Vet.Med.J.,Giza. Vol.40,NO.2 (1992):83-88.

POTANCY TEST FOR FOOT AND MOUTH DISEASE VACCINE BATCHES IN GUINEA PIGS

By

S.EL-KILANY* ; N.METWALLY* ;A.OMAR* ; M.ABD EL-SAMEA* ; S.SABER** ; I.REDA** and A.A.MOUSSA***

* Vet.Serum & Vaccine Res.Inst.,Abbassia,Cairo,Egypt.
** Fac.Vet.Med.,Cairo University
*** General Organization for Vet. Services

(Received: 19.1.1992)

INTRODUCTION

Traditional methods used for control of Foot and Mouth Disease (FMD) have included vaccination of cattle and buffaloes with tissue culture-formalin inactivated-aluminium hydroxide gel vaccine (Moussa et al., 1974). Successful immunization of livestock is known to depend on many factors including the method of vaccine evaluation. The choice of suitable method is also dependant on its effect , on the general tolerance of the vaccine and its safety (Gill et al., 1959). (It is also clear that, a serious economic loss brought about FMD vaccine when tested in cattle). However, here is not altogether surprising as there is a general dearth of readily accessible data on the benefit cost aspects of FMD control in any of the sectors of livestock industry.

In the following study, comparative study and standardization of different methods used for FMD vaccine evaluation.

MATERIAL AND METHODS

Material:

a) Twenty male mixed breed calves, 12-18 months old and susceptible to FMD virus.

b) Baby mice, 2-3 days old were used for isolation and titration of FMD virus.

c) Guinea pigs, 500 grams body weight were used for preparation of the hyperimmune sera and FMD vaccine potency.

d) FMD virus type , (strain O_1/2/72-Egypt)was used.

e) Guinea pig hyperimmune serum, was prepared according to Traub and Manso (1944).

f) Formaldehyde, 30.03 molecular weight was obtained from BDH Chemical Ltd. Poole, England.

g) Aluminium hydroxide gel, 2% strength and 1.3% Al_2O_3 dry

matter was produced from Suprex Copenhagen Denmark.

h) Saponin was used as 10% solution and obtained from BDH, England.

i) merthiolate (Thiomersal was used at a concentration of 10,000 as a bacteriocidal agent.

Methods:

Potency test in guinea pigs:

Five groups of guinea pigs, each of 5 animals, were inoculated with 1 ml. of 4 fold dilution of the prepared FMD vaccine S/C and one group left as a control. All animals and the controls were challenged with 10^4 MID_{50} guinea pig adapted virus and the guinea pig protective dose fifty ($GPPD_{50}$) was calculated according to **Karber's Method** (1931).

FMD vaccine preparation:

Five batches of the vaccine were prepared according to **Moussa et al. (1979).**

Serum neutralization test (SNT):

The micro-plate technique was used as described by **Hable (1969).**

Passive haemagglutination test (PHA): The test was done as described by Reda and **Wittmann (1972).**

EXPERIMENTS AND RESULTS

- Safety of FMD vaccine batches:

The innocuity test was checked by intradermolingual inoculation of 1 ml. in 10 sites of the tongue of susceptible calf (**Henderson**, 1953) and by inoculation of 0.1 ml. of each batch via intraperitoneal route in 7 groups of unweaned baby mice, also by inoculation in tissue cultures. The vaccine was safe by showing no lesions or cytopathic effect.

Evaluation of FMD vaccine batches was by estimation of guinea pig protective dose ($GPPD_{50}$) in guinea pigs and cattle as shown in table (1).

Immune response of cattle vaccinated with the prepared FMD vaccine batches then challenged with the virulent FMD strain:

Five groups of susceptible calves (each of 4 calves) were vaccinated subcutaneously with 5 ml. dose of each prepared vaccine. Sera from each gorup were collected periodically at 7, 14, 21 days post-vaccination (DPV). The immune response of vaccinated calves were studied using SNT and PHA tests and the achieved data were shown in table (2).

