A Trial For Developing Of Inactivated Pneumo-4 Vaccine By Using Of An Immunostimulatory Complex (ISCOM)

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ABSTRACT

The current study was conducted for the developing the vaccinal product with patent name, Pneumo-4, a combined inactivated respiratory virus vaccine adjuvanted with al- hydragel using immunestimulatory complex (ISCOM) in the form of Quill-A saponin.

This study was performed by preparation of three formulae of Pneumo-4 vaccine (adjuvented with Al-hydra-gel) and pneumo-s (adjuvented with Quill-A saponin only) while the third was Pneumo-s gel. (adjuvented with Al-hydra-gel incorporated with Quill-A saponin).

For evaluation of the prepared vaccine formulae, testing of purity, safety as well as potency and using of laboratory animals and apparently healthy calves.

This study proved that the three formulae of the prepared vaccines were pure and fully safe to be used in calves without any local or systemic post vaccinal reaction.

Serological investigation using serum neutralization test and ELISA revealed that the vaccinated calves developed serum neutralizing antibody against all fractions of the prepared vaccine. This study proved that the prepared formulae of vaccines (Pneumo-s gel) giving a long lasting duration of immunity extends up to 30 weeks post vaccination and higher than of the locally produced pneumo-4 vaccine which giving up to 24 weeks only.

The best of choice is the vaccine formulae, which contains Al-hydra-gel participated with Quill-A saponin, as it is a balanced formulae and being pure, fully safe and highly potent and is recommended for field application for bovine.

INTRODUCTION

Bovine pneumo enteritis complex syndrome continuous to be a major enemy to beef and dairy industries of paramount economic importance because of losses and high death rates. So, respiratory and enteric diseases are often cited as the significant cause of economic losses probably more costly than all other diseases *(1,2)*.

Several viruses linked to development of Pneumo-enteric complex syndrome including, bovine viral diarrhea *(3)*, infectious bovine rhino-tracheitis *(4)* and Para influenza 3 viruses *(5)*.

Moreover, Bovine respiratory syncytial virus (BRSV) was also incriminated as a major viral, causing of respiratory disease in cattle with closal impact *(6)*.

In Egypt since the mid of last century, much attention was drawn to these viral infection as the most significant causes of great economic losses due to high mortalities, abortion and persistent infection, particularly in newly borne calves, prolonged feeding periods, severe weight loss and highly costs of treatment and prevention program *(7,8)*.

There is no another choice for using of vaccine for control of pneumo-entritis complex syndrome, where the vaccination is by far the most efficient and cost effective method for controlling such viral infections.

A locally combined inactivated respiratory virus vaccine, Pneumo-4 adjuvented with Al-hydra -gel is already produced. Aluminium hydroxide gel is a mineral salt adjuvant, but the using of adjuvant is to maximize the effectiveness of poorly immunogenic viral antigens and for obtaining a great enhancement of the immune response. The use of immunostimulatory complex in form of Quill-A saponin to incorporate the vaccine formulation for getting a maximum enhancement of the animal Immune response to vaccination.

So, the aim of this study was the upgrading of already produced pneumo-4 vaccine by using Quill-A saponin as an immunostimulatory complex incorporated with al-hydra-gel in a newly developed vaccine formula for enhancement the immunogenicity of viral antigens for combating and controlling such viral infection with strong immune response and long lasting immunity.

MATERIAL AND METHODS

Viruses

Bovine viral diarrhea Virus (BVDV)

Egyptian Bovine Viral Diarrhea Virus "Iman-strain" with a titer of 7 logs $_{10}$ $TCID_{50}$ /ml.

It was firstly isolated at Tahrir province *(9)*.

Infectious Bovine Rhinotracheitis (IBR)

A reference Egyptian strain of Infectious Bovine Rhinotracheitis Virus (IBR) "AbouHammad Strain ", with a titer of 8 log_{10} $TCID_{50}$ /ml, was isolated and identified *(4)*.

