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TRIAL TO INCREASE THE DURATION OF NEUTRALIZING ANTIBODIES IN CATTLE VACCINATED WITH INACTIVATED FMD VACCINE

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SUMMARY

Inactivated FMD vaccine is weakly immunogenic and multiple vaccinations at four-month intervals are necessary for the prevention of the disease. Our results revealed that in cyclophosphamide treated animals, the antibody titers remained protecting up to 35 weeks post vaccination ,while in non treated group, the animals became susceptible to infection 19 weeks post vaccination. So, it is possible to increase the duration of neutralizing antibodies in serum when a low single dose of cyclophosphamide is administered four days before vaccination with aluminum hydroxide-saponin FMD vaccine and this will increase the intervals between vaccinations and decrease the commercialization costs of vaccination programes.

INTRODUCTION

Foot and mouth disease is a highly contagious disease that affects both domestic and wild cloven-hoofed animals leading to the appearance of vesicles on the feet, in the oral cavity, and on the mammary glands of females. Mortality from myocarditis is most commonly seen in young animals and healing of vesicular lesions usually takes place in 2-3 weeks (Ivonne et al., 1995). It is caused by seven types of foot and mouth disease virus (FMDV) in the genus Aphthovirus; family Picornaviridae, with at least 70 subtypes (Davies 2002).

Vaccination constitutes an important control policy for foot and mouth disease in affected areas with advanced eradication programs, as well as, in free regions that decide to use immunization as

control measure after recent introduction of the disease (Bergmann et al., 2003).

The immunity acquired through infection or use of current vaccine is strictly type specific and to a lesser degree, subtype-specific (Frederic et al., 1999).

Inactivated FMD vaccine is poorly immunogenic (Doel, 1999) and multiple vaccinations at six-month intervals are necessary for the prevention of the disease .Usually, cattle receive two to eight vaccine inoculations before slaughter and this will increase the cost of repeated vaccination. (Portiansky et al., 1996).

In addition to the high costs of the repeated vaccination, the oil adjuvant can be harmful and frequent appearance of subcutaneous abscesses will decrease the economic value of cattle (Fondevila et. al., 1993).

There are several trials to increase the level of immune status of farm animals vaccinated against FMDV by using immunomodulators.

When cyclophosphamide, a well - known antimitotic drug, is administered in a very low dose and before antigenic challenge, it can display an immunomodulating activity and enhancing antibody synthesis probably by its action on the suppressor cascade (Dray and Mokyr 1984). If a low dose of cyclophoshpamide used before antigenic challenge, it can be a potent immune enhancer and this augmentation has been attributed to a greater toxicity for suppresor T cells than T helper lyphocytes (Daniel et al., 1997).

The purpose of this work is to study the effect of cyclophophamide on the life span of neutralizing antibodies in the serum of cattle vaccinated with inactivated aqueous FMD vaccine.

MATERIALS AND METHODS

1. **Cyclophosphamide:** $C_7H_{15}Cl_2N_2O_2P.H_2O$ F.W. 279.1., Cat . No. C-0768 lot. 091k1176 Sigma - Aldrich CO., USA.
   A well known antimitotic drug and characterized by its rapid clearance from the blood due to its short half-life time (2-4 hours) (Kawabata et. al., 1990)
2. **Calves:** Two groups (each contained three calves) of 6-8 month old with 200-300Kg body weight were used. These calves were clinically healthy and free from antibodies against FMD virus as proved by using SNT and ELISA.
3. **Virus:** The virus used in this study was the locally isolated FMD virus type O_2/1993. Viral stock of O_2 was prepared by infecting BHK_{21} cell monolayer. Virus particles were collected from the supernatant and cleared by centrifugation (3000 rpm). The cytopathic effect of FMDV on BHK_{21} cells 24 hours after infection was determined by $TCID_{50}$ of (Reed and Muench 1938) analysis. Viral stock were maintained at -70°C.

4- **Baby hamster kidney cells (BHK_{21} clone 13):** The cells were propagated at FMD department, Abbasia, Cairo, using Minimum Essential Medium (MEM) with Earls salts with 8-10% sterile newborn calf serum.

