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DETERMINATION OF THE BEST IMMUNIZING DOSE OF PARAINFLUENZA-3 VIRUS VACCINE (MONOVALENT OR COMBINED) AND ITS VALIDITY

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SUMMARY

The influence of various doses of inactivated PI-3 virus vaccine on the serological response of guinea pigs and calves were studied. When testing vaccine in groups of guinea pigs revealed single dose 200 HA units elicited a response of 32 HI units, 400 HA units induced response 128 HI units, 600 HA units induced a response of 256 HI units after 14 days. This response was not improved by further increasing the dose of antigen on challenge all the vaccinated animals proved resistant but the controls developed pneumonia.

The 3ml and 5ml dose of monovalent PI-3 and combined inactivated respiratory virus vaccine (Pneumo-3) respectively gave better and higher immune response than the other studied dose (1 and 2ml) of monovalent PI-3 vaccine and 3, 10ml of Pneumo-3.

Moreover, the amount of Alhydrogel which has to be added to the vaccine as well as the validity and sterility of the vaccine were also examined.

INTRODUCTION

Respiratory diseases of animals are extremely economic importance because of the losses and high death rates. PI-3 beside causing respiratory illness induce poor weight gains loss of meat production due to stunting lengthening of the feeding period and death of the animal (Hamdy et al., 1965).

Samira (1992) prepared inactivated PI-3 vaccine. El-Sabbagh et al. (1995) used binary ethyleneimine as inactivant and its application for vaccine production.

Hussien et al. (1996) and Samira et al. (1995) prepared an inactivated combined respiratory virus

vaccine (Pneumo-3) for the vaccination of cattle and buffalo in El-Serw and Mehalet Mousa stations.

Later on, they carried out other modifications on this tissue culture vaccine either by the addition of stabilizers as gelatin, alhydrogel, DEAE-Dextran and oil or by lyophilizing the end product and study the action of some immunopotentiator as levamisole (El-Sabbagh , under press). Samira et al. (1996) concluded that the using of DEAE-Dextran as adjuvant give the highest titre and best potency when tested in calves.

El-Sabbagh (1997) and Ghaly (1998) investigated the growth of IBR and BVD viruses in different cell culture system. Monolayer, suspension and roller systems, as well as DEAE dextran to increase the yield of IBR and BVD viruses propagated in different cell culture systems.

Therefore, the purpose of the present work is to test the relation between the dose and the seroconversion manifested by the vaccinated animals were studied, the amount of Alhydrogel which has to be added and the validity of the vaccine.

MATERIAL AND METHODS

1. Virus :

PI-3 virus : Strain 45 (10^{-8} $TCID_{50}$/ml).

These local strains were isolated and identified by Singh and Baz (1966) and used in the preparation of vaccines.

Vaccines :

1. **Local inactivated PI-3 vaccine :** containing PI-3 (strain 45) 8 log10 $TCID_{50}$ (Samira, 1992).

2. Local combined inactivated respiratory vaccine (Pneumo-3) containing PI-3 (strain 45) 8 log10 $TCID_{50}$/ml (Samira, 1992), IBR (Abou Hammad strain) 8 log10 $TCID_{50}$/ml (El-Sabbagh, 1993) and BVD-MD (Iman strain) 7 log10 $TCID_{50}$/ml (Ghaly, 1993).

The vaccines were produced in the Department of Rinderpest Like Diseases and Blue Tongue in Vet. Serum and Vaccine Research Institute, Abbasia, Cairo Egypt.

3. Tissue culture :

Madin Darby bovine kidney (MDBK) cell line culture (Marcus and Moll, 1968) tested to be free from the non cytopathic (NPC) BVD-MD virus was used in this study.

4. Alhydrogel solution :

It was used as a stabilizer and adjuvant of the preparation of vaccines. It was sterilized by autoclaving for 20 minutes at 140°C and the pH was adjusted to 6.6 then it was bottled and stored at 4°C.

EXPERIMENT AND RESULTS

1. Adsorption of inactivated PI-3 virus to choice optimum concentration of adjuvant :

Choice of the optimum concentration of Alhydrogel as adjuvants for virus adsorption.

