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ORIGINAL ARTICLE

Preparation of FMD type A87/IRN inactivated vaccine by gamma irradiation and the immune response on guinea pig

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Abstract FMD is one of the most economically damaging diseases that affect livestock animals. In this study FMD Virus type A87/IRN was multiplied on BHK21 cells. The virus was titrated by TCID50 method, it was $10^{7.5}$/ml. The FMD virus samples were inactivated by gamma ray from 60Co source at –20°C. Safety test was done by IBRS2 monolayer cell culture method, also antigenicity of irradiated and un-irradiated virus samples were studied by Complement Fixation Test. The Dose/Survival curve for irradiated FMD Virus was drawn, the optimum dose range for inactivation of FMDV type A87/IRN and unaltered antigenicity was obtained 40–44 kGy. The inactivated virus samples by irradiation and ethyleneimine (EI) were formulated respectively as vaccine with Al(OH)3 gel and other substances. The vaccines were inoculated to Guinea pigs and the results of Serum Neutralization Test for the normal vaccine and radio-vaccine showed protective titer after 8 months. The potency test of the inactivated vaccines was done, PD50 Value of the vaccines were calculated 7.06 and 5.6 for inactivated vaccine by EI and gamma irradiation respectively.

Keywords Vaccine · Foot and Mouth Disease · Virus · gamma irradiation

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Introduction

Foot and Mouth Disease (FMD) is the most contagious disease of cloven-hoofed animals. In unvaccinated herds, usually the mortality in adult animals is negligible, but it may be considered in young animals. Milk production highly decreases and animals used for traction can become useless [1]. FMD virus is a genus of the *Picornaviridae* family called *Aphtovirus*, this genus contains seven serotypes: *A, O, C, Asia1*, and three types of South African Territory: *SAT1, SAT2 and SAT3* [2].Vaccination is the most important control and the eradication strategy for animal virus diseases. The different processes for preparing vaccines against viral diseases are comprised by a sequence of steps which, although different in accordance with particular virus and processes selected, may be classified as follows: virus production, virus inactivation and vaccine formulation. In this study, radiation technology is incorporated in the principal steps of a viral vaccine preparation. Inactivation of viruses by ionizing radiations has been studied by Pollard, Dertiger and etc [3 and 4]. Irradiated, inactivated viruses have been reported to retain most of their antigenicity [5]. As a good result of the application of ionizing radiations for virus inactivation, which was used successfully as antigen in the preparation of a antiviral radio vaccine was of the order of one million doses which effectively protected the inoculated cattle against the disease [6].

Material and methods

1) Virus multiplication: FMDvirus type A87/IRN was propagated in BHK21 suspension culture, by Earl's media and 0.5% bovine serum which were treated by PEG 6000 at

37 °C incubator without CO_2 for 12 h. Then the Cytopathic Effect (CPE) was observed on BHK21 cells as lyses and separated cells. The virus suspensions were centrifuged at 1000 ×g for 15 min, the supernatants was stored at -70 °C [2 and**7**].

2) Virus titration: Tissue Culture Infection $Dose_{50}$ /ml ($TCID_{50}$ /ml) means virus particles per ml which can make CPE at 50% inoculated cells and it was calculated **[8].**

3) Inactivation of FMDV by gamma irradiation and ethylenimine: A gamma cell instrument Issledovapel, PX-30 model with dose rate: 0.551 Gy/sec and activity: 3652 Ci was used. Different doses of gamma ray: 10, 20, 25.35,40,45 and 50 kGy were used for irradiation of virus samples, and the duration of the virus samples irradiation were 5h, 12 min 10 h, 20 min 13 h, 9 min 18 h, 24 min 20 h, 42 min 23 h, 2 min and 26 h, 2 min respectively, for each dose of gamma ray 10 viral vials and 70 vial vials for 7 gamma doses were irradiated. The irradiation was done at low temperature about –20°C [5]. Other virus samples were treated by ethylenimmine 0.035 M/L at 30 °C during 24 h for virus inactivation then the inactivation was stopped by 0.04 M sodium thiosulphate.

4) Safety test and Complement Fixation test (CF test): Infectivity of irradiated virus samples by different doses of gamma ray was determined by cell culture methods. All of the irradiated virus samples were inoculated on IBRS2 cell, also their titration was obtained by $TCID_{50}$ methods. Antigenecity of irradiated and un-irradiated virus samples were studied by CF test [9and10].