5- **FMD vaccine:** FMD virus type O_1/93 was inactivated by binary ethylenimine and adjuvenated by aluminum hydroxide, batch no.12/2003, each dose contains 3 cattle PD50 and was produced at Veterinary Serum and Vaccine Research Institute, FMD Department, Abbasia, Cairo, Egypt.

6- **Serum neutralization test (SNT):**

It was performed using the microtechnique described by (Ferreira 1976) in wich serial dilutions of the sera, in triplicate, were mixed with 10^4 $TCID_{50}$. The neutralization titer of the tested sera was obtained according to the (Reed and Muench 1938).

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

It was carried out according to the method described by (Hamblin et al., 1986).

EXPERIMENTAL DESIGN

Two groups of calves (each contained three animals)were used in the experiment. Four days before vaccination, a group of calves received cyclophosphamide (5 mg/Kg body weight dissolved in 5 ml of double distilled water) by intraperitoneal route. Both groups were immunized with FMD vaccine by a subcutaneous route. Serum samples were collected weekly till 5 weeks then every 2 weeks post vaccination. The immune response was evaluated using SNT and ELISA techniques.

RESULTS

Table 1: Comparative serum neutralizingg antibody titers of cattle treated with cyclophosphamide before vaccination and non treated gorup.

weeks post vaccination	cy-treated group **				non treated group **			
	cattle no.			Mean	cattle no.			Mean
	1	2	3		4	5	6	
Prevaccination	0.3*	0.5	0.4	0.4	0.3	0.6	0.4	0.4
1	0.9	1.0	0.8	0.9	0.9	0.9	0.8	0.9
2	1.4	1.5	1.3	1.4	1.4	1.2	1.3	1.3
3	1.5	1.9	1.7	1.7	1.7	1.5	1.5	1.6
4	1.75	2.25	2.0	2.0	2.0	1.8	1.6	1.8
5	2.1	2.7	2.0	2.25	2.3	2.1	2.1	2.2
7	2.0	2.4	2.3	2.2	2.4	2.1	2.1	2.25
9	1.9	2.1	2.0	2.0	2.2	1.9	1.9	2.0
11	1.85	2.1	2.0	2.0	2.0	1.7	1.6	1.8
13	1.7	1.9	1.9	1.8	2.0	1.5	1.6	1.7
15	1.7	1.8	1.7	1.7	1.8	1.3	1.5	1.5
17	1.6	1.75	1.7	1.7	1.75	1.3	1.2	1.4
19	1.6	1.75	1.7	1.7	1.75	1.0	0.8	1.2
21	1.4	1.6	1.5	1.5	1.4	0.7	0.6	0.9
23	1.3	1.5	1.4	1.4	1.0	0.5	0.5	0.7
25	1.3	1.4	1.4	1.4	0.8	0.4	0.5	0.5
27	1.3	1.3	1.4	1.4				
29	1.2	1.3	1.3	1.25				
31	1.2	1.25	1.3	1.25				
33	1.0	1.25	1.3	1.2				
35	1.0	1.25	1.25	1.2				
37	0.7	1.0	0.9	0.9				
39 weeks	0.6	0.6	0.7	0.6				

* Values expressed in log 10 of the reciprocal of the 50% serum end-point dilution.
** Cattle treated with cyclophosphamide four days before vaccination.

Figure (1): Comparative means of serum neutalizing antibody titers of cattle treated with cyclophosphamide before vaccination and non treated group.