Para-Influenza 3 Virus (PI-3)

A reference Egyptian strain of Para-Influenza 3 "strain 45" with a titer of 8 log_{10} $TCID_{50}$/ml, it was firstly isolated and identified *(10)*.

Bovine Respiratory Syncytia Virus (BRSV)

Bovine Respiratory syncytia Virus (BRSV) strain 375L, with a titer of 6 log_{10} $TCID_{50}$/ml was kindly supplied by Smith Kline Beecham, Animal health Nordon laboratories, U.S.A.

All viruses were propagated and adapted, and titrated on Madin Darby Bovine Kidney (MDBK) cell culture. MDBK cell line has been proved to be free from any adventitious agent, especially non-cytopathic strain of bovine viral diarrhea virus (BVDV) by using of Infectivity method *(11)*.

All viruses were gently supplied by Rinder Pest like Diseases Research Department, Veterinary Serum and Vaccine Research Institute (VSVRI), Abbassia, Cairo.

Inactivators

Ten percentage of Binary Ethylene amine (BEI) was used as virus inactivators.

It was prepared by 99% of 2-bromethylene amine hydrobromide (BEI) (Br CH_2CH_2 NH_2 HBr) of molecular weight 204.9 dissolved in alkaline solution of sodium hydroxide (NaOH) N/15 and incubated at 37°Cin water bath for 1 hour, it was used as 1 N/ virus inactivator in vaccine preparation.

It was obtained from Panreac, Sintesia, Quimica, SA. and was used according to previous method *(12)*.

Sodium thiosulphate

Sodium thiosulphate pure pentahydrate ($Na_2S_2O_3$-$5H_2O$)–Lobachmie-PVT.LTD, Mumbai-India, It was prepared as 20% solution and added to the inactivated virus solution to stop the action of BEI.

Merthiolate (Thiomersal)

It was used as vaccine preservative, used as (0.1 % in double distilled water) after

sterilization by autoclaving. It was used as a final concentration of 1:10,000.

It was obtained from park scientific limited north Hampton, M.K, ($C_2H_5Hg.S.C_6H_4COONa$).

Adjuvants

Aluminium hydroxide gel (Al-hydra- gel)

Al-hydro-gel (2%gel Hanil limited London U.K.)

It was used at 30% final concentration.

Quil-A Saponin

It was obtained as a powder from Super FosBiosector a/s Denmar /T and used as 10% solution in double distilled water in vaccine preparation ***(13,14)***. It was kept overnight at 4 °C then filtered through Seitz filter (EKs) and used as 0.34 mg/1ml of vaccinal dose.

Preparation of combined inactivated respiratory viruses' vaccine

The prepared combined inactivated vaccine, containing of BVDV, IBR, PI-3 and BRSV, was prepared ***(15)***.

Virus inoculation

All viruses were initially propagated for 5 successive passages in Madin Darby Bovine Kidney (MDBK) cell culture. Each virus suspension was harvested when inoculated cell culture showed 70% Cyto-Pathic Effect (CPE), by rapid freezing and thawing at 37°C ***(16)***.

Virus inactivation

Each harvested virus was inactivated by addition of 10% of 0.1 molar of Binary Ethylene Imine (BEI) with stirring at 37°C for optimum times of each virus ***(12, 15, 17,)***.

Then adding sodium thiosulphate 20% with a final concentration 2% to stop the action of BEI. The inactivated viruses was mixed in equal volumes under aseptic condition so that each vaccinal dose should contain at least the following titers:

BVD: $10^6 TCID_{50}$/ml, IBR: 10^6 $TCID_{50}$/ml, PI-3: 10^6 TCID50/ml and BRSV: $10^{5.5}$TCID50/ml according to previously recorded method ***(18)***.

Vaccines formulation

Three types of vaccine formulation were used as follows:-

Formulated vaccine, adjuvanted with Al-hydra-gel

The vaccinal virus's suspension was mixed at together with 30% of aluminum hydroxide gel, Al-hydra-gel solution and stirred on a magnetic stirrer for obtaining a homogenized solution and termed as pneumo-4 gel vaccine.