5) Vaccine Formulation: The inactivated virus samples were treated by 6/1000 chloroform, absorbed on AL $(OH)_3$ gel and formulated by saponin, glycine, phenol red and phosphate buffer [2]. Therefore two kinds of the vaccines against *FMD Virus type A 87/IRN* were prepared, the first one which is inactivated by BEI as normal vaccine (NV) and the 2nd one which is inactivated by gamma irradiation as radio vaccine (RV).

6) Immune response of the inactivated vaccines by gamma irradiation and ethylenimine in Guinea Pigs: At first stage 35 Guinea Pigs 450–500 g body weight were selected and divided in 7 groups. Three routes of inoculation were used for two types of the vaccines: 1) subcutaneously and infraaxillary. 2) Intraperitoneally. 3) Subcutaneously on back foot, also one control group (unvaccinated) [11 and 12]. Three groups were inoculated by NV; the other three groups were inoculated by RV and one group as control group (non-vaccinated).

The second stage 30 guinea pigs in 3 groups were vaccinated by radio vaccine and normal vaccine and unvaccinated group. Each Guinea Pig was inoculated with 0.5 ml of the vaccines subcutaneously and infraaxillary, the booster dose was injected at 21st d and the animals were bled 14 d after the booster. The last 3 groups of guinea pigs were bled after 1, 3, 6 and 8 months. The sera were separated from the blood samples. The complement factors of the sera were inactivated at 56°C for 30 min. Then Sera were tested for the presence of antibodies against *FMD virus* by serum neutralization test [8].

7) Serum Neutralization Test (SNT): The sera were diluted in Eagle's maintenance medium in a 2-fold dilution stating from 1:4 to 1:128. The SNT was done according to the Kraber protocol [13]. This test was done in flat bottom-96 microplates, in which the monolayer of BHK21 was grown. Any well in which the virus has been neutralized and the cells remain intact was as a positive well and other wells in which the virus has not been neutralized and CPE could be shown were as negative wells. Antibody titres were expressed as the logarithm of the reciprocal of the final dilution of serum in the virus/serum mixture that neutralized an estimated 100 $TCID_{50}$ at the 50% endpoint.

8) Challenge study for protective response: The guinea pigs adapted by *FMD serotype A87/IRN* at fifth passage level were prepared in a 5 ml suspension after clarification, passaged freshly in two Guinea Pigs by intra dermoplantar tunneling route and the pads have been collected from the animals which showed the primary lesions were used as a source of challenge virus. Approximately 1g of pad materials was triturated in 5 ml Eagl's medium to obtain a homogenous suspension, centrifuged at 3000 rpm, 15 min and the supernatant was collected and used as neat virus [7]. Challenge experiment in guinea pigs was carried out following the method of Lucam etal [14]. Both of the two types vaccines (inactivated by Gamma-irradiation and Ethylenimine) were diluted in carbonate-bicarbonate buffer pH=8.2 in a 2-fold dilution from 1:1 to 1:16. Each dilution of the vaccines was inoculated to each group of animal via subcutaneously and infraaxillary 0.5 ml for each dose. Booster dose was given with the same dose of vaccine at 21st d. After two weeks 10 groups of vaccinated animals with two types of vaccines, and 2 groups of unvaccinated (control) animals, each group contains 5 animals were challenged with Guinea Pig adapted virus(sixth passage) at 100 LD_{50} ($Lethal\ Dose_{50}$) [11]. The animals were checked after 4–5 d of challenge for the development of primary and secondary lesions. If the virus generalizes in the guinea pig's body, the vesicles on the un-inoculated feet and the tongue are observed and it is positive reaction for *FMD* infection in guinea pigs. The observations were recorded and the Protection $Dose_{50}$ (PD_{50}) calculated in both of the vaccinated groups (NV and RV)

and control groups by Karber method [13]. The virus Generalization has been described by Leucam et al [14].

