	1.5	1.5	2.1	2.1	2.4	2.1	0.3
16	1.2	1.2	1.2	1.2	2.1	2.1	2.1	2.1	0.3
18	1.2	1.2	1.2	1.2	1.8	1.8	2.1	1.8	0.3
20	0.9	1.2	1.2	1.1	1.8	1.5	2.1	1.8	0.3
22	0.9	0.9	1.2	1.0	1.2	1.2	1.5	1.2	0.3
24	0.9	0.9	0.9	1.0	1.2	1.2	1.5	1.2	0.3
26	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.3
28	0.9	0.9	0.9	0.9	0.6	0.6	0.6	0.6	0.0
30	0.6	0.9	0.9	0.9	0.6	0.6	0.6	0.6	0.0

A1, A2 and A3 = vaccinated calves no.

SNT = serum neutralization test.

\* = The results of SNT expressed as log<sub>10</sub> TCID<sub>50</sub>/ml.

N.B.: The permissible protective level is 1.2 SNT titer.

**Table 2.** Immune status (ELISA titer) of calves vaccinated with Alhydrogel and modified Ginseng FMD vaccines against O<sub>1</sub>/3/93-Egypt virus.

Weeks post vaccination	Type of vaccines								Control group of calves*
	Alhydrogel FMD vaccine				Ginseng FMD vaccine				
	A1	A2	A3	Average*	A1	A2	A3	Average*	
0	0.6	0.3	0.9	0.9	0.6	0.3	0.3	0.6	0.3
1	1.0	1.2	1.0	1.0	1.5	1.2	1.5	1.4	0.3
2	1.5	1.5	1.5	1.5	2.1	1.5	1.8	1.8	0.3
3	2.1	1.8	1.8	1.9	2.1	1.8	2.1	2.0	0.3
4	2.1	2.1	1.8	2.0	2.7	2.1	2.4	2.4	0.6
6	2.4	2.7	1.8	2.3	2.7	2.4	2.7	2.6	0.9
8	2.1	2.4	2.1	2.2	2.7	2.4	3.0	2.7	0.9
10	1.8	2.1	1.8	1.9	2.4	2.4	2.4	2.4	0.9
12	1.8	1.8	1.8	1.8	2.4	2.1	2.4	2.3	0.6
14	1.8	1.8	1.8	1.8	2.1	2.1	2.4	2.2	0.6
16	1.5	1.8	1.5	1.6	2.1	2.1	2.4	2.2	0.6
18	1.5	1.5	1.5	1.5	2.1	1.8	2.1	2.0	0.6
20	1.2	1.5	1.2	1.3	1.8	1.5	2.1	1.8	0.6
22	0.9	0.9	1.2	1.0	1.8	1.5	1.8	1.8	0.3
24	0.9	0.9	1.2	1.0	1.2	1.2	1.5	1.3	0.3
26	0.9	0.9	0.9	0.9	1.2	0.9	1.2	1.1	0.3
28	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.3
30	0.6	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.3

A1, A2 and A3 = vaccinated calves no.

\* = The results of ELISA expressed as log<sub>10</sub> TCID<sub>50</sub>/ml.

N.B.: The permissible protective level is 1.5 ELISA titer

**Table 3.** Immune status (SNT titer) of calves vaccinated with Alhydrogel and modified Ginseng FMD vaccines against A<sub>1</sub>/Egypt/2006 virus.

Weeks post vaccination	Type of vaccines								Control group of calves*
	Alhydrogel FMD vaccine				Ginseng FMD vaccine				
	A1	A2	A3	Average*	A1	A2	A3	Average*	
0	0.3	0	0.6	0.3	0.3	0.0	0.0	0.2	0.0
1	0.9	1.2	0.9	1.0	1.2	1.2	1.2	1.2	0.0
2	1.5	1.5	1.2	1.4	1.8	1.5	1.5	1.6	0.3
3	1.8	1.5	1.5	1.6	2.1	1.5	1.8	1.8	0.3
4	1.8	2.1	1.5	1.8	2.4	1.8	2.1	2.1	0.3
6	2.1	2.4	2.1	2.2	2.4	2.1	2.4	2.3	0.6
8	1.8	2.1	2.1	2.0	2.4	2.1	2.7	2.4	0.6
10	1.8	1.8	1.8	1.8	2.1	2.4	2.4	2.3	0.6
12	1.5	1.8	1.8	1.7	2.1	2.4	2.4	2.3	0.3
14	1.5	1.5	1.5	1.5	2.1	2.1	2.4	2.1	0.3
16	1.2	1.2	1.2	1.2	2.1	2.1	2.1	2.1	0.3
18	1.2	1.2	1.2	1.2	1.8	2.1	2.1	2.0	0.3
20	0.9	1.2	1.2	1.1	1.8	1.5	2.1	1.8	0.3
22	0.9	0.9	1.2	1.0	1.2	1.5	1.5	1.4	0.3
24	0.9	0.9	0.9	1.0	1.2	1.2	1.5	1.2	0.3
26	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.3
28	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.0
30	0.6	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.0

A1, A2 and A3 = vaccinated calves no.