Formulated inactivated respiratory viruses vaccine with Quil-A saponin

It was carried out by adding of Quil-A saponin to the fore mentioned mixed inactivated respiratory viruses vaccine ,adjuvanted at a concentration of 0.34mg Quil-A saponin per ml of vaccinal dose according to previously cited method ***(13,19)***, this formulated vaccine termed as pneumo-4-S.

Formulated inactivated respiratory viruses vaccine with Al-hydra-gel and Quil-A saponin

Addition of Quil-A saponin to previously formulate inactivated respiratory viruses' vaccine adjuvanted with Al-hydra-gel at a concentration of 1 mg saponin per vaccinal dose (1ml of vaccinal dose following the cited method ***(13, 19)***.

Then the pH was adjusted to 7.9 and thiomersal was added as vaccine preservative at a final concentration 0.001% for each of the fore mentioned formulated vaccines. The final vaccine product was aseptically distributed in sterile bottles of 100ml capacity, (of 20 vaccinal doses) then capsulated and labeled.

Evalution of the prepared combined inactivated respiratory viruses' vaccine formula

Purity test (sterility test)

Product testing numbers 113-26, 27, 30 for testing the freedom of the three formulae of combined inactivated respiratory viruses vaccine, was done (tabled) by culturing random samples of such vaccine formula on tryptose

phosphate broth, thioglycolate media, Sabaroud agar media and PPLO media.*(18)*.

Safety test

In laboratory animal

It was conducted in adult albino Swiss mice of total number 30 mice {injected intraperitonil (I/P) or intracranial (I/C) with 0.1ml dose for each} and guinea pigs injected (I/P) with 0.5ml dose for each *(20)*. All animals were kept under observation for 15 days post inoculation for detection of any abnormalities (table 2).

In the original host (calves)

Safety tests in calves was achieved using eight apparent healthy male adult calves free from antibodies against the viruses included in vaccine. Each formula of the prepared combined inactivated Respiratory vaccine (pneumo- 4 gel and pneumo-4-s and pneumo-4-s gel), two calves were used for each formula, inoculated intramuscularly with 10 times of the vaccinal dose (50ml), *(18)* of product testing code number 113:41, two calves were inoculated with 50ml of physiological saline solution at the same time and kept under strictly observation as non-vaccinated control.

All animals were kept under observation for 2 weeks post inoculation.

Potency test

Potency evaluation of the prepared combined inactivated Respiratory Viruses vaccine was carried out *(21)*.Total of twelve, cross. Breed (local and fresian. Holeshtein hybrid).Adult male apparently healthy calves of around 9 months old, were used in this study, all calves were housed in a special isolator and free from antibodies (Veterinary Serum and Vaccine Research Institute).

The animals were randomely assigned into 4 groups each group.consists of 3 calves as follow:

Group 1: Pneumo -4 vaccinated group

Each calf of this group was vaccinated by I/M inoculation with 5ml pneumo-4 vaccine (locally produced combined inactivated Respiratory Virus Vaccine adjuvanted with aluminum hydroxide gel, by two doses, two weeks apart.

Group 2: Pneumo- 4 S vaccinated group

Each calf of this group, was vaccinated with combined inactivated Respiratory viruses vaccne adjuvant with Quil-A saponin, with 5ml inoculated I/M in 2doses two weeks apart.

Group 3: Pneumo-4 S gel vaccinated group

Each calf of this group was vaccinated with prepared combined inactivated Respiratory viruses vaccine adjuvant inactivated Respiratory viruses vaccine adjuvant with the participated al. hydra-gel with Quil-A saponin, and inoculated with 5ml I/M by 2 doses 2 weeks apart as in group 1 and 2.

Group 4: Non vaccinated control group

This group consisted of three calves, kept as a non vaccinated contact control group.