For this purpose, to each 100 ml amounts of the previously titrated inactivated PI-3 vaccine; the following amounts of Alhydrogel were added 10, 20, 30, 40, 50 and 100ml. The mixtures were kept overnight at 4oC with continuous stirring. Following this each of these mixture was centrifuged at 3000 rpm for 10 minutes. The sediment was kept aside and the infectivity of the residual non adsorbed virus in the supernatant fluid was assayed haemagglutination test and cytopathogenesis in monolayer MDBK cell culture. The titre was expressed in $\log_{10}$ $TCID_{50}$/ml.

Results as shown in table (1) revealed that most of the virus adsorbed when using 30% concentration of Alhydragel since the least residual virus was detected at this concentration.

2. Stability of haemagglutinin of inactivated virus suspension :

Virus inactivated with BEI was stored at 4°C for 2 months and HA titre checked at intervals of 7 days for 2 months. A further sample was stored at room temperature for 2 weeks and at 37°C for one week and HA content checked daily.

Samples of 25ml of binary inactivated virus were adjusted to different pH levels by an appropriate addition of N/1 hydrochloric acid or N/1 sodium hydroxide. The samples were incubated at 37°C for 4 hours after which HA tests were done and any alteration in haemagglutinin content noted.

Table (1) : Titration of residual non adsorped PI-3 virus after being mixed with various quantities of Alhydrogel

Volume of alhydrogel added/100ml	Residual PI-3 virus titre in log10 TCID50/0.1 ml	HA titre	Conatrol
10	10^3	64	1024
20	$10^{2.5}$	32	1024
30	$\leq 10^1$	8	1024
40	$\leq 10^1$	8	1024
50	$\leq 10^1$	8	1024
100	$\leq 10^1$	8	1024

N.B. The original virus titre used was $\log_{10}$ 8 $TCID_{50}$/ml.

The results in table (2) revealed that the BEI inactivated PI-3 virus suspension was incubated at different pH levels at 37°C for 4 hours. The haemagglutinin was preserved at pH 5.0 to 8.0 but completely destroyed at pH 4.0.

PI-3 virus inactivated with BEI was stored at pH 6.0 for 2 months at 4°C. No change in the HA content was observed during this time. Sample stored at room temperature for 7 days showed no alteration in HA content, but after this time a slow decline inactivity was noted.

3. Adsorption of inactivated PI-3 virus adjuvant at varient pH:

25ml of BEI inactivated virus suspension were adjusted to different pH levels and 30% Alhydrogel added and this suspension was agitated for 12 hours at 4°C and the efficiency of adsorption checked by estimating the HA content of the supernatant controls with no adjuvant were run in parallel (Table 2).

The results obtained in table (2) indicated that PI-3 antigen adsorbed completely to Alhydrogel at pH 6.0.

4. Stability of PI-3 vaccine :

Antigen adsorbed to Alhydrogel was stored at room temperature for 7 days and stored at 4°C for 6 months and injected into calves.

Table (2) : PI-3 antigen adsorbed to Alhydrogel at varient pH

pH	HA titres in supernatant after adsorption	
	Control without Alhydrogel	with Alhydrogel
8	1024	1024
7	1024	1024
6.5	1024	1024
6.0	1024	16
5.5	1024	8
5.0	1024	8
4.5	512	8
4.0	256	--

1000 HA units elicited a response of 128 HA units which compared well with freshly prepared vaccine.

Results obtained revealed no effect of storage for 6 months in 4°C or at room temperature for 7 days.

5. Vaccine evaluation :

The vaccines were evaluated according to the following :

A. Purity :

In accordance with the US Code of Federal Regulations (1987) testing 9, CFR 113-26, 113 - 27, 113.27, 11.3, 30 and 113.25.

B. Safety :

According to 9.CER (1987) testing 113-41 in calves and 9 CFR 113.38 in guinea pigs were used in this study.

C. Potency (Immunological response) :

1. Effect of varying doses of PI-3 vaccine in Guinea pigs :

Groups of 5 Guinea pigs were vaccinated with PI-3 vaccine of varying doses of vaccine (table 3) whereas 200 units elicited a response of 32 HI units after 14 days, 400 HA units induced a response 128 and 600 HA units induced a response of 256 HI units. This response was not improved by further increasing the dose of antigen. On challenge, all the vaccinated animals proved resistant, but the controls developed pneumonia. Control animals in contact showing negligable antibody response.

