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A STUDY ON THE IMMUNE RESPONSE OF SHEEP TO FOOT AND MOUTH DISEASE VIRUS VACCINE TYPE 'O' PREPARED WITH DIFFERENT INACTIVANTS AND ADJUVANTS

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Summary. – Foot and mouth disease virus (FMDV) type 'O' was inactivated either with formaldehyde or binaryethyleneimine (BEI). Vaccines were prepared with inactivated virus incorporating aluminium hydroxide gel or mineral oil as an adjuvant. The antibody response in sheep was monitored by serum neutralization and ELISA test for a period of six months. Significant difference in antibody response was not observed between vaccines inactivated with formaldehyde or BEI. On the other hand significant difference in the antibody response was noticed between alhydrogel and oil vaccines. The high titer of antibodies stimulated by oil adjuvant vaccines persisted longer than those of alhydrogel vaccines within the period of study.

Key words: foot and mouth disease virus; killed vaccine; adjuvants; antibodies; sheep

Introduction

Foot and mouth disease (FMD) is an economicaly significant disease in India. The control of the disease is effected at present by vaccination. For effective control of the disease whole susceptible population should be vaccinated including the small ruminants but there is no much data as regards the dose of the vaccine, type of the vaccine and the vaccine and the immune study of the vaccinated sheep.

Even though the disease in sheep is mild and inapparent (Geering, 1967; Burrows, 1968) it frequently produces a prolonged carrier state. For effective control of FMD in endemic areas systematic vaccination programme is a must in sheep to minimize its incidence. According to All India Coordinated Research Project for Epidemiological Studies on Foot and Mouth Disease report 1990, in recent years the outbreak of the disease with FMD type 'O' is maximal in this country. Keeping this in view a systematic study on the antibody response of

FMD type 'O' vaccine prepared by using different inactivants and adjuvants, were carried out in sheep.

Materials and Methods

Virus strain. Madras strain of type 'O' virus isolated in 1975 from Tamilnadu and maintained at the Indian Veterinary Research Institute (IVRI), Mukteswar was used.

Hyperimmune sera were prepared against type 'O' in guinea pigs.

Experimental animals. Albino guinea pigs of either sex weighing 450–500 g were obtained from IVRI, Yelahanka, Bangalore. Albino rabbits weighing 4–5 kg obtained from private breeding farm were used.

BHK-21 cells. Both monolayer and suspension cultures were used. The monolayer cultures were prepared in test tubes for serum neutralization test and suspension cultures were prepared for virus preparation as described by Kadoi *et al.* (1975).

Preparation of seed virus. Virus isolated from cattle tongue and adapted in BHK-21 cells (8 passages) was used for the preparation of seed virus in BHK-21 suspension cultures.

Virus assay. The infectivity titration of virus samples was performed in BHK-21 clone 13 monolayer cells and the $TCID_{50}$ titer was calculated as usual.

Complement fixation test. The CF antigen titer was determined by a micro methods as described by Telling (1975).

Preparation of alhydrogel vaccine. The virus was harvested from BHK-21 suspension cells 20 hr after infection. Suitability of the virus for vaccine preparation was assessed by determining infectivity and CF titer after clarification. Then the virus was inactivated by using formaldehyde or BEI.

The vaccine was formulated to contain 70 parts of virus and 30 parts of alhydrogel in glycocol buffer at final pH 8.7 when inactivated with 0.06 % formalin for 48 hr at 26 °C. The vaccine was concentrated after 14 days and saponified (Kumar *et al.*, 1979).

The inactivation of virus with BEI (0.001 mol/l) was done according to Bahnemann *et al.* (1974). The inactivation was stopped by adding 20 % sodium thiosulphate and the vaccine was formulated with alhydrogel as described above.

The oil adjuvant vaccine was prepared by mixing 60 parts of inactivated virus, 30 parts of mineral oil and 10 parts of lanolin and by blending for 5 min in a sterilized blender. When the correct viscocity of the vaccine was achieved a simple test was conducted to know whether a stable emulsion was formed.

Quality control vaccines. The vaccines were tested for sterility in various bacteriological media as described by Cruickshank *et al.* (1975). Safety tests of the vaccines were conducted according the British Veterinary Codex (1965). Potency tests of the vaccines were conducted in guinea pigs (Lucam *et al.*, 1964).

Vaccination of sheep. 24 non-vaccinated sheep against FMD type 'O' were purchased from organised farm. The animals were screened against FMDV type 'O' antibodies. Vaccines prepared under different treatments were compared by vaccinating the sheep in different group and the antibody response was monitored by serum neutralization (SN) test and ELISA.

