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Original Research Article

Immunological response of locally prepared oil adjuvanted pneumo-5 vaccine in calves

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ABSTRACT

The present study aimed to prepare a combined inactivated vaccine containing bovine viral diarrhea genotype-1 (BVD-1), bovine viral diarrhea genotype-2 (BVD-2), infectious bovine rhinotracheitis (IBR), parainfluenza-3 (PI-3) and bovine respiratory syncytial virus (BRSV) and adjuvanted with montanide oil ISA 206. Quality control results proved that the pneumo-5 vaccine was pure and completely safe to be used in calves without abnormalities. Potency test was performed on two groups of calves three for each group, where the first group was vaccinated with pneumo-5 vaccine adjuvant with montanide oil ISA 206 and the second group was left as non-vaccinated control group. In group (1), serum neutralization test revealed that the serum neutralizing antibody titers in BVD-1 and BVD-2 developed more higher than the minimal acceptable titer of the protective level (\log_{10} 0.9), while \log_{10} 0.6 was protective against IBR, PI-3 and BRS viruses at one month of vaccination and remained protective till the end of experiment compared to group (2) that showed no neutralizing antibody response. The prepared vaccine proved to be highly potent as the developed BVD-1, BVD-2, IBR, PI-3 and BRSV antibodies remained within the protective level for 9 months post vaccination.

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1. Introduction

Bovine viral diarrhea genotype-1, bovine viral diarrhoea genotype-2, infectious bovine rhinotracheitis, parainfluenza-3 and bovine respiratory syncytial viruses have all been incriminated in the etiology of acute respiratory diseases in cattle and calves (Durham and Hassard, 1990).

Pneumoenteritis viruses such as (BVD-1), (BVD-2), (IBR), (PI-3) and (BRS) represent the most important and severe illness among calves (Barranco et al., 2003; Yavru et al., 2005) and are widely spread through the world including Egypt.

Respiratory disease complex has a major economic impact on the beef industries due to annual losses in calves and adults in millions of dollars.

Control of bovine respiratory disease complex (BRD) is a major focus of veterinary health programs (Stokka and Edwards, 1990). Viral respiratory diseases usually reach their peak during the early housing season in months of October, November and December (Verhoeff and Van Nieuwstadt, 1984). During this period, climatic conditions appear to favor the dissemination of these viruses. Environmental risk factors include hunger, thirst, extreme, hot and cold climatic temperature, fear and anxiety during transportation, weaning, dehorning, castration, highly parasitism, deficiency of vitamins such as vitamin A, poor ventilation, dust, ammonia and overcrowding (Schwabe et al., 1977).

The present work was performed to prepare a safe potent polyvalent inactivated vaccine containing BVD-1, BVD-2, IBR, PI-3 and BRS viruses by using montanide oil ISA 206 as adjuvant.

2. Materials and methods

2.1. Viruses

2.1.1. Bovine viral diarrhea virus genotype-1 (BVD-1)

A local Egyptian strain (Iman strain) with a titer of $10^{6.5}$ TCID₅₀/ml. was isolated from Friesian calves with severe pneumoenteritis at Tahrir Province (Baz, 1975).

2.1.2. Bovine viral diarrhea virus genotype-2 (BVD-2)

A cytopathic (strain 125) with a titer of $10^{6.5}$ TCID₅₀/ml, Infectious Bovine Rhinotracheitis virus (IBRV): A local isolate of "Abou Hammad strain" with a titer of $10^{7.5}$ TCID₅₀/ml was firstly isolated and identified by (Hafez et al., 1976).

2.1.3. Parainfluenza-3 virus (PI-3V)

Reference Egyptian strain of PI-3 (Strain 45) with a titer of 10^8 TCID₅₀/ml. was firstly isolated and identified by Singh and Baz (1966).

2.1.4. Bovine respiratory syncytial virus

Reference strain of BRSV (375L) with a titer of $10^{6.5}$ TCID₅₀/ml was identified (Smith et al., 1975).

All viral strains were propagated and tittered on Madin Darby Bovine Kidney (MDBK) cell culture, which has been proved free from infectious agents especially non cytopathic strain of BVD virus. These viruses were supplied by Rinderpest Like Diseases Research Department, Veterinary Serum and Vaccine Research Institute Abbasia, Cairo (VSVRI) and used in vaccine preparation and SNT.