21 days post vaccination, all the vaccinated animals and the control group were challenged by inculation of 10^4 LD_{50} FMD virus via intralingual route then examined daily for 7 days for any local reations.

Table (1): Results of estimation of FMD vaccine potency in guinea pigs and cattle ($GPPD_{50}$).

Batch No.	Dilution of FMD vaccine* undiluted	1/4	1/16	1/64	1.256	Calculated $GPPD_{50}$ expressed in 1 ml.	Calculated $GPPD_{50}$ per 5 ml. dose	Cattle protection percentage
1	0/4**	0/4	1/4	3/4	1/4	0.156	158.10	100%
2	0/4	0/4	1/4	4/4	4/4	0.227	125.60	100%
3	0/4	0/4	0/4	1/4	4/4	0.056	500.00	100%
4	0/4	0/4	0/4	2/4	4/4	0.08	315.5	100%
5	0/4	0/4	0/4	1/4	4/4	0.056	500.00	100%

$GPPF_{50}$ = Guinea pig protective dose fifty in 5 ml vaccine dose.

* = Dilution of FMD vaccine inoculated in guinea pigs and challenged 21 days post - vaccination

** = Number of guinea pigs showed generalization over total number of chal lenged animals.

*** = Four cattle vaccinated with FMD vaccine and challenged with virulent FMD virus.

Table(2): Results of determination of FMD antibody level from sera of calves vaccinated with 5 batches of vaccine then challenged with FMD virus strain O_1 /2/72 Egypt.

Batch No	Animal No	Weeks post vaccination						Resuts of challenge	
		1st WPV		2nd WPV		3rd WPV		FMD lesions	
		SNT	PHAT	SNT	PHAT	SNT	PHAT	Primary	Secondary
1	1	0.3	0.6	0.6	0.9	1.2	1.2	-	-
	2	0.45	0.6	0.6	0.9	1.35	1.5	-	-
	3	0.6	0.9	0.75	1.5	1.35	1.65	-	-
	4	0.6	0.9	0.9	1.2	1.35	1.35	-	-
	Average	0.49	0.71	0.71	1.01	1.31	1.42		
2	1	0.3	0.6	0.6	0.9	0.9	0.9	+	-
	2	0.3	0.75	0.75	0.6	1.05	1.2	-	-
	3	0.6	0.6	0.6	1.2	1.5	1.2	-	-
	4	0.3	0.6	0.6	0.9	1.65	1.5	-	-
	Average	0.38	0.64	0.64	0.9	1.28	1.2		
3	1	1.05	1.35	1.35	1.05	1.65	1.5	-	-
	2	1.05	1.2	1.2	1.05	1.5	1.05	-	-
	3	0.9	1.35	1.35	1.05	1.6	1.65	-	-
	4	0.3	1.35	1.35	1.05	1.6	1.65	-	-
	Average	0.58	1.31	1.31	1.05	1.64	1.76		
4	1	0.9	1.5	1.35	1.5	1.65	1.8	+	-
	2	1.2	1.2	1.5	1.65	1.8	1.8	+	-
	3	0.9	1.5	1.2	1.35	1.65	1.65	-	-
	4	0.6	1.5	1.2	1.2	1.35	1.5	-	-
	Average	0.9	1.42	1.31	1.42	1.61	1.69		
5	1	0.9	1.5	1.5	1.65	1.65	1.8		
	2	1.2	1.35	1.8	1.85	1.95	1.8	-	-
	3	1.2	1.35	1.8	1.5	2.1	1.65	-	-
	4	0.6	0.9	0.9	1.2	1.05	1.2	-	-
	Average	0.89	1.03	1.5	1.55	1.65	1.65		
Control								+ +	+ +

Table (3): The comparison between $GPPD_{50}$,infectivity titer, CFT , SNT and PHAT.