Results

The antibody response of sheep to the formaline inactivated gel vaccine is presented in Table 1. An average SN antibody titer of 1.48 was noted at the first week post vaccination. The titer steadily increased and reached a maximum of 1.93 at the 4th week and then declined. An average ELISA titer of 41 was noted

Table 1. Immune response of sheep to formalin inactivated FMD vaccine

A. Aluminium hydroxide gel as adjuvant

Sheep No.	Type of assay	Titer								
		Weeks post vaccination								
		1	2	3	4	8	12	16	20	24
124	SN	1.35	1.40	1.40	1.86	1.95	1.56	1.40	1.26	1.10
	ELISA	25	25	50	50	100	50	25	25	Nil
126	SN	1.46	1.70	1.86	1.95	1.70	1.65	1.70	1.40	1.26
	ELISA	50	50	50	100	50	50	50	25	25
131	SN	1.65	1.86	1.95	2.00	1.95	1.56	1.26	1.26	1.10
	ELISA	50	50	100	200	100	50	25	Nil	Nil
	Average SN titer	1.48	1.65	1.73	1.93	1.86	1.59	1.45	1.30	1.15
	Average ELISA titer	41	41	66	116	83	50	33	16	8

B. Mineral oil as adjuvant

133	SN	1.46	1.65	1.70	1.86	2.00	2.30	2.30	2.00	2.16
	ELISA	25	50	50	50	100	200	200	100	100
136	SN	1.86	2.25	2.16	2.25	2.16	2.16	2.16	2.00	2.00
	ELISA	50	200	200	200	200	100	100	100	50
137	SN	1.35	1.70	1.86	1.70	2.16	2.16	2.25	2.00	1.95
	ELISA	25	50	50	50	100	100	200	100	100
	Average SN titer	1.55	1.86	1.90	1.93	2.10	2.20	2.23	2.00	2.03
	Average ELISA titer	33	100	100	100	100	133	166	100	83

Vaccine dose 1.25 ml.

at the first week post vaccination and reached a maximum of 116 at the 4th week and thereafter declined.

The results with the formaline inactivated oil vaccine are presented in Table 1. An average SN antibody titer of 1.55 was noted after the first week post vaccination and reached a maximum of 1.23 at the 16th week post vaccination

and then declined. ELISA titers showed an identical pattern.

The results with the BEI inactivated gel vaccine are presented in Table 2. An average SN antibody titer of 1.86 was recorded after the first week post vaccination. The titer increased to 2.09 at the 3rd week and then steadily declined to 1.39 at the 24th week post vaccination.

Table 2. Immune response of sheep to BEI inactivated FMD vaccine

A. Aluminium hydroxide gel as adjuvant

Sheep No.	Type of assay	Titer								
		Weeks post vaccination								
		1	2	3	4	8	12	16	20	24
135	SN	1.70	1.70	1.95	2.00	1.86	1.86	1.56	1.35	1.26
	ELISA	50	50	100	100	50	50	25	25	Nil
140	SN	1.95	1.95	2.16	2.00	2.16	2.16	1.86	1.65	1.35
	ELISA	50	50	100	100	100	100	50	50	25
141	SN	1.95	2.00	2.16	2.00	1.95	1.95	1.86	1.70	1.56
	ELISA	50	100	200	200	100	100	50	50	25
	Average SN titer	1.86	1.88	2.09	2.00	1.99	1.99	1.76	1.56	1.39
	Average ELISA titer	50	66	133	133	83	83	41	41	16

B. Mineral oil as adjuvant

123	SN	1.05	1.56	1.70	2.00	2.16	1.95	1.86	1.95	1.86
	ELISA	25	50	50	100	100	100	50	50	50
129	SN	1.40	1.56	1.95	2.16	2.16	2.00	2.00	1.86	1.86
	ELISA	50	50	100	100	200	100	100	50	50
138	SN	1.05	1.40	1.56	1.95	sic	died	-	-	-
	ELISA	Nil	25	50	50	-	-	-	-	-
	Average SN titer	1.16	1.50	1.73	2.03	2.16	1.97	1.93	1.90	1.86
	Average ELISA titer	25	41	66	83	150	100	75	50	50

Vaccine dose 1.25 ml.

The results with the BEI inactivated oil vaccine are shown in Table 2. An average SN antibody titer of 1.16 was noted at the first week post vaccination. The titer increased to 2.16 by the 8th week and then declined to 1.86 after 24 weeks post vaccination. The ELISA titers showed an identical pattern of antibody response.

Repeated experiments with BEI inactivated vaccines with both types of adjuvants yielded similar results.

Discussion

In many countries for the preparation of FMD vaccine formaldehyde is used as an inactivant. It has been reported that the degree of inactivation by formaldehyde is not clearly known (Graves, 1963; Wesslen and Dinter, 1966) and subsequently derivatives of ethyleneimine have received attention for the inactivation of FMDV (Brown *et al.*, 1963).

Formaldehyde and BEI were used on a comparative basis in the present study for FMDV type 'O' inactivation. The vaccines were prepared and tested in guinea pigs and sheep. The results showed that there was no significant difference in the immune response between these inactivants.

The immunogenicity of FMD vaccine could be considerably improved using suitable adjuvants. Aluminium hydroxide gel, saponin or mineral oil are used for this purpose. In the present investigation these adjuvants were employed in the preparation of vaccines and the antibody response was monitored by SN test and ELISA for a period of six months. According to Cardassis *et al.* (1966) there was correlation between the antibody titer and the degree of immunity in sheep. According to them antibody titer of 1.80 and above conferred absolute protection.

Considering these standards the oil adjuvant vaccine conferred absolute protection for a period of six months whereas the gel vaccine only for 3–4 months.

In the present study the appearance, the persistence and decline of virus neutralizing antibody titer was monitored by ELISA and SN test. A positive correlation between these two tests was observed in all the tests. De Simone *et al.* (1981) observed a good correlation between the virus neutralizing antibodies and ELISA antibodies.

From this limited study it can be concluded that the inactivation of FMDV by formaldehyde and BEI has no influence on the quality of the vaccine, while there is significant difference between vaccines with different adjuvants, aluminium hydroxide gel and mineral oil.

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