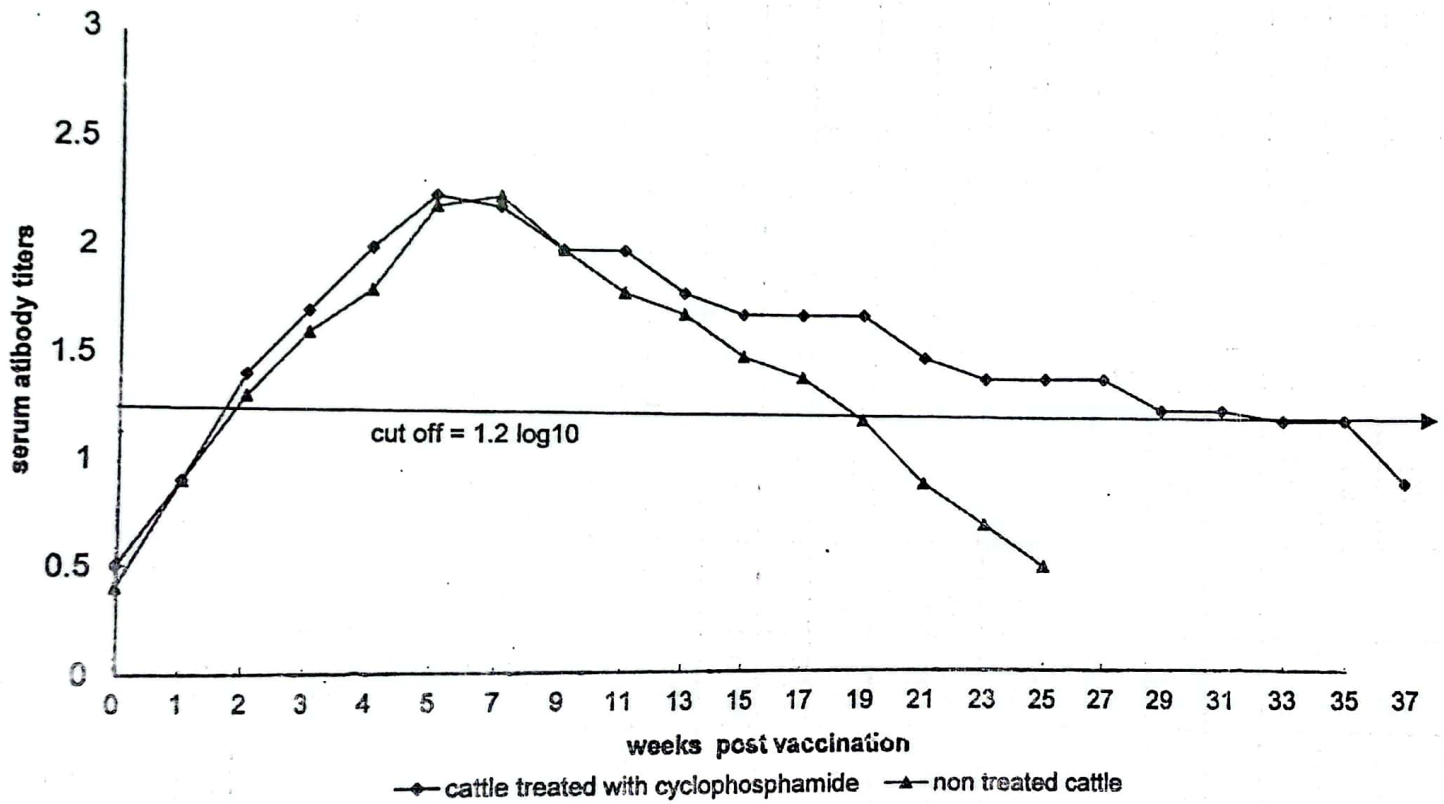

Table 2: Comparative ELISA antibody titers of cattle treated with cyclophosphamide before vaccination anf non treated gorup.