Batch No.	$GPPD_{50}$/ml.	Cattle protection percentage	Infectivity titer /ml.	FMDV Particle dose	CFT /ml.	SNT in Log 10	PHAT in Log 10
1	1.156	100 %	$10^{8.55}$	$316 * 10^6$	1.9	1.31	1.42
2	0.227	100 %	$10^{7.75}$	$57 * 10^6$	1.3	1.28	1.20
3	0.056	100 %	10^8	$100 * 10^6$	1.6	1.64	1.76
4	0.08	100 %	$10^{8.5}$	$316 * 10^6$	1.6	1.61	1.69
5	0.056	100 %	10^8	$100 * 10^6$	1.6	1.65	1.62

The comparative studies between vaccine potency test in guinea pigs, cattle and different methods by using different batches of FMD vaccines are shown in table (3).

DISCUSSION

From the data achieved in this study, values of 0.227 to 0.056 $GPPD_{50}$/ml., were obtained for all 5 prepared FMD vaccine batches. Accordingly, the tested FMD vaccines could be accepted for cattle immunization. Current literatures of **Moussa et al. (1974) and Awad et al. (1979)** showed that locally prepared conventional FMD vaccine proved to contain 0.21-0.3 ml. $GPPD_{50}$. Also, **Mackowiak et al (1966)** found that by statistical analysis of data obtained from using 1500 guinea pigs and 1000 cattle, a value of 0.15-0.4ml. $GPPD_{50}$ were satisfactory for successful immunization of cattle with such vaccines. **Similarly, Mowat (1974)** showed that, the minimum accepted $GPPD_{50}$ varied from 0.35-0.5 ml. The superiority in $GPPD_{50}$ obtained by this study over that in the current literatures could be attributed to the higher antigen content incorporated in the FMD vaccines under test.

Furtherly, and to complete the evaluation of the prepared FMD vaccines in guinea pigs, its immune response was studied using different serological technique. Passive haemagglutination (PHA) and serum neutralization (SN) test were used to assess the immune response of susceptible calves to prepared FMD vaccines. The results achieved showed that, the prepared FMD vaccines were able to evoke SN and PHA types of antibodies to a considerable titers over a period of three weeks after which the animals were challenged and proved to be protected. The SNT revealed also that, SN antibdey levels were in general running parallel to that of PHA antibody level but in

slightly lower titre.

The level of antibody titres acieved at 3 weeks postvaccination were favourable to protect animals if exposed to virus infection. Passive haemagglutination test (PHA) was quite sensitive as SNT (Moreau et al., 1973).

Van Bekkum (1970) observed a high degree of correlation for type "O" between the $GPPD_{50}$ and the average neutratization titres in groups of 50 or more of cattle. Between these methods a correlation coefficient of 0.93 was found for 8 vaccines which also suggested that, the $GPPD_{50}$ can be reliable tool for evaluating vaccine potency in cattle. Also, the relationship between the titre of neutralizing antibody in cattle and protection against intradermolingual challenge is well established (Van Bekkum, 1969; Brooksby, 1968; Lucam et al., 1969 and Mackowiak et al. 1959).

SUMMARY

Five batches of different antigenic FMDV titrations were tested in guinea pigs using guinea pig protective dose fifty ($GPPD_{50}$). all propared batches gave 100% protection when inoculated into susceptible cattle and challengec with virulent FMD virus.

The FMD vaccine batches were inoculated into susceptible calves and examined for 3 weeks. The SNT revealed that the SN antibody levels were in general running parallel to that of PHA but in slightly lower titer.

The comparison between the evaluation of the prepared FMD vaccine batches both in vivo and vitro showed that vaccines with varying parameters could induce good immune response in both laboratory and field animals.