2.2. Inactivant

Bromo-ethyleneimine hydrobromide (BEI) was used for inactivation of the vaccinal viruses according to Bahnemann (1975).

2.3. Sodium thiosulphate

It was used to stop the action of BEI according to Bahnemann (1975). It was prepared as 20% solution in double distilled water, sterilized by autoclaving and added to the inactivated virus suspension as 2%

2.4. Montanide Oil ISA 206

It is a mineral oil based adjuvant in water emulsion or a double emulsion. It was obtained from Seppic, Paris, France (2002)

2.5. Thiomersal

It was used as a vaccine preservative added to the vaccine at final concentration of 0.01%.

2.6. Animals

2.6.1. White Swiss mice

Ten Albino Swiss weaned mice of 10-15g body weight were used for safety test of the prepared vaccine. They were obtained from Laboratory Animals Department, Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo.

2.6.2. Calves

A total of 12 susceptible healthy male native breed calves aged 6 months were used to study the safety and potency of the prepared vaccine. These animals were found to be free from antibodies against the used viruses using SNT.

2.7. Vaccine preparation

The used viruses were propagated in MDBK cell line and the virus suspensions were harvested then subjected to two cycles of freezing and thawing and inactivated by 0.01M of binary ethylenimine according to EL-Sabbagh et al. (1993) and Zeidan et al. (1999). Sodium thiosulphate 2% of 20% was

added to stop the action of BEI then equal volumes of the inactivated virus fluids were mixed together according to Samira et al. (2001) and thoroughly mixed with montanide oil ISA 206 at a ratio 1:1 (vol/vol) (Barteling et al., 1990). The PH was adjusted to 7.5. Thiomersal was added as a vaccine preservative at final concentration of 0.001% and distributed in sterile bottles of 50 ml capacity.

2.8. Quality control of the prepared vaccine.

2.8.1. Sterility test

It was performed in accordance to USA Code of Federal Regulations, CFR (1987) to confirm the freedom of the prepared vaccine from bacteria, mycoplasma, fungi and extraneous viruses as non-cytopathic strain of BVDV.

2.8.2. Safety tests

2.8.2.1. In mice

Ten Albino Swiss mice were used, where five mice were inoculated intraperitoneally (IP) with 0.2 ml/mouse of the prepared vaccine and the other five mice were inoculated by the same dose and route with physiological saline and kept under observation as control. All mice were kept under observation for two weeks for the detection of any clinical abnormalities.

2.8.2.2. In calves

Safety test in calves was applied using six male calves divided into 2 groups (Three calves/group), The first group was inoculated I/M with ten times of the vaccinal dose of the prepared oil adjuvanted vaccine (50 ml) according to USA Code of Federal Regulations, CFR (1987). The other three calves were inoculated with the same dose and route by physiological saline solution. All animals were kept under observation for two weeks post inoculation for detection of abnormalities.

2.8.3. Potency test

Potency evaluation of the prepared polyvalent inactivated respiratory viral vaccine adjuvant with montanide oil ISA 206 vaccine was carried out according to USA Code of Federal Regulations, CFR (1987). Potency evaluation was determined by immune response of calves post vaccination to estimate the permissible limit of protective level against each viral component of the tested vaccine as well as the duration of immunity. Six male calves were used in this study and divided into 2 groups, three calves for each group as follow:

Group (1). Each calf was intramuscularly immunized with 5 ml of the locally oily prepared vaccine containing (BVD-1, BVD-2, IBR, PI-3 and

BRS) viruses by two injections, with three weeks interval.

Group (2). This group consisted of three calves and kept as non-vaccinated contact control.

2.9. Serum samples

Serum samples were collected from all calves on the first day of vaccination (0 day), the day of booster vaccination (21 days post vaccination) then every month. All serum samples were collected and inactivated at 56°C for 30 minutes in a water bath for inactivation of nonspecific substances, then stored at - 20°C until used in detection of specific neutralizing antibodies against all virus components of the prepared vaccine.

2.10. Serum neutralization test

It was performed on MDBK cell line using the micro titer technique. The sera were expressed as the Log10 of the inverse dilution, which protected 50% of the wells as calculated by Reed and Muench (1938).