weeks post vaccination	cy-treated group **				non treated group **			
	cattle no.			Mean	cattle no.			Mean
	1	2	3		4	5	6	
Prevaccination	0.7*	0.9	0.9	0.8	0.7	0.9	0.7	0.75
1	1.2	1.45	1.2	1.3	1.2	1.3	1.25	1.25
2	1.9	1.9	1.75	1.85	1.8	1.7	1.75	1.75
3	1.9	2.4	2.0	2.1	2.1	1.9	2.0	2.0
4	2.1	2.7	2.45	2.4	2.45	2.25	2.25	2.3
5	2.6	3.1	2.45	2.7	2.75	2.5	2.6	2.6
7	2.35	2.75	2.75	2.6	2.9	2.5	2.8	2.7
9	2.2	2.5	2.45	2.4	2.6	2.25	2.45	2.4
11	2.2	2.5	2.45	2.4	2.45	2.1	2.2	2.25
13	2.0	2.3	2.3	2.2	2.45	1.9	2.1	2.15
15	2.0	2.25	2.1	2.1	2.1	1.75	1.8	1.9
17	2.0	2.2	2.1	2.1	2.0	1.75	1.7	1.8
19	1.9	2.2	2.1	2.1	2.0	1.4	1.65	1.7
21	1.7	2.1	1.9	1.9	1.75	1.2	1.2	1.4
23	1.65	1.85	1.9	1.9	1.45	0.75	0.75	1.0
25	1.65	1.85	1.8	1.9	1.2	0.6	0.75	0.85
27	1.65	1.85	1.8	1.9				
29	1.65	1.7	1.75	1.7				
31	1.65	1.7	1.75	1.7				
33	1.4	1.7	1.75	1.65				
35	1.4	1.7	1.65	1.65				
37	1.2	1.4	1.2	1.3				
39 weeks	1.0	0.9	1.2	1.0				

* Values expressed in log 10 of the reciprocal of the 50% serum end-point dilution.
** Cattle treated with cyclophosphamide four days before vaccination.

Figure (2): Comparative means of ELISA antibody titers of cattle treated with cyclophosphamide before vaccination and non treated group.

DISCUSSION

The immunization of cloven hoofed animals with killed FMD vaccine requires periodic vaccination due to low vaccine immunogenicity. Therefore, FMDV antigens need to be combined with adjuvants such as aluminum hydroxide, saponin or oil emulsion. Animal handling for periodic inoculation and the repeated doses of vaccines increase the commercialization costs. Moreover the use of adjuvants may induce adverse effects (Portainsky et. al., 1996).Therefore the search for increasing the duration of neutralizing antibodies in the serum of cattle vaccinated with inactivated FMD vaccine is justified to decrease the numbers of inoculation and consequently the cost of vaccination .

In table (1) and (2) neutralizing and ELISA antibody titers could be detected as early as 7 days after vaccination then increased rapidly till reached a protective level at 3rd week .These results are agreed with (Kardiasis et. al., 1964), (Wisniewski et. al ., 1972) and (Bengelsdroff , 1989) who found that more than 95% of the vaccinated cattle with SN titers of greater than 1.2 were protected from generalized FMD, 61.5% of vaccinated cattle with SN titers less than or equal 1.2 were not protected and developed generalized infection . At 3rd week post vaccination, no significant difference were found in neutralizing antibody titers against the O1 serotype between the cyclophoshpamide treated and non treated animals. Statistically significant ($p<0.005$) difference between the two groups were observed after 19 weeks post vaccination. 19 weeks post vaccination the neutralizing antibody titer while in non treated group was reduced almost completely and animals became susceptible to infection, the cyclophosphamide treated animals showed significant higher antibody titers at a level that can protect them from infection with the homologous virus up to 35 weeks post vaccination. Theses results are agreed with (Portiansky et. al ., 1996) who found that pretreatment with cyclophoshpamide increased the duration of anti-FMDV neutalizing antibodies in cattle vaccinated with the commercial aluminium hydroxide-saponin FMD vaccine. Also these results are supported by (Portainsky et .al., 1989) who found that when adult mice were administered with a low dose of cyclophosphamide 4 days before infection with FMDV, viral replication and pancreatic damage can be prevented and the immune response against virus was enhanced.

Also our results are in agreement with (Hamblin et. al., 1986) who found a positive correlation between ELISA and virus neutralization titers for sera either vaccinated or involved in outbreaks of FMDV. The protective level was 1.2 log 10 by means of SN test which equivelent to 1.65 log 10 by means of ELISA.

CONCLUSION

It is possible to increase the duration of neutralizing antibodies in serum when a single dose of cyclophosphamide is administered four days before vaccination with aluminum hydroxide-saponin FMD vaccine. So increasing the intervals between vaccinations will decrease the commerciaiization costs of vaccination programs.

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