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STUDIES ON TRIVALENT IBR, BVD AND PI-3 INACTIVATED VACCINE

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SUMMARY

Three types of combined respiratory vaccine containing Infectious Bovine Rhinotracheitis (IBR), Bovine Viral Diarrhoea (BVD) and Parainfluenza -3 (PI-3) viruses were prepared from the local isolates grown in MDBK cell line, inactivated by binary ethyleneimine (BEI) and adsorbed with different adjuvants, DEAE-Dextran, alhydragel and mineral oil adjuvants were compared immunologically for the choice of the most preferable one for vaccine production.

All types of the prepared vaccines were safe when tested in guinea pigs and calves. Potency test was performed by immunization of susceptible calves which were divided into 3 groups each group was inoculated by one type of vaccine and the third group was kept as control. Two intramuscular injections of each vaccine were administered at an interval of two weeks.

The results indicated a variation in kinetics of the development of serum neutralizing (SN) against all component of the vaccine and haemagglutinating antibodies against PI-3virus in sera of all vaccinated calves 6 months after inoculation.

The protective capacity of each vaccine was studied when the vaccinated animals were challenged one month post vaccination, neither thermal reactions nor virus isolation could be detected. Under the conditions of these experiments a significant higher degree of immunogenicity was demonstrated with DEAE dextran adjuvant vaccine.

INTRODUCTION

The efficiency of inactivated vaccines depends on different factors among them the type of inactivator and the type of adjuvant which are considered the most important factor as they play a significant role in evaluating potency of the vaccine (Wittman, 1972 and 1978). Thomas et al. (1986) reported that inoculation of vaccines with adjuvant led to significantly higher antibody response than when an adjuvant was excluded from the vaccine. Laboratory studies for the efficacy of the prepared vaccines was evaluated in terms of studying potency, duration of immunity after vaccination and with regard to resistance of infection after experimental challeng with virulent viruses as reported by Zuffa and Feketeova (1980). The production of a safe efficacious non infective vaccine that would be

valuable in immunizing cattle without threat of adverse reactions that have followed the use of attenuated vaccines (Kolar et al., 1971).

Infectious Bovine Rhinotracheitis (IBR), Bovine Viral Diarrhoea (BVD) and Perainfluenza - 3 viruses are the main causative agents of pneumoenteritis complex of cattle and buffalo calves which constitute a serious disease problem in Egypt (Hafez et al., 1975).

In Egypt, Samira (1992); El-Sabbagh (1993) and Ghali (1993) succeeded in preparing a safe and potent freeze dried combined inactivated vaccine using gelatin as adjuvant against IBR, BVD and PI-3 viruses. However , there is always concern regarding a possible hard technically and high costly nature of freeze drying would not accept a freeze dried inactivated vaccines as an appicable vaccine in a massive vaccination programmes. Thus the purpose of this work is to study the efficiency of various types of vaccines with three different adjuvants and evaluating the immunogenic potencies of the three vaccines by experimental immunization of calves.

MATERIAL AND METHODS

1. Viruses:

IBR virus : Abou Hammad strain(10^{-7}/ $TCID_{50}$ /ml).

PI-3 virus : Strain 45 (10^{-8} / $TCID_{50}$ / ml).
BVD virus :Iman strain (10^{-6} / $TCID_{50}$ / ml).

These local strains were isolated and identified by Hafez et al. (1976), Singh and Baz (1966) and Baz (1975) respectively, were used in the preparation of inactivated vaccine. Virulent virus was used in challenge exposure test in calves.

2. Cell Culture:

Madin Darby BovineKidney (MDBK) cell line (Marcus and Moll, 1968) tested to be free from the non cytopathic (NCP) BVD-MD virus.

3-Vaccine Production:

The three batches of combined inactivated respiratory virus suspensions inactivated separately with Binary ethyleneimine were used according to El-Sabbagh et al., (1995) And just before the addition of adjuvants, thiomersal was added as a vaccine preservative at final concentration of 0.01%.

a. DEAE-Dextran adjuvant vaccine:

According to Anderson et al .(1971), to each 100 ml inactivated virus fluid 100 ml of autoclaved 20% Di-ethylamino ethyl(DEAE) dextran MW 500,000, Pharmacia. Fine chemical Sweden in 0.25 M tris HCl buffer of pH 7.6-7.8 was mixed.

b. Alhydragel adjuvant vaccine:

To each 120 ml inactivated virus fluid , 80 ml of Alhydrogel suspension were added and stirred for 24 hours at 4°C.

c. Mineral oil adjuvant vaccine:

96 ml marcol oil were mixed slowly with 4 ml Tween 20 and 0.2 ml Span 80, then the mixture

was sterilized by autoclaving. To each 50 ml inactivated virus fluid , 100 ml of prepared oil adjuvant were added and emulsified.