Results and discussion

Table 1 indicates the virus titration for irradiated and un-irradiated samples after safety test and also the figure 1 shows Dose/survival curve for irradiated samples. The virus titration was decreased gradually by increasing of gamma ray doses (Table 1 and Fig. 1); also D_{10} value factor (dose of gamma ray which can decrease one logarithmic cycle of virus population) was obtained 5.3-5.88 kGy. The optimum dose range of gamma ray for *FMD Virus* inactivation with virus titration $10^{7.5}$ $TCID_{50}$/ml was obtained between 40–44 kGy that the virus antigenecity unalterated. The results of safety test for irradiated samples with gamma ray doses: 40, 45 and 50 kGy were suitable because CPE was not visible after three times blind culture on cell culture. The results of CF test for irradiated and un-irradiated samples show the antigenecity of irradiated *FMD virus* from 0–45 kGy was not changed [9]. The result of CF test was showed in table 2. After the vaccines inoculated subcutaneously on back foot in two groups of guinea pigs, just two of ten animals were showed antiserum titration above the protective titration (PT= 1.2) and other 8 animals were below the PT. Also the results of SNT for the vaccines were inoculated intraperitoneally show this rout is not suitable for the vaccine inoculation, because most of the animal's antisera titration were less than protective titration. The anti *FMD Virus* sera titration of the Guinea pigs which were vaccinated subcutaneously and infraaxillary are according to the table 3; it shows the irradiated *FMD* vaccine can immunize guinea pigs as well as the inactivated *FMD* vaccine by ethylenimine. Therefore the best rout of injection is subcutaneously and infraaxillary [15]. The Protective $Dose_{50}$ (PD_{50}) was calculated by the method of Karber [13]. The virus generalization in control animals was described by Leucam and et al [14]. The results of PD_{50} for two different vaccines are summarized in table 4; it shows three dilutions of the radio-

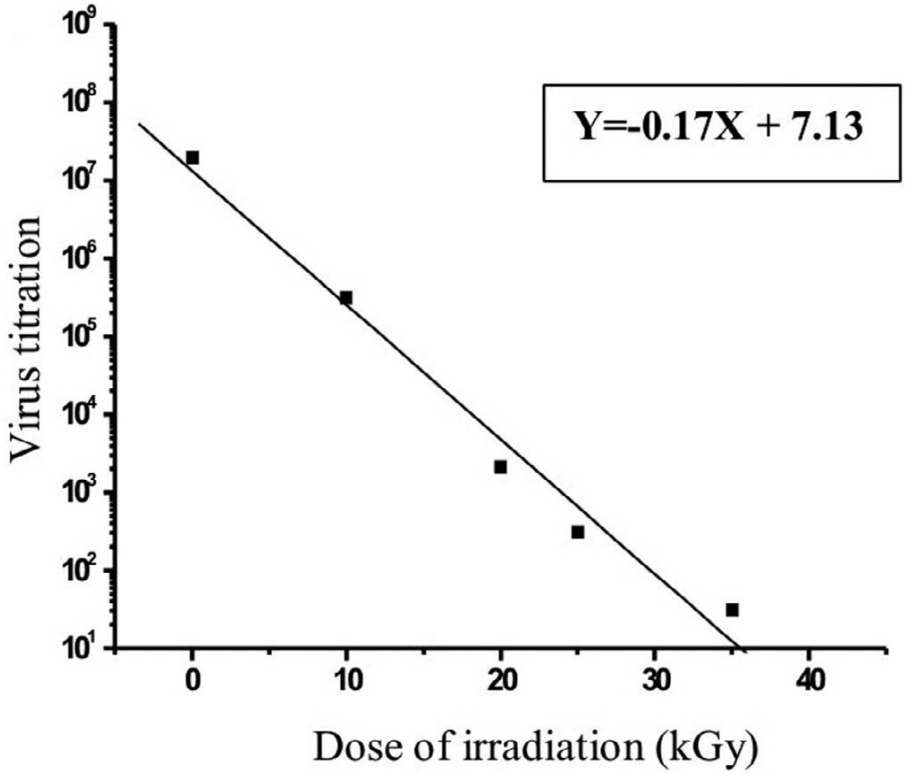

Fig. 1 Dose/Survival curve for irradiated FMDV typeA87/IRN

Table 1 The result of virus titration for irradiated and unirradiated FMDtypeA87/IRN