Sampling

Blood samples were obtained from all groups for serum separation before vaccination (Zero time), then at the day of booster vaccination (2weeks post 1st vaccination, then at 2 weeks regular intervals for 36 weeks. Sera were collected and inactivated at $56^{o}C$ for 30 minutes in a water bath to destroy most of its viral inhibitory activity, then stored at - $20^{0}C$ until assayed for evaluation of immune responses and duration of immunity.

Serological investigation

Serum neutralization tests (Beta procedure)

Serum neutralization test was used for detection of specific neutralizing antibodies against each antigen fraction contained by the formulae of the prepared vaccines. S.N.T was carried out using the micro-titer technique (Beta procedure) *(22, 23)*. The serum neutralizing antibody titers of the tested sera were expressed in $\log_{10}$ $TCID_{50}$/ml following the calculation formula *(24)*.

Enzyme linked Immunosorbent assay

It was performed as described previously *(25-27)*.

RESULT AND DISCUSSION

Bovine viral diarrhea virus (BVDV), Bovine herpes virus-type 1 or Infection bovine rhinotrachetitis virus (IBR), para-influnza-3 and bovine respiratory syncytial (BRSV) virus have been all incremented in the etiology of Pneumoentritis complex syndrome which is considered a great problem in cattle industry as proved in Egypt *(32-34)*.

So, vaccination is by fact, the most efficient and cost effective method for controlling infectious diseases particularly viral diseases. In addition the use of vaccine, which continue till now to be a basic tool to reduce the risk of infection, basically it produces a prophylactic broad spectrum of protection against a range of viral agents, and high level of specific antibodies prior to infection may ensure reduced risk of disease for obtaining life survival of animals *(35)*.

In fact, the ideal vaccine to be used, should be cheap, stable and available for mass vaccination, at the same time it must be safe, sterile, potent and should be free of any adverse side effect either locally at the site of inoculation or systemically.

In addition to the requirement listed above, the ideal vaccine for active immunization should therefore induce strong immunization with long lasting duration of prophylactic protection. This required part of an ideal vaccine, entirely dependent on the choosing of adjuvant *(36, 37)*.

So the aim of this work was the using of advanced innovative immunostimulatory complex Quil-A sponin plus al-hydra-gel to upgrade of locally produced and registered inactivated combined respiratory viruses vaccine (pneumo-4).

Regarding, the evaluation of the prepared formulae of the combined inactivated vaccine, in the form of pneumo-4 (vaccine adjuvanted or pneumo-s formula adjuvanted with saponin only) and finally pneumo S- gel (formulae of vaccine adjuvanted with Quil-A saponin pluse alhydra-gel). All formulae of vaccines were tested *(18, 20)*.

Purity test of the prepared 3 formulae of vaccines (Table 1) showed complete absence of any bacterial, fungal or mycoplasma contamination. Also, the results purity tests revealed that the prepared 3 vaccines were also free from any extraneous viral contamination. Table 2 showed the safety of the different formulae of the vaccine showed neither local nor systemic post vaccinal reaction. When inoculated with 10 times the vaccinal dose were obtained. All these results come in agreement with those obtained by several researchers *(38-40)*.

Regarding the potency evaluation of the prepared vaccine formulae, the estimation of the immune response was carried out by serological tests includes serum neutralization test (Table 3) and ELISA test (Table 4).

The obtained results in Table 3, showed that the mean serum neutralizing antibody titers developed in vaccinated calves with the prepared vaccine formulae, as in group 1 which vaccinated by pneumo-4 vaccine (adjuvanted with Al.hydra.gel).

The protective level of SNT titer start from 4 weeks post 1st vaccination and reach the peak at the 8 2.0, 2.20, 2.20 and 2.0, As expressed in log10 against BVD, IBR, PI-3 and BRSV respectively, in calves vaccinated with locally produced pneumo-s gel vaccines (group 3).

