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PREPARATION AND EVALUATION OF COMBINED INACTIVATED RESPIRATORY VIRAL VACCINE PNEUMO-3 BY USING OF MONTANIDE OIL ISA 206 AS AN ADJUVANT

El-Bagoury, G.F.[a], **El-Sabbagh,M.M** [b] **and El-Hawary,R.I.** [b]

[a] *Departments of Virology, Faculty of Veterinary Medicine, Benha University,* [b] *Veterinary Serum and Vaccine Research Institute، Abbassia, Cairo.*

A B S T R A C T

The present study aimed for preparation of combined inactivated vaccine containing bovine viral diarrhoea virus, infectious bovine rhinotracheitis and parainfluenza-3 viruses adjuvant with Montanide oil ISA 206. The prepared vaccine was evaluated for purity, safety and potency tests. The study proved that the prepared vaccine adjuvanted with Montanide oil ISA 206 was pure and completely safe to be used in calves without any local or systemic post-vaccinal reaction. Potency test was performed on two groups of calves four for each group, first group was vaccinated with combined inactivated respiratory viral vaccine adjuvant with Montanide oil ISA 206 and the second group was left as non-vaccinated control group. Explaining of humoral immune response by using of serum neutralization test revealed that the serum neutralizing antibody titres were developed more higher than the minimal acceptable titre of protective level at one month post vaccination and lasts for 12 months post vaccination to IBRV, 9 months to BVDV and 10 months to PI-3V. In conclusion, the prepared combined inactivated vaccine being pure, completely safe and perfectly potent and effective control of pneumo-enteritis disease complex syndrome.

Key Words: Montanide oil ISA 206, Pneumo-3, SNT

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1. INTRODUCTION

Bovine viral diarrhea, Infectious bovine rhinotracheitis and Parainfluenza-3 viruses have all been incriminated in the etiology of acute respiratory disease in cattle and calves (**1**).

Respiratory disease complex has a major economic impact on the beef industries which cause losses in calves and adult cattle with millions of dollars loss each year to the cattle industry controlling bovine respiratory disease complex (BRD) which is a major focus of veterinary health programmes (**2**).

Viral respiratory diseases usually reaching their peak during the early housing season in the months of October, November and December (**3**). At this time of year, the climatic conditions appear to favour the dissemination of these viruses. Environmental risk factors includes 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 over-crowding (**4**).

So, the aim of this work was performed to prepare a safe effective combined inactivated vaccine containing BVD, IBR,

PI-3 virus by using Montanide oil ISA 206 as an adjuvant.

2. MATERIALS AND METHODS

2.1. *Viruses:*

A. Bovine viral diarrhoea virus (BVDV):

BVDV is a local Egyptian strain (Iman strain) with a titre of $10^{6.5}$ $TCID_{50}$/ml. It was firstly isolated from a Freisian calves with sever pneumoenteritis at Tahrir Province by (**5**).

B. Infectious Bovine Rhinotracheitis Virus (IBRV):

IBRV is a local isolate "Abou Hammad strain" with a titre of $10^{7.5}$ $TCID_{50}$/ml. It was firstly isolated and identified by **(6)** from calves suffering from respiratory disorders.

C. Parainfluenza-3 virus (PI-3V):

Reference Egyptian strain (Strain 45) with a titre of 10^{8} $TCID_{50}$/ml. It was firstly isolated and identified by **(7)** from Egyptian buffaloes.

These viral strains were propagated and titrated on Madin Darby Bovine Kidney (MDBK) cell culture, which has been proved free of any infectious agents, specially non-cytopathic strain of BVD virus. These viruses were supplied by Rinderpest Like Diseases Research Dept., Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo (VSVRI).

2.2. *Inactivant:*

Two bromo-ethyleneimine hydrobromide (BEI). It was purchased from Sigma Company and used for inactivation of the vaccinal viruses of the prepared vaccine.

2.3. Sodium Thiosulphate:

It was purchased from Sigma Company and prepared as 20% solution in double distilled water and it was sterilized by autoclaving. It was used to stop the action of BEI.