3. Results

3.1. Sterility test

The prepared polyvalent vaccine was found to be free from bacterial, fungal, mycoplasma contamination and no extraneous viral agents (Table 1).

3.2. Safety tests

3.2.1. In mice

There were no clinical abnormalities or deaths observed during two weeks of observation period (Table 2).

3.2.2. In calves

Neither elevation of body temperature nor development of clinical signs of illness was recorded in calves inoculated with 10 doses of the vaccine during two weeks post vaccination.

3.3. Clinical findings post vaccination

The specific antibody titers were estimated by using serum neutralization test (SNT) (Tables 3,4 and Figs. 1,2). The mean serum neutralizing antibody titer remained at the protective level till the end of experiment at (9 months post vaccination) against for (BVD-1, BVD-2, IBR, PI-3 and BRS) viruses respectively. The control non vaccinated group showed no neutralizing antibody response.

Table 1. Sterility test of the prepared pneumo-5 vaccine.

Medium	Examined microorganism	Result
Nutrient agar	Aerobic bacteria	No colonies
Thioglycolate broth	Anaerobic bacteria	No turbidity
Sabarouds-agar	Fungus	No colonies

Table 2. Safety test of the prepared pneumo-5 vaccine.

Signs	Animals observed	
	Mice	Calves
Local reaction	Negative	Negative
Systemic reaction	Negative	Negative
Mortalities	Negative	Negative

Table 3. Mean BVD-1 and BVD-2 serum neutralizing antibody titers of the prepared pneumo-5 vaccine.

Time post vaccination	Mean BVD-1 and BVD-2 antibody titers*		
	BVD-1	BVD-2	Non vaccinated control Group
0 day**	0.20	0.21	Respond negatively lower than protection a ranged (between log ₁₀ 0.20-0.40)
21 day***	0.35	0.29	
1M	1.20	1.35	
2M	1.60	1.65	
3M	1.80	1.95	
4M	1.65	1.60	
5M	1.45	1.45	
6M	1.25	1.25	
7M	1.06	1.00	
8M	0.98	0.95	
9M	0.91	0.91	

*BVD antibody titer expressed a log₁₀ 0.9 was considered protective. **1st dose: 0day of vaccination. ***2nd dose: 21days post vaccination

Table 4. Mean antibody titer of IBR, PI-3 and BRS viruses in sera of the prepared pneumo-5 vaccine.

Time post vaccination	Mean IBR, PI-3 and BRS antibody titers*			
	IBR	PI-3	BRS	Non vaccinated control Group
1 st dose	0.35	0.25	0.20	Respond negatively lower than protection (a ranged between log ₁₀ 0.20-0.40)
2 nd dose	0.50	0.28	0.35	
1M	1.35	1.30	1.50	
2M	2.05	1.80	1.70	
3M	1.95	1.75	1.60	
4M	1.65	1.60	1.55	
5M	1.45	1.45	1.15	
6M	1.25	1.15	0.98	
7M	1.06	0.95	0.88	
8M	0.98	0.91	0.75	
9M	0.85	0.75	0.66	

*IBR, PI-3 and BRS antibody level log₁₀ 0.6 was protective. **1st dose: 0 day of vaccination ***2nd dose: 21days post vaccination

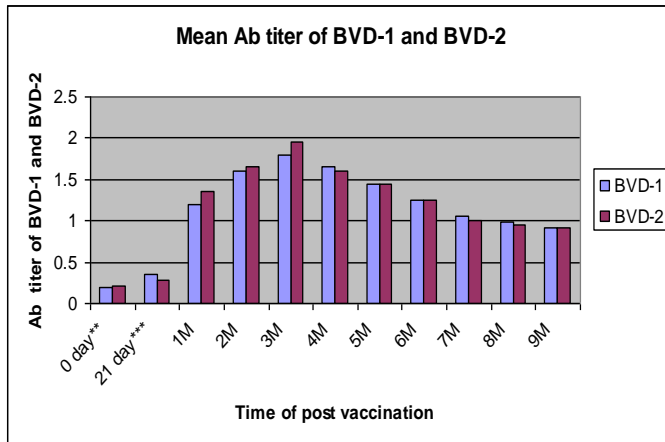


Fig 1. Mean BVD-1 and BVD-2 serum neutralizing antibody titers of the prepared pneumo-5 vaccine