While protective SNT titer start from 4 weeks post 1st Vaccination and reach the peak at the 8 1.85, 1.90, 1.90 and 1.75 as expressed in log10 against BVD ,IBR ,PI-3 and BRSV respectively, in calves vaccinated with locally produced pneumo-s vaccines (group 2)

Otherwise the protective of SNT titer start from 4 weeks post 1st Vaccination and reach the peak at the 8 2.00, 2.20, 2.05 and 2.00.. as expressed in $\log_{10}$ against BVD, IBR, PI-3 and BRSV respectively in calves vaccinated with locally produced pneumo-4 vaccines (group 1) the current findings was also obtained by several authers *(41,42)*.

Similar magnitude of SN antibody responses to BVD, IBR, PI-3 and BRSV was

obtained sufficiently to protecte and combate of infection of such viruses of calves vaccinated with locally produced pneumo-4 vaccines *(15,43)*, but its peak reached after 6 weeks post vaccination and remained for more than 6 months post vaccine. Regarding to the duration of immunit, induced by the pneumo-S gel vaccine, it lasts for 8 months post vaccination.

Similar results were obtained by ELISA (Table 4) which showed the highest level of S/P ratio (2.60, 2.50, 2.40 and 2.60) in cattle vaccinated with Pneumo-S gel by the 8 while other groups showed S/P ratio (2.20, 2.25, 2.15, 2.10 and 2.00, 1.90, 1.90, 1.75) in cattle vaccinated with Pneumo-4 and Pneumo-S respectively.

From the above results, the use of Pneumo-s-gel vaccine prolonged the period of immunity about 6 weeks over the period of immunity induced by Pneumo-4 vaccine.

So, it could be concluded that the incorporation of Quil-A saponin with Al.hydra-gel as immunostimulant complex (ISCOM) develops strong immune response with long lasting duration immunity up to more than 30 weeks post vaccination and is the best of choice for using in field application.

Table 1. Sterility of the different prepared inactivated respiratory viruses vaccine formulae

Vaccine formula	Used media			
	Tryptose phosphate broth at 37°C for 7 days	Thioglycolated media at 37°C for 7 days	Sabaraud's media at 25°C for 7 days	PPLO (Mycoplasma media)
Pneumo-4-gel adjuvanted with Al.hydra.gel	No growth	No growth	No growth	No growth
Pnemo-4-S adjuvanted with saponin	No growth	No growth	No growth	No growth
Pneumo-4-S-gel adjuvant with Quil-A saponin	No growth	No growth	No growth	No growth

Table 2. Safety tests

Observed	Animal		
	Laboratory animals		Original host (calves)
	Mice	Guinea pig	
Body temperature	-	Negative	38.5^0C
Local reaction	Negative	Negative	Negative
Systemic reaction	Negative	Negative	Negative
Clinical signs	Negative	Negative	Negative

Table 3.Mean serum neutralizing antibody titers against BVDV, IBR, PI-3 and BRSV in sera of calves following vaccination with pneumo-4, Pneumo-S and Pneumo-S gel vaccines