2.4. Montanide oil ISA 206:

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

2.6. Animals:

A. White Swiss mice:

Ten mature White Swiss mice of 10-15 gm weight used for safety test of the prepared vaccine obtained from VSVRI.

B.Calves:

A total of fourteen, cross breed, apparently healthy male calves of about 6-9 months old were used in this study. Calves were housed in an isolation facility at VSVRI.

Eight calves of them were used for potency evaluation, while six calves were used for safety test.

2.7. Vaccine preparation:

Viruses were propagated in MDBK cell line and inactivated by 0.001 M of BEI according to (**8**), then pooled according to (**9**) and thoroughly mixing with montanide ISA 206 at ratio 1:1 vol/vol according to (**10**). The pH was adjusted to 7.5. The vaccine was produced and provided by department of the Rinderpest like diseases, VSVRI.

2.8. Quality control of the prepared Vaccine

2.8.1. Purity test:

It was performed in accordance with **(11)** testing to be proved free from bacteria, mycoplasma, fungi and extraneous viruses as non-cytopathic strain of BVDV.

2.8.2. Safety tests:

Safety test in mice:

Five mice for the prepared vaccine were inoculated intraperitoneally (IP) with 0.2 ml, other five mice were kept as control. All mice were kept under observation for 16 days for the development of any clinical abnormalities.

Safety test in calves:

Safety test in calves was applied using six adult male calves divided into 2 groups (three calves for each). The first group was inoculated I/M with ten times of the vaccinal dose of the prepared vaccine (with oil adjuvant). Fifty ml were inoculated I/M in different musculature of animal body.

The safety test in calves was according to **(11).**

The other three calves were inoculated with the same dose (50 ml) and same times and same route (I/M) by physiological saline solution and kept under observation as non-vaccinated contact control group. All animals were kept under observation for 2 weeks post inoculation for detection of any abnormalities.

2.8.3. Potency test:

Potency evaluation of the combined inactivated respiratory viral vaccine adjuvant with Montanide oil ISA 206 vaccine was carried out according to **(11)**.

Potency evaluation was determined by immune response post vaccination over the permissible limit of protective level against each viral component of tested vaccine, as well as the duration of immunity.

Eight adult male cross-breed calves were used in this study and divided into 2 groups, four calves for each group:

Group I: Each calf was intramuscularly immunized with 5 ml of the locally produced oily prepared vaccine (BVD, IBR and PI-3) by two injections. This group was used for studying the duration of immunity.

Group II: Consists also of four calves and this group was left as non-vaccinated contact control group.

2.9. Serum samples:

Serum samples were collected from calves in three groups periodically examined for 12 months. The sera were inactivated at 56°C for 30 minutes, and then stored at -20°C till used in detection of specific antibodies for BVDV, BHV-I and PIV-3 using SNT.

2.10. Serum neutralization test (SNT):

It was performed on MDBK cell line using the micro technique as described by [**12**].

Fig (1): Neutralizing antibody titer of IBR virus in sera of calves vaccinated with combined inactivated respiratory viral vaccine adjuvant with Montanide oil ISA 206

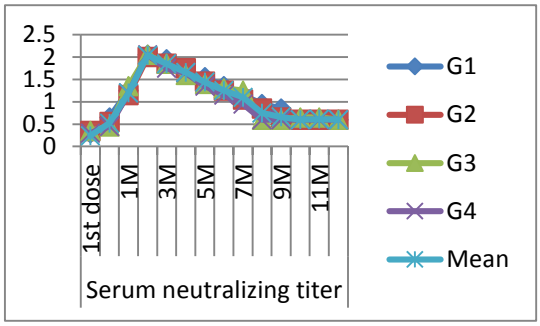


Fig (2) : Neutralizing antibody titer of BVD virus in sera of calves vaccinated with newly improved vaccine adjuvant with Montanide oil ISA 206