2. Effect of varying doses of PI-3 vaccine and Pneumo-3 vaccine in calves :

Eighteen calves were chosen for this purpose. The animals were tested before vaccination and proved to have no specific antibodies against PI-3. They were divided into 2 groups according to the type of vaccine (Monovalent PI-3 and combined inactivated respiratory virus vaccine (Pneumo-3). Each group was subdivided into 3 subgroups according to the inoculated dose and each dose was inoculated into 3 animals. The doses used were either 1, 2 or 3ml from monovalent (PI-3) vaccine and 3, 5, 10ml of combined inactivated respiratory vaccine (Pneumo-3) given intramuscularly and repeated at the same pattern after 2 weeks apart. Sera from vaccinated animals were collected at 21 days, 1, 2, 3 and 4 months interval post vaccination. These sera were tested for the seroconversion using the serum neutralization

Table (3): Effect of dose variant of inactivated PI-3 vaccine on Guinea pigs (5 animals/group).

Group No.	Dose (HI units)	(HI units) prevaccination	HI unit post vaccination	Challenge lung lesion
1	200	≤8	32	None
2	400	≤8	128	None
3	600	≤8	256	None
4	1000	≤8	256	None
5	control	≤8	≤8	Severe pneumonia

N.B. The original virus titre used was $\log_{10}$ 8 $TCID_{50}$/ml.

Table (4) : Average neutralizing titres of calves at different doses of monovalent PI-3 vaccine and combined inactivated respiratory virus vaccine (Pneumo-3).

Interval post vaccination	Type of vaccine received	Geometric means of neutralizing titre expressed as log10 of different doses of vaccines		
		1 ml	2 ml	3 ml
Prevacc.	Monovalent PI-3 vaccine	0	0	0
21 DPV		0.55	1.0	1.25
1 MPV		1.35	1.75	1.95
2 MPV		1.20	1.65	1.85
3 MPV		1.05	1.55	1.70
4 MPV		0.90	1.4	1.50
	Combined inactivated respiratory virus vaccine (Pneumo-3)	3 ml	5 ml	10 ml
Prevacc.		0	0	0
21 DPV		0.80	0.95	0.65
1 MPV		1.3	1.65	1.55
2 MPV		1.4	1.65	1.60
3 MPV		1.2	1.55	1.40
4 MPV		1.0	1.4	1.20

The minimum accepted protective SN titre for PI-3 virus is 0.60 $\log_{10}$ or 1:4 (Mihajlovic et al., 1979).

DPV: Days post Vaccination.
MPV: Months Post Vaccination.

Table (5): Average haemagglutination inhibition titres of calves sera vaccinated with 1, 2, 3 ml doses of inactivated PI-3 vaccine and 2, 5, 10 ml of combined inactivated respiratory virus vaccine (Pneumo-3).

Interval post vaccination	Type of vaccine received	Geometric means of neutralizing titre expressed as log10 of different doses of vaccines		
		1 ml	2 ml	3 ml
Prevacc.	Monovalent PI-3 vaccine	0	0	0
21 DPV		1.5	1.8	1.95
1 MPV		2.1	2.5	2.6
2 MPV		2.0	2.2	2.4
3 MPV		1.95	2.0	2.25
4 MPV		1.85	1.9	2.0
	Combined inactivated respiratory virus vaccine (Pneumo-3)	3 ml	5 ml	10 ml
Prevacc.		0	0	0
21 DPV		1.45	1.75	1.45
1 MPV		2.0	2.50	2.25
2 MPV		1.95	2.25	2.00
3 MPV		1.85	1.85	1.70
4 MPV		1.60	1.70	1.50

The minimum accepted protective HI titre for PI-3 virus is 1.2 $\log_{10}$ or 1:80 (Mohanty and Lillie, 1964)

DPV:Days Post Vaccination.

MPV: Months Post Vaccination.

(SNT) and micro-haemagglutination inhibition test (HI) tests according to the methods described by Kone (1969) and Cho et al. (1985), respectively.

The results of seroconversion in calves vaccinated with 1, 2 and 3ml doses of monovalent inactivated PI-3 vaccine and 3, 5 and 10ml doses of combined inactivated respiratory virus vaccine (Pneumo-3) are presented in table (4) and (5) and Fig. (1, 2) showed that all tested doses induced protection titre against PI-3 virus.

The data presented demonstrated that the SN titre increase from the 21th day post vaccination to reach the maximum by one month post vaccination and remained high till 4 months post vaccination (table 4).