SNT = serum neutralization test.

\* = The results of SNT expressed as  $\log_{10}$  TCID<sub>50</sub>/ml.

N.B.: The permissible protective level is 1.2 SNT titer.

**Table 4.** Immune status (ELISA titer) of calves vaccinated with Alhydrogel and modified Ginseng FMD vaccines against A<sub>1</sub>/Egypt/2006 virus.

Weeks post vaccination	Type of vaccines								Control group of calves
	Alhydrogel FMD vaccine				Ginseng FMD vaccine				
	A1	A2	A3	Average*	A1	A2	A3	Average*	
0	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.0
1	1.2	1.5	1.2	1.3	1.8	1.5	1.8	1.7	0.0
2	1.8	1.5	1.5	1.6	2.1	1.8	1.8	1.9	0.3
3	2.1	2.1	1.8	2.0	2.4	2.1	2.4	2.3	0.3
4	2.1	2.4	2.1	2.3	2.7	2.4	2.7	2.6	0.3
6	2.4	2.7	2.4	2.6	3.0	2.7	3.0	2.9	0.6
8	2.4	2.4	2.4	2.4	3.0	2.7	3.0	2.9	0.6
10	2.1	2.1	2.1	2.1	2.7	2.7	3.0	2.8	0.6
12	2.1	2.1	2.1	2.1	2.4	2.1	2.4	2.3	0.3
14	1.8	1.8	1.8	1.8	2.1	2.1	2.4	2.2	0.3
16	1.8	1.8	1.8	1.8	2.1	2.1	2.4	2.2	0.3
18	1.5	1.5	1.5	1.5	2.1	2.1	2.4	2.2	0.3
20	1.2	1.5	1.2	1.3	2.1	1.8	2.4	2.1	0.3
22	1.2	1.5	1.2	1.3	1.8	1.8	2.1	1.9	0.3
24	0.9	1.2	1.2	1.1	1.8	1.5	1.8	1.7	0.3
26	0.9	0.9	0.9	0.9	1.5	1.2	1.2	1.2	0.3
28	0.9	0.9	0.9	0.9	1.2	0.9	0.9	1.0	0.0
30	0.6	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.0

A1, A2 and A3 = vaccinated calves no.

\* = The results of ELISA expressed as log<sub>10</sub> TCID<sub>50</sub>/ml.

N.B.: The permissible protective level is 1.5 ELISA titer.



## ENHANCING EFFECT OF GSLS ON VACCINATED CALVES WITH FMD

Table 5. Cell-mediated Immune response of calves Vaccinated with Alhydrogel and modified Ginseng FMD vaccines.

Time post vaccination	Calves vaccinated with Alhydrogel FMD vaccine.				Calves vaccinated with Alhydrogel and Ginseng FMD vaccine				Control group of calves*	
		1	2	3	Average*	1	2	3		Average*
prevaccination	PHA	0.070	0.070	0.073	0.071	0.074	0.076	0.078	0.076	0.060
	POK	0.012	0.011	0.016	0.013	0.023	0.024	0.025	0.024	0.011
	V	0.018	0.020	0.025	0.021	0.048	0.049	0.050	0.049	0.020
3 <sup>rd</sup> day	PHA	0.148	0.150	0.158	0.152	0.230	0.232	0.234	0.232	0.070
	POK	0.107	0.108	0.115	0.110	0.190	0.191	0.192	0.191	0.015
	V	0.125	0.127	0.132	0.128	0.308	0.309	0.310	0.309	0.022
1 <sup>st</sup> Week	PHA	0.178	0.180	0.182	0.180	0.293	0.294	0.295	0.294	0.070
	POK	0.123	0.124	0.128	0.125	0.201	0.203	0.205	0.203	0.012
	V	0.195	0.197	0.202	0.198	0.340	0.340	0.343	0.341	0.026
2 <sup>nd</sup> Week	PHA	0.257	0.258	0.265	0.260	0.358	0.360	0.362	0.360	0.080
	POK	0.228	0.229	0.236	0.231	0.339	0.341	0.343	0.341	0.014
	V	0.312	0.313	0.317	0.314	0.488	0.490	0.492	0.490	0.027
3 <sup>rd</sup> Week	PHA	0.278	0.279	0.283	0.280	0.412	0.412	0.415	0.413	0.090
	POK	0.302	0.303	0.307	0.304	0.441	0.440	0.445	0.442	0.019
	V	0.359	0.360	0.364	0.361	0.523	0.522	0.527	0.524	0.028
4 <sup>th</sup> Week	PHA	0.252	0.253	0.257	0.254	0.294	0.296	0.298	0.296	0.010
	POK	0.314	0.315	0.319	0.316	0.357	0.360	0.363	0.360	0.011
	V	0.322	0.321	0.326	0.323	0.560	0.558	0.562	0.560	0.027
5 <sup>th</sup> Week	PHA	0.221	0.222	0.227	0.224	0.292	0.294	0.296	0.294	0.090
	POK	0.264	0.263	0.268	0.265	0.333	0.332	0.337	0.334	0.015
	V	0.282	0.283	0.283	0.284	0.428	0.430	0.432	0.430	0.024
6 <sup>th</sup> Week	PHA	0.225	0.224	0.229	0.226	0.282	0.283	0.287	0.284	0.080
	POK	0.248	0.250	0.254	0.251	0.266	0.264	0.268	0.266	0.016
	V	0.237	0.236	0.241	0.238	0.339	0.340	0.344	0.341	0.023
7 <sup>th</sup> Week	PHA	0.198	0.199	0.203	0.200	0.254	0.252	0.256	0.254	0.070
	POK	0.218	0.219	0.223	0.220	0.241	0.240	0.245	0.242	0.012
	V	0.197	0.198	0.202	0.199	0.319	0.320	0.324	0.321	0.021
8 <sup>th</sup> Week	PHA	0.120	0.118	0.122	0.120	0.197	0.197	0.200	0.198	0.080
	POK	0.131	0.131	0.134	0.132	0.229	0.230	0.234	0.231	0.013
	V	0.149	0.149	0.152	0.150	0.300	0.300	0.303	0.301	0.023
9 <sup>th</sup> Week	PHA	0.099	0.100	0.101	0.100	0.206	0.204	0.208	0.206	0.070
	POK	0.173	0.175	0.177	0.175	0.251	0.253	0.255	0.253	0.011
	V	0.114	0.116	0.118	0.116	0.268	0.270	0.272	0.270	0.020
10 <sup>th</sup> Week	PHA	0.098	0.100	0.102	0.100	0.132	0.134	0.136	0.134	0.070
	POK	0.133	0.135	0.137	0.135	0.180	0.180	0.183	0.181	0.011
	V	0.114	0.116	0.118	0.116	0.160	0.163	0.168	0.165	0.020