Dose of irradiation(KGy)	0	10	20	25	30	35	40	45	50
Virus titration/ml ($TCID_{50}$)	$10^{7.5}$	$10^{5.5}$	$10^{3.3}$	$10^{2.5}$	10^{2}	$10^{1.5}$	0	0	0

Table 2 The result of CF test

Dose of irradiation (kGy)	Dilution of FMDV typeA87/IRN ($10^{7.5}$ $TCID_{50}$ /ml)					
	1	1/2	1/4	1/8	1/16	1/32
0	4	4	4	3.5	Tr	–
10	4	4	4	3	1	–
20	4	4	4	3	1.5	–
25	4	4	4	3	1	–
30	4	4	4	2.5	0.5	–
35	4	4	4	1.5	Tr	–
40	4	4	4	3	1	–
45	4	4	4	2.5	0.5	–
50	4	4	4	0.5	–	–

Table 3 The results of SNT for titration of anti FMDV sera in the guinea pigs who were injected subcutaneously & infraaxillary (If the SN titre is more than 1.2, it is protective)

Time of serum titration	Anti FMD sera titration	mean	results	Time of serum titration	Anti FMD sera Titration		
1 month	NV	1.8	P	6 months	NV	1.74	P
	RV	1.8	P		RV	1.74	P
	unvaccinated	0.6	NP		unvaccinated	0.6	NP
3 months	NV	1.8	P	8 months	NV	1.77	P
	RV	1.8	P		RV	1.74	P
	unvaccinated	0.6	NP		unvaccinated	0.6	NP

NV: Normal Vaccine, RV: Radio-Vaccine, P: Protective, NP: Not protective

Table 4 PD50 of the inactivated FMD vaccine

Type of vaccine	**Dilution of vaccine**	**No of Guinea pigs**	**Percentage of generalization**	**PD_{50}**
Normal vaccine	1:1	5	0	7.06
	1:2	5	0	
	1:4	5	0	
	1:8	5	60	
	1:16	5	100	
	Control	5	100	
Radio-vaccine	1:1	5	0	5.60
	1:2	5	0	
	1:4	5	0	
	1:8	5	100	
	1:16	5	100	
	Control	5	100	

vaccine (1:1, 1:2 and 1:4) can immunize the guinea pigs as well as the normal vaccine.

The inactivation methods of *FMD Virus* are: 1) Inactivation by formaldehyde 2) Inactivation by aziridines such as acetylethylenimine, ethylenimine and propylenimine. Both of them have some residues in the final products, also some of them are toxic and some of them make allergic responses in the animals and it is possible to escape some viruses during the chemical inactivation routs [2]. Known methods of virus inactivation are based on the chemical action of some substance such as acetylethylenimine, betapropolactone, glycidalaldehyde, formaldehyde, etc. In such a process

the viral suspension should be kept at room or higher temperatures for 24–48 h. Under these conditions, physical and chemical agents act to degrade the virus antigenic proteins. On the contrary with ionizing radiation at low temperatures, the treatment dose not cause such degradation allowing the study of different viral functions [6].

Irradiated inactivated viruses have been reported to retain most of their antigenicity. Some of the researchers from Argentina studied for the production of some inactivated vaccines by ionizing irradiation of some viruses such as: *FMD virus* and *Herpes Simplex virus* [5]. Particularly Frescura.T and et al have observed that the antigenicity of type C lyophilized *FMD virus* which was inactivated by gamma radiations keeps unaltered by Complement Fixation method [8]. In this study the optimum dose range of gamma ray for inactivation of *FMD Virus typeA87/IRN* at -20 °C, without any change in antigenicity was obtained 40-44 kGy. Therefore the irradiated inactivated *FMD virus* with unalterated antigenicity character and good safety test results can be used to prepare of inactivated radio-vaccine. Also we formulated the vaccines by the Alhydrogel as adjuvant and other substances. The formulated vaccines were inoculated to Guinea pigs and the vaccinated animals were studied by SNT method and it shows the inactivated *FMD* vaccine by gamma irradiation can immunize guinea pigs as well as the inactivated *FMD* vaccine by ethylenimine. The result of potency test was showed in table 3, PD_{50} were 7.06 and 5.60 for inactivated vaccine by EI and gamma irradiation respectively, and therefore it shows three dilutions of the radio-vaccine (1:1, 1:2 and 1:4) can immunize the guinea pigs as well as the normal vaccine.

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