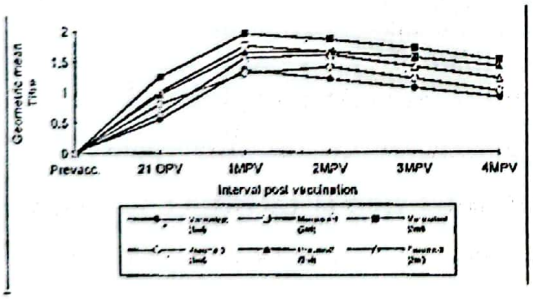

Fig. (1): Average neutralizing titres of calves at different doses of monovalent PI-3 vaccine and combined inactivated respiratory virus vaccine (Pneumo-3).

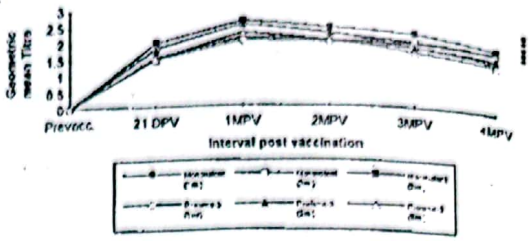

Fig. (2): Average haemagglutination inhibition titres of calves sera vaccinated with 1, 2, 3ml doses of inactivated Pi-3 vaccine and 2,5, 10ml of combined inactivated respiratory virus vaccine (Pneumo-3).

Examination of calves sera at various doses by the HI test demonstrated that the peak of haemagglutinating antibody titre was generally reached after one month post vaccination (table 5).

DISCUSSION

The vaccine used compared of 30% Alhydrogel and 70% of the inactivated virus mixed together which proved to be needed for complete adsorption of the virus particle during 24 hours (table 1).

Stability of vaccine, storage of vaccine at room temperature for 7 days or storage at 4°C for 6 months revealed no effect of efficacy of the vaccine.

Guinea pigs are valuable for assessing potency in that tests can be completed in 14 days and they are of course much were economical to use than calves. Table (3) as Tribe et al. (1968) who evaluate PI-3 vaccine in Hamster and Guinea pigs.

For studying the immune response of cattle immunized with that vaccines, two parameters were considered; the inoculated dose and the type of vaccine. Moreover, the level of antibodies of each animal was traced for 4 months using the serum neutralization and haemagglutination inhibition test as criteria for the level of protection.

Concerning the effect of different doses on the immune response irrespective of type of vaccine. It appeared that the 3ml and 5ml doses generally gave the highest immune response throughout the investigation period from monovalent PI-3 and Pneumo-3 vaccine.

These results agreed with Knezevic et al. (1990) who applied vaccination of heifers and cows with two injections either 2ml or 5ml of the oil inactivated combined IBR and PI-3 vaccine and they found that HI antibody titre to PI-3 was greater in animals given 5ml than in animals given 2ml of the vaccine.

Matsuoka et al. (1972) immunized group of vaccine by 2 doses of 10ml of the combined inactivated respiratory virus vaccine which containing PI-3, IBR killed pasteurella multocida and pasteurella haemolytica vaccine with 3 weeks apart, gave significant increase in serum neutralizing antibodies to IBR and haemagglutinating inhibiting antibody to PI-3 after one month post booster dose.

The low immune response noticed in animals can't be explained to the difference in the amount of antigen rather than the effect of adjuvant, which is two folds higher in the 10ml than in the 5ml dose in Pneumo-3 vaccine. Such amount of adjuvant into vaccine has an adverse effect on its immune response as evidenced by Rakhmanov (1972) who reported that the incorporation of aluminium hydroxide gel in FMD vaccine at a ratio of 50%, might cause plasmocytosis depression. On the other hand, the better response of 3ml of monovalent vaccine (large dose) on the vaccine is proportional to the increase in body weight and the well developed immune system as well as due to the immunopotentiating effect of this adjuvant rather than to the antigen content, and agreed with Anderson et al. (1971) who indicated that higher antigen doses maintained serum antibody for a longer period and this associated with the change from IgM to IgG production.

The presence of even little amounts of neutralizing antibodies may be protective against the disease possibly higher levels of antibody could be stimulated by increasing the dose. On the other hand, less vaccine might stimulate the same level of antibody it is used as permissible dose of vaccine.

Finally, for the vaccination of vaccine, it is recommended to inoculate animals with 2 ml dose of monovalent PI-3 vaccine and 5ml dose from combined inactivated vaccine Pneumo-3 and second

dose apart 2-3 weeks from the first dose is recommended to increase the protective titre and duration of immunity and the vaccine stored at 4°C for at least 6 months.

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