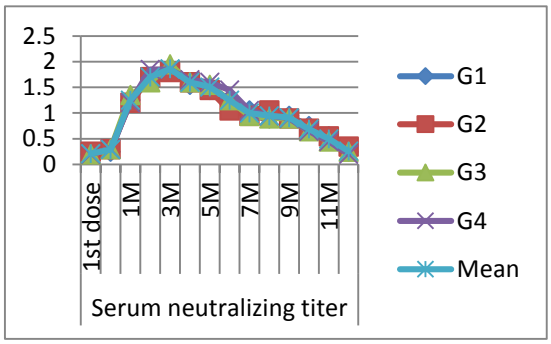
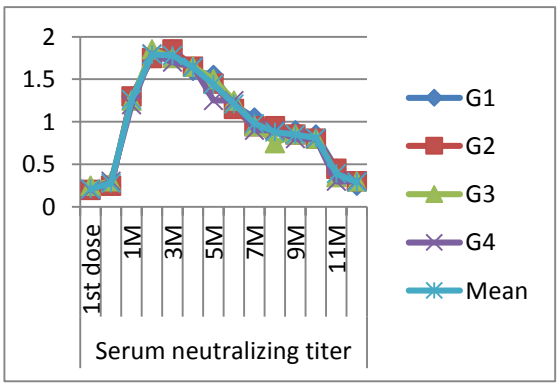


Fig (3) : Neutralization of antibody titer of PI-3 virus vaccinated with newly improved vaccine adjuvant with Montanide oil ISA 206



3. RESULTS

Table (1): Mean serum neutarlizing antibody titers in calves vaccinated with newly improved vaccine and preumo-3 vaccine:

Time post vaccination	*Group I*			*Group II*
	IBR	BVD	PI-3	
1st dose	0.24	0.21	0.21	Non vaccinated control group respond negatively lower than protection and ranged between 0.20-0.40
2nd dose	0.52	0.29	0.28	
1M	1.20	1.25	1.26	
2M	2.04	1.70	1.79	
3M	1.85	1.86	1.78	
4M	1.66	1.60	1.64	
5M	1.44	1.51	1.44	
6M	1.25	1.25	1.21	
7M	1.10	1.00	0.98	
8M	0.75	0.95	0.88	
9M	0.66	0.91	0.85	
10M	0.65	0.69	0.81	
11M	0.61	0.50	0.39	
12M	0.60	0.26	0.29	

First dose: 0 day of vaccination, Second dose: 14 days post vaccination for gel and 21 days for oil, Protective serum neutralizing (SN) antibody titre against BVD is 0.90 according to **(13)**, IBR is 0.60 according to **(14),** PI-3 is 0.60 according to **(15)**.

4. DISCUSSION

The appearance of respiratory disease may be due to stress factors as bad environment, transportation, accumulation of ammonia and excessively high humidity in closed areas which lower the resistance of animal which enhanced the multiplication of microorganisms **(16).** Infectious agents associated with bovine respiratory diseases including three viruses which are BVDV, IBRV and PIV-3 **(9).** Vaccination programs for breeding herds are integral parts of preventive health programs designed to lessen the effects of infectious respiratory diseases in cattle (**17**).

Oil adjuvant vaccines are commercially available for a wide variety of viral diseases. Oil emulsions trap antigen and release it over a larger period producing a more pronounced increase in the immune response after one dose than do alum adjuvant. Oil emulsions increase the circulation and trap of lymphocytes in draining lymphoid tissue as well as oil adjuvant may affect the immune response by enhancing the physical presentation of the antigen to macrophages (**18**). The present study was planned for preparation and evaluation of combined inactivated respiratory viruses' vaccines from: BVD, IBR and PI-3 viruses' adjuvant with Montanide oil ISA 206 for using in calves for controlling of such infectious diseases. Currently for evaluation of the prepared vaccine for purity, safety and potency testing. The purity testing of the prepared vaccine showed complete absence of any bacterial, fungal or mycoplasma contamination on inoculated media for 15 days post inoculation, at the same time, the results of purity test revealed that the prepared vaccine was also free from any infectious or extraneous viral contamination.