REFERENCES

Awad, F., Reda, I.; Moussa, A.A.; Elmulla, A. A.; Daoud, A. ; Hussein, K. and Marzouk, M. S. (1979): Studies on foot and Moouth disease in buffaloes. 14th Arab. Vet. cong., 24-29/3

Brooksby, J. B. (1969):Some data on the dose response relationahip. Meeting of the FAO Res. GR. of the Eur. comm. for the control of FMD. Brescaia, 24-26 Sept. 1969. Rep. No. AN. EUF. MD/69/11, P. 32-37, Rome-FAO.

Gill, E.; Sullivan, J. F.; Stone, H. D. and Hundwmann, A. S. (1959): Role of adjuvants in immunogenicity of killed Newcastle disease vaccine. Am. J. Vet. Res., 20: 357-365.

Habel, K. (1969): Virus neutralization test, fundemental techniques in virology. Acad. Press. N.Y.

Henderson,W.M. (1953): The use of virus culture in Foot and Mouth Disease research: Proc. XVth Inst. Vet. Congr. Stockholm, 1: 191-195.

Karber, G. (1931): Beitrag zur kollektiven Behandlung pharmakologischer reihenversuche. Arch. Exp. Path. Pharmak., 126: 480-483.

Lucam, F.; Fedida, M.; Dannacher, G. and Perraud, J. (1969): Evaluation et duree de l'immunite antiaphtheuse chez le boeuf primo vaccine. Bull. Off. Int. Epiz., 71, 463 484.

Mackowiak, C., Lang, R.; Fontaine, J. and Petermann, H.G. (1959): Technique de control due vaccin antiaphtheux par serum neutralization sur culture de tissue Control of FMD by serum neutralization in tissue culture. Ann. Int. Pasteur, 97: 571 582.

Mackowiak, C.; Fontaine, J.; Terre,

J.S.; Stellmann, C.; Roumiantzeff, M. and Petermann, H.G. (1966): Control quantitatif du vaccin antiaphteux. Etude de la Loi dose effect at correlation entre less doses vaccinated 50 pour 100 chez les Coboys et les bovides. Bull. Off. Int. Epiz., 65 (1-2): 131-171.

Morcau, Y.; Stellmann, C. Terre, J. (1973): Assay of FMD serum antibodies by passive haemagglutination: Application to the control of vaccines. Bull. Off. Int. Epiz., 79: 265-281.

Moussa, A.Y.; Banoub, S.M.; El Said, T.E.; Fahmy, F. and Bohm, H.O. (1974): Typing and subtyping of a strain of FMD virus isolated from Sharquia province in 1970. J. Egypt. Vet. Med. Ass., 34 (3-4), 413-419.

Moussa, A.A.M., Daoud, A.; Hussein, K.; Fahmy, F.; El-Kilany, S. and El Shehawy, L. (1979): Prevalence of FMD in Egypt. 14th Arab. Vet. Med. Cong., 14: 39

Mowat, G.N. (1974): Potency of BHD produced FMD vaccines after storage. Bull. Off. Int. Epiz., 81: 1151-1154.

Reda, I.M. and Wittmann, G. (197 The use of $CrCl_3$ for coopling of FMD virus erythrocytes adn the ability of these sensitiz erythrocytes to react in passive haemagglutin tion and passive immunohemolysis. Arch Ge Virusforsch, 38: 205 215.

Traub, E. and Manso, I.R. (1944): Ue die herstallung complement bindendere meer chweinchon sera, fur die typen diagnose be Maul und klavenseuche. Zbl. Bakt. I. org 151: 380 392.

Van Bekkum, J.G. (1969): Correlation be tween serum antibody level and protection against challenge with Foot and Mouth Dis ease virus. Meeting of the Fao RG. of the Eur Comm. for the control of FMD. Brescia, 24 26, Rep. No. An. EUF. MD/29/11P, 38 4 Rome FAO.

Van Bekkum, J.G. (1970): Experience with various methods for the evaluation of vac cine potency. European Comm. Cpmtrp FMD, Mrg. Res. Gp. Stund. Tech. Copen Ankara, Turkey. App. IV: 64-66.