\* = $\Delta$  Optical densites

\*\* = type of mitogen

POK= Pokeweed

PHA = Phytohaemagglutinin V =FMD Virus

N.B.: The permissible protective level is 0.250 Delta Optical Density

## DISCUSSION

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.

So, this study is to improvement of inactivated FMD Alhydrogel vaccine with adding Ginseng stems and leaves saponin as an adjuvant.

From **Tables (1 and 2)** the results revealed that SNT and ELISA titers for Alhydrogel FMD vaccines, go in hand with the results obtained are 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.

From **Tables (3 and 4)** the results revealed that SNT and ELISA titers for Alhydrogel and Ginseng FMD vaccine agreed with (**Rivera *et al.*, 2003; Hu *et al.*, 2003; Sun *et al.*, 2005; Yan *et al.*, 2007 and Song and Hu, 2009**) who showed that adjuvant properties of Ginseng

Extract as potent adjuvant induced higher antibody titers than the vaccine adjuvanted with Al(OH)<sub>3</sub> and improved the potency of adjuvants. Results supported also by (**Scaglione *et al.*, 1996, Rivera *et al.*, 2003 and Wang *et al.*, 2009**) who found that ginseng might help the vaccine work more effectively, increasing antibody production.

From **Table (5)** the results of evaluation of cell mediated immune response using lymphocyte proliferation test for all animal groups expressed by ΔOD (Delta Optical Density). Supported by (**Soos *et al.*, 1984, Knudsen *et al.*, 1979; Sharma, 1981**) who reported that cell mediated immune response was a constitute of immune response against FMD virus. And in agreement in some points with (**Mercedes *et al.*, 1996; El-Watany *et al.* 1999; Abeer, 2001; Mansour, 2001 and Samir, 2002**) that FMD vaccine stimulated the cellular immune response and lymphocyte stimulation by FMDV was greater than by mitogens (PHA) and (POK) and appeared increased in 1<sup>st</sup> and 2<sup>nd</sup> weeks post vaccination. While disagreed with **El-Watany *et al.* (1999)** and **Mansour (2001)** in that cell mediated immune response reach its highest level on the 14<sup>th</sup> day.

The obtained results were in agreement with **Song *et al.* (2002)**, **Chen *et al.* (2008)** and **Sun *et al.* (2009)** who stated that Ginseng extract act as an activator of the TH1 response. The Th<sub>1</sub> type is characterized by the production of antigen-specific IgG2a a Th<sub>1</sub> and the secretion of gamma interferon, interleukins which favor cellular immunity.

Our results also were supported by **Xie *et al.* (2004)** and **Wang *et al.* (2007)** who mentioned that Ginseng extract enhanced interleukins which enhance cell mediated immune response.

Finally, it can conclude that: The usage of Saponin extracted from ginseng stems and leaves in inactivated FMD vaccine gave long lasting immunity than that which with Alhydrogel adjuvant alone GSLS and improve both cellular and humoral immunity and gave earlier and more long lasting immunity.

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