4. Serological Methods:

1. Serum Neutralization Test:

It was carried out following the technique of Kone (1969) against PI-3, BVD and IBR viruses.

2. Micro Haemagglutination Inhibition Test:

It was carried out according to the technique of Cho et al. (1985) against PI-3 virus.

Laboratory testing of the three vaccines:

According to American Codic.

1. Safety test in Guinea pigs (Bittle,1968).
2. Safety test in calves (Phillips 1968).
3. Potency test in calves (Fernelius , 1972).
4. Post vaccinal challenge (Syurin et al., 1983).

1. Safety test in Guinea pigs:

A total of 8 adult Guinea pigs weighing about 250-300 grams were used, 2 were inoculated intraperitonially with 0.5 ml of each type of vaccines and 2 were inoculated with sterile saline using the same route and dose (control group).

2. Safety test in calves:

6 susceptible clinically normal calves were observed 3 days before vaccination, each 2 calves were inoculated intramuscularly with 10 field doses of each type of vaccines.

3- Immunization of calves:

For each tested vaccine, four seronegative calves were inoculated intramuscularly with 5 ml of vaccine. A booster dose of 5 ml of the same vaccine was given intramuscularly 2 weeks after the primary dose. One month after vaccination, two calves from each vacinated group were challenged with virulent pooled virus of IBR, BVD and PI-3 by intravenous and intramuscular injections, according to Zuffa and Feketeova (1980). The other two calves of each vaccinated group were left for studying the duration of immunity against each component of the tested vaccine for months post vaccination. Three animals of the control group were also infected at one month from the beginning of the experiment with virulent pooled virus. IBRV, BVDV and PI-3 virus-neutralizing antibodies were assayed by serum neutralization test. Also, PI-3 virus-haemagglutinating antibodies were determined by haem-agglutination inhibtion test as well as thermal reaction and virus isolation form blood, nasal, conjuctival and rectal swabs which were followed for 14 days post challenge (El-Trabili et al., 1983).

RESULTS

All the prepared vaccines were safe in Guinea pigs and calves, neither thermal reaction nor virus detection was recorded in all inoculated calves.

Potency tests for the different vaccines in IBR, BVD and PI-3 viruses- seronegative calves

Table (1) : Results of studies on immune response in calves vaccinated by three types IBR, BVD and PI-3 viruses vaccines.

Time post vaccination		Serum neutralizing antibody titres expressed Log_{10}								
		DEAE-Dextran adjuvant inactivated vaccine			Alhydragel adjuvant inactivated vaccine			Oil adjuvant inactivated vaccine		
		IBR	BVD	PI-3	IBR	BVD	PI-3	IBR	BVD	PI-3
Zero day	First	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1 WPV	vaccination	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2 WPV	"Initial dose"	0.55	0.40	0.50	0.45	0.35	0.40	0.35	0.25	0.25
3 WPV		1.50	1.20	1.35	1.10	1.05	1.00	0.85	0.65	0.70
4 WPV		2.15	1.90	2.00	1.70	1.55	1.75	1.35	1.20	1.30
6 WPV	Second	2.25	2.00	2.10	1.70	1.60	1.75	1.50	1.45	1.50
8 WPV		2.25	2.00	2.15	1.70	1.60	1.65	1.55	1.45	1.55
12 WPV	vaccination	2.20	1.95	2.20	1.60	1.50	1.55	1.45	1.30	1.45
16 WPV		1.95	1.70	1.90	1.50	1.50	1.40	1.20	1.15	1.25
20 WPV	"Booster	1.65	1.50	1.75	1.20	1.25	1.25	0.95	1.05	1.00
24 WPV	dose"	1.45	1.20	1.35	0.95	0.95	0.90	0.65	0.90	0.75
28 WPV		1.25	0.95	1.10	0.75	0.65	0.80	0.55	0.45	0.60

Contact control group showing no neutralizing antibody during the experimental period.

WPV = Week post vaccination.

1. The minimum accepted protective SN titre for IBR virus is 0.60 Log_{10} or 1:4 (Zuffa and Feketeova , 1980).
2. The minimum accepted protective SN titre for BVD virus is 0.90 Log_{10} or 1:8 (Bittle et al. , 1968).
3. The minimum accepted protective SN titre for PI-3 virus is 0.60 Log_{10} or 1:4 (Mihajlovic et al. , 1979).