WPV	Mean serum neutralizing antibody titres expressed in log 10											
	Pneumo -4				Pneumo-S				Pneumo-S gel			
	BVDV	IBR	PI3	BRS	BVDV	IBR	PI3	BRS	BVDV	IBR	PI3	BRS
0day	0.35	0.30	0.20	0.30	0.2	0.3	0.3	0.25	0.3	0.30	0.30	0.25
2	0.55	0.60	0.65	0.40	0.5	0.6	0.6	0.55	0.70	0.80	0.75	0.70
4	1.55	1.70	1.77	1.30	1.2	1.50	1.50	1.40	1.60	1.70	1.45	1.55
6	1.90	2.1	2.05	1.90	1.40	1.60	1.70	1.90	2.00	2.20	1.90	2.00
8	**2.00**	**2.20**	**2.05**	**2.00**	**1.85**	**1.90**	**1.90**	**1.75**	**2.10**	**2.20**	**2.20**	**2.00**
10	2.00	2.05	2.00	1.90	1.75	1.70	1.70	1.60	2.00	2.10	2.00	1.90
12	1.85	1.95	1.90	1.90	1.45	1.50	1.40	1.40	1.90	2.00	1.80	1.90
14	1.65	1.77	1.60	1.60	1.20	1.25	1.30	1.25	1.85	2.00	1.90	1.80
16	1.50	1.60	1.55	1.40	0.95	1.00	1.10	1.15	1.65	1.90	1.80	1.45
18	1.40	1.50	1.45	1.40	0.90	0.70	0.65	0.70	1.50	1.70	1.70	1.75
20	1.33	1.30	1.30	1.32	0.90	0.60	0.55	0.55	1.40	1.70	1.75	1.65
22	1.15	1.20	1.15	1.05	0.60	0.50	0.40	0.30	1.40	1.60	1.50	1.45
24	0.90	0.90	0.75	0.70	0.60	0.30	0.30	0.30	1.25	1.45	1.45	1.35
26	0.60	0.70	0.65	0.55	0.30	0.25	0.30	0.20	1.15	1.30	1.15	1.25
28	0.30	0.30	0.30	0.40	0.25	0.30	0.30	0.20	0.90	0.90	0.85	0.90
30	0.25	0.30	0.30	0.25	0.30	0.30	0.20	0.25	0.90	0.70	0.70	0.65
32	0.30	0.30	0.20	0.25	0.30	0.30	0.25	0.20	0.60	0.45	0.40	0.40
34	0.30	0.30	0.30	0.20	0.20	0.25	0.30	0.30	0.30	0.30	0.25	0.30
36	0.30	0.25	0.20	0.30	0.30	0.25	0.30	0.30	0.30	0.30	0.30	0.30

WPV= week post vaccination
*= just before 1st vaccination.
**= 2nd dose of vaccination.
Control group showed no protection neutralizing antibody titers.
N.B.
Minimum acceptable titer of protective level expressed in $\log_{10}$ antibody against
BVDV = 0.9 *(28)*.
IBR = 0.6 *(29)*.
PI-3 = 0.6 *(30)*.
BRSV = 0.6 *(31)*.

Table 4. Mean S\P ratio of ELISA to BVDV, IBR, PI-3 and BRS antibodies in sera of calves following vaccination with pneumo-4, pneumo-S and Pneumo-S gel vaccines

WPV	Mean S\P ratio of ELISA											
	Pneumo -4				Pneumo-S				Pneumo-S gel			
	BVDV	IBR	PI3	BRS	BVDV	IBR	PI3	BRS	BVDV	IBR	PI3	BRS
0 day*	0.20	0.10	0.20	0.10	0.10	0.15	0.10	0.10	0.15	0.10	0.30	0.25
2**	0.60	0.70	0.65	0.50	0.70	0.65	0.50	0.60	0.70	0.60	0.75	0.70
4	1.70	1.75	1.80	1.50	1.50	1.55	1.60	1.65	1.80	1.70	1.75	1.75
6	2.00	2.20	2.15	1.95	1.70	1.65	1.75	1.80	2.20	2.10	1.90	2.00
8	**2.20**	**2.25**	**2.15**	**2.10**	**2.00**	**1.90**	**1.90**	**1.75**	**2.60**	**2.50**	**2.40**	**2.60**
10	2.30	2.15	2.20	2.00	2.15	1.70	1.70	1.60	2.70	2.60	2.70	2.55
12	2.10	1.90	2.00	1.95	1.95	1.80	1.70	1.80	2.60	2.40	2.65	2.50
14	2.00	2.25	1.20	2.10	1.80	1.85	1.70	1.65	2.50	2.45	1.50	1.40
16	1.90	1.85	1.95	1.70	1.75	1.70	1.60	1.65	2.20	2.10	2.10	2.10
18	1.70	1.60	1.75	1.60	1.60	1.40	1.65	1.50	1.90	1.85	1.80	1.95
20	1.50	1.10	1.20	1.10	1.20	0.10	1.15	1.15	1.70	1.75	1.65	1.60
22	1.20	1.10	1.25	1.15	1.00	0.90	0.90	0.85	1.40	1.45	1.40	1.45
24	1.00	0.90	0.95	0.90	0.85	0.70	0.60	0.75	1.30	1.30	1.35	1.25
26	1.00	0.90	0.95	0.85	0.60	0.45	0.50	0.60	1.20	1.10	1.15	1.15
28	0.70	0.70	0.65	0.75	0.55	0.50	0.50	0.40	1.00	1.10	0.85	0.90
30	0.65	0.60	0.60	0.55	0.50	0.40	0.50	0.30	1.00	0.90	0.90	0.95
32	0.50	0.50	0.45	0.50	0.45	0.40	0.35	0.30	0.90	0.85	0.80	0.85
34	0.40	0.40	0.35	0.30	0.30	0.15	0.20	0.20	0.60	0.50	0.55	0.60
36	0.35	0.30	0.25	0.25	0.20	0.15	0.10	0.20	0.40	0.35	0.40	0.40