These results support that obtained of safety testing when applied on mice also, when calves vaccinated with 10 times of the detected vaccinal dose for safety test in calves and the results showed neither local nor systematic post vaccinal reaction, also, there is no development of any clinical signs or elevation of rectal temperature during the whole experimentation period. All the above mentioned results supporting the purity and safety of the prepared newly developed vaccine. All these results are go

in accordance with the results obtained by **(19)**.

The potency evaluation of the prepared newly developed vaccine in calves in table (1) and Fig. (1,2,3) results revealed that all vaccinated animals developed serum neutralizing antibody titres (SN antibody) when reached their peak at one month and remained stable higher than the minimal acceptable titre of protective level which lasts for 12 months post vaccination. Such data are similar to that obtained by **(13)** who recorded that the BVD antibody level of 1:8 dilution (log10 0.9) was protective, **(14) and (15).** Those authors reported that the minimal acceptable titre of neutralizing antibodies was 1:4 dilutions or 0.6 log10 was protective against PI-3 and IBR viruses.

Serum neutralizing antibody titers in group 1 which vaccinated with the vaccine adjuvanted with Montanide oil ISA 206 showed the highest level among all viruses at one month post vaccination. The titers remained stable in the protective level at 12 months for IBRV, 9 months for BVDV and 10 months for PI-3V then the titers began to decrease gradually until reach to the minimal protective level in all viruses. While the control non vaccinated group showed no neutralizing antibody response.

Now it is found the oil vaccine is good and the enhanced action observed with this 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 (**20**).

In conclusion, The prepared combined inactivated respiratory viral vaccine containing of BVD, IBR and PI-3 viruses and adjuvanted with Montanide oil ISA 206 gives a considerable and highly immunogenic in vaccinated calves. Combined inactivated vaccine adjuvanted with Montanide oil ISA 206, was proved to be pure, fully safe and potent.

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FACULTY OF VETERINARY MEDICINE
مجلة بنها للعلوم الطبية البيطرية

تحضير وتقييم لقاح الأمراض التنفسية الجماعي المثبط المحسن (النيمو-3) باستخدام زيت المانتونيد اى اس ايه 206

جبر فكرى الباجورى[1]، مجدى محمد على الصباغ[2] ، رشا إبراهيم أحمد الهوارى[2]

[1]قسم الفيرولوجيا – كلية الطب البيطرى – جامعة بنها، [2]معهد بحوث الامصال واللقاحات البيطرية بالعباسية–القاهرة

الملخص العربى

استهدف البحث محاولة تحضير لقاح محسن جامع مخمد يحتوى على فيروسات الاسهال البقرى الفيروسى (بى فى دى فيروس) والتهاب القصبة الهوائية الرغامى المعدى (أى بى ر فيروس) وفيروس البارا-أنفلونزا-3 (بى أى – 3 فيروس) ومحمل على زيت المانتونيد اى اس ايه 206 كعامل مساعد للتحفيز المناعى. تمت معايرة اللقاح المحضر لاختبارات النقاوة والأمان وايضاً اختبارات فاعلية اللقاح. وقد ثبت من هذه الدراسة أن اللقاح المحضر والمحمل على مادة زيت المانتونيد اى اس ايه 206 لقاح نقى تماماً وأمن تماماً لاستخدامه فى العجول. فتم تحصين المجموعة الأولى بلقاح المحضر و المحمل على زيت المانتونيد اى اس ايه 206. بينما تركت المجموعة الثانية كمجموعة غير محصنة وضابطة للتجربة . وجاءت نتائج تتبع الاستجابة المناعية باستخدام اختبار المصل المتعادل لتثبت ان المستوى المناعى للاجسام المضادة المتعادلة قد تكونت فى العجول التى تم تحصينها باللقاح المحضر لتعطى مستوى مناعى استمر حتى 12 شهر فى (اى بى ر فيروس)و9 اشهر فى (بى اى-3 فيروس) بينما استمر حتى 10 اشهر فى(بى فى دى فيروس). وفى الخلاصة يمكن القول أن اللقاح المحضر الجامع المثبط هو لقاح نقى وأمن وبفاعلية كاملة.

(مجلة بنها للعلوم الطبية البيطرية: عدد 24(2)، 2013 :116–122)