Table (2) : Results of haemagglutination test in vaccinated calves,

Time post vaccination	Log_{10} Haemagglutinating inhibiting titres post vaccination		
	DEAE-Dextran adjuvant inactivated vaccine	Alhydrogel adjuvant inactivated vaccine	Oil adjuvant inactivated vaccine
Zero day	0.20	0.15	0.00
1 WPV	0.40	0.25	0.30
2 WPV	1.20	1.00	0.95
3 WPV	1.80	1.60	1.30
4 WPV	2.40	2.10	1.80
6 WPV	2.50	2.15	1.90
8 WPV	2.50	2.10	1.85
12 WPV	2.40	2.00	1.65
16 WPV	2.10	1.95	1.45
20 WPV	2.00	1.80	1.35
24 WPV	1.95	1.60	1.20
28 WPV	1.65	1.50	1.00

Contact control group showing no Haemagglutinating inhibiting antibody titre.

WPV = Week post vaccination.

* The minimum accepted protective HI titre for PI-3 virus is 1.20 Log_{10} or 1:80 (Mohanty and Lillie , 1964).

revealed that all tested vaccines were potent. Vaccinated calves were resistant to challenge at the 14th day after boostering. No clincial abnormalities or viraemia or virus shedding within the 2 weeks post challenge. Tables (1 and 2) showed that all tested vaccines induced protective titers against IBRV, BVDV and PI-3 virus-neutralizing antibodies; starting with a mean Log_{10} values of (1.50, 1.20, 1.35), (1.10, 1.05, 1.00) and (0.85, 0.65, 0.7) at the 3rd WPV, reaching to their maximal values of (2.25, 2.00, 2.20), (1.70, 1.60, 1.75) and (1.55, 1.45, 1.55) between the 4th and 12th WPV; and declined to their minimal accepted titers of (1.25, 0.95, 1.10) at the 28th WPV (0.95, 0.95, 0.85) and (0.65, 0.90, 0.75) at the 24th WPV respectively in sera of calves vaccinated with DEAE-dextran, Alhydragel and mineral oil adjuvant vaccines. Also, PI-3 virus-haemagglutinating antibodies of acceptable titers were recorded at mean Log_{10} values of (1.20 and 1.65) at the 2nd and 28th WPV; (1.60 and 1.50) at the 3rd and 28th WPV; and (1.30 and 1.20) at the 3rd and 24th WPV respectively in sera of calves vaccinated with DEAE- dextran, alhydragel and mineral oil adjuvant vaccines.

DISCUSSION

Trivalent inactivated respiratory vaccines were prepared under the most appropriate selected condition in MDBK cell line using three different adjuvants, viz 40% alhydragel, 10% DEAE-Dextran in a final concentration 100mg/ml and oil adjuvants (2 part of oil + 1 part of inactivated virus suspensions. They were evaluated for their safety in Guinea pigs and calves as well as for their potencies and protection for calves.

Safety test carried out in Guinea pigs as well as susceptible calves proved that, all vaccines were safe, potent. Determination of the immune response to IBR, BVD and PI-3 viruses by using serum neutralization and haem-agglutination inhibition tests is considered to be valied when challenged vaccinated animals manifested no clinical signs of the diseases (thermal reaction or viraemia) within the 2 weeks observation period post challenge. The results are represented in Tables (1 and 2). All prepared vaccines induced protective antibodies against IBR, BVD and PI-3 viruses for a period between (the 3rd to 28th WPV), (the 3rd - 24thWPV) and (the 4th - 24th WPV) respectively in sera of calves vaccinated with DEAE-dextran, Alhydragel and mineral oil adjuvant vaccines.

Dextran as an adjuvant for enhancing the antibody production was mixed with vaccine batch to a final concentration 100mg/ml which has proved to be the minimum efficient concentration capable to adsorb all virus particles in the vaccine mixture (Pagana and Vaheri, 1965). This phenomenon was explained by Kaplan et al. (1967) as that the polycations could possibly act by binding to the cellular surface (thereby creating of favourable ionic charge for virus attachment. Another explanation is by complexing with the virus particles (thereby allowing them to attach to the cell surface more efficiently (Pagana and Vaheri, 1965).

The conclusion which can be uptaken from results of this study the fact that, DEAE-dextran adjuvant BEI inactivated trivalent vaccine is absolutely efficient and applicable.

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