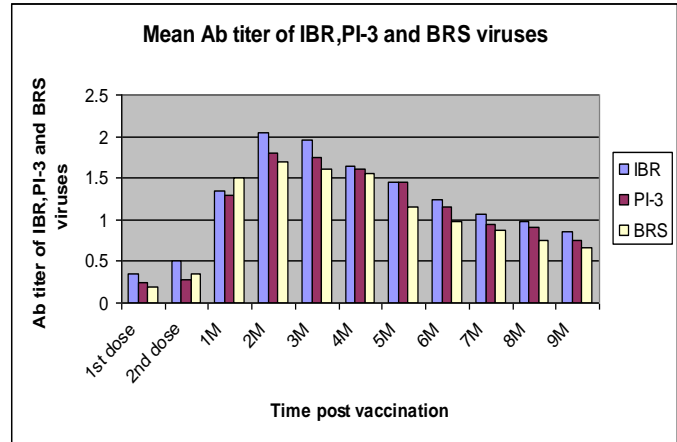


Fig 2. Mean antibody titer of IBR, PI-3 and BRS viruses in sera of the prepared pneumo-5 vaccine.

4. Discussion

Respiratory disease occurs due to stress factors as bad environment, transportation, accumulation of ammonia and excessively high humidity in closed areas which lower the resistance of animal and enhance the multiplication of microorganisms (Bickert and Herdt, 1985). Infectious agents associated with bovine respiratory diseases include five viruses; BVD-1, BVD-2, IBR, PI-3 and BRS (Samira et al., 2001). Vaccination programs for breeding herds are integral parts of preventive health programs designed to lessen the effects of infectious respiratory diseases in cattle (Knezevic et al., 1990).

Currently the prepared vaccine showed complete absence of any bacterial, fungal or mycoplasma contamination on specific media for 15 days post inoculation. Moreover, the results of sterility test revealed that the prepared vaccine was also free from any infectious or extraneous viral contamination (Table 1).

The obtained results supported safety tests when applied on mice and calves vaccinated with 10 times of vaccinal dose, where there was neither local nor systemic post vaccinal reaction and, there was no development of any clinical signs or elevation of

rectal temperature during the whole experimentation period (Table 2). Such findings agreed with those obtained by Urban et al. (1995) and Parker et al. (2009) who stated that in cattle trials for use of the montanide gel based prepared vaccine showed no adverse post vaccinal reaction.

The potency evaluation of the prepared polyvalent viral vaccine in calves (Tables 3,4 and Figs. 1,2) revealed that all vaccinated animals developed serum neutralizing antibody titers (SN antibody) reached their peak at three month (Table 3) and two months (Table 4) and remained stable higher than the minimal acceptable titer of protective level until the end of experiment. Such data are similar to those given by Bittle (1968) who recorded that the BVD antibody level of 1:8 dilution (\log_{10} 0.9) was protective (Mihajlovic et al., 1979; Zuffa and Fekeotova, 1980) who reported that the minimal acceptable titer of neutralizing antibodies was 1:4 dilution (\log_{10} 0.6) was protective against IBR, PI-3 and BRS viruses compared with the control non vaccinated group (group 2) that showed no neutralizing antibody response.

It is obvious that the enhanced action observed with the prepared vaccine was said to be due to a

gradual and continuous release of antigen to stimulate antibody production. Oil is a material for transport of the antigen throughout the lymphatic system and finally a stimulus for accumulation of immunologically important cells (Bahnmann et al., 1988). Montanide oil ISA 206 has a great safety margin to be inoculated without any local or systemic allergic reaction; also its mode of action to be used as an adjuvant was trap antigen and releases it over a larger period producing a more increase in the immune response. Oil emulsions increase the circulation and trap of lymphocytes in draining lymphoid tissue as well as may affect the immune response by enhancing the physical presentation of the antigen to macrophages (Vanselow, 1987).

5. Conclusion

It is concluded that the prepared polyvalent inactivated respiratory viral vaccine containing of BVD-1, BVD-2, IBR, PI-3 and BRS viruses adjuvanted with montanide oil ISA 206 was proved to be pure, fully safe and highly potent.

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