*= just before 1st vaccination.

**= 2nd dose of vaccination.

N.B:

Serum positive ratio (S/P) ≥ 1.0 is considered positive.

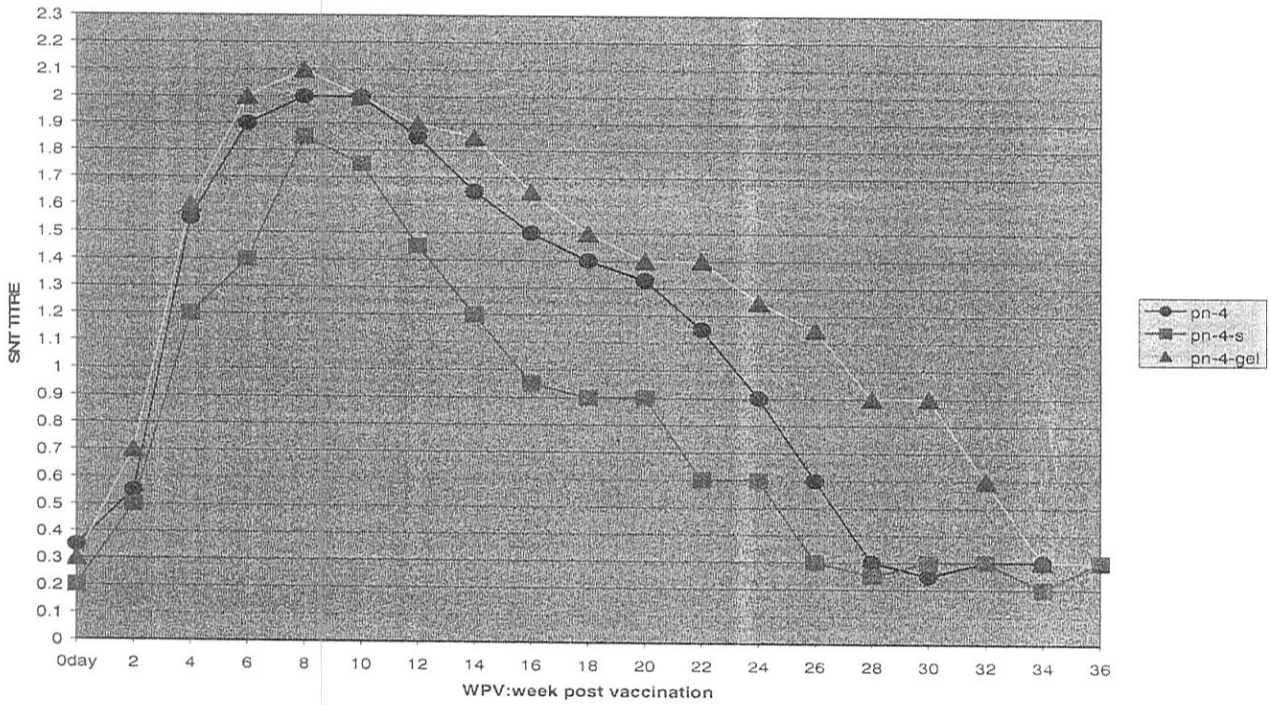

Fig.1. Mean SNT against BVDV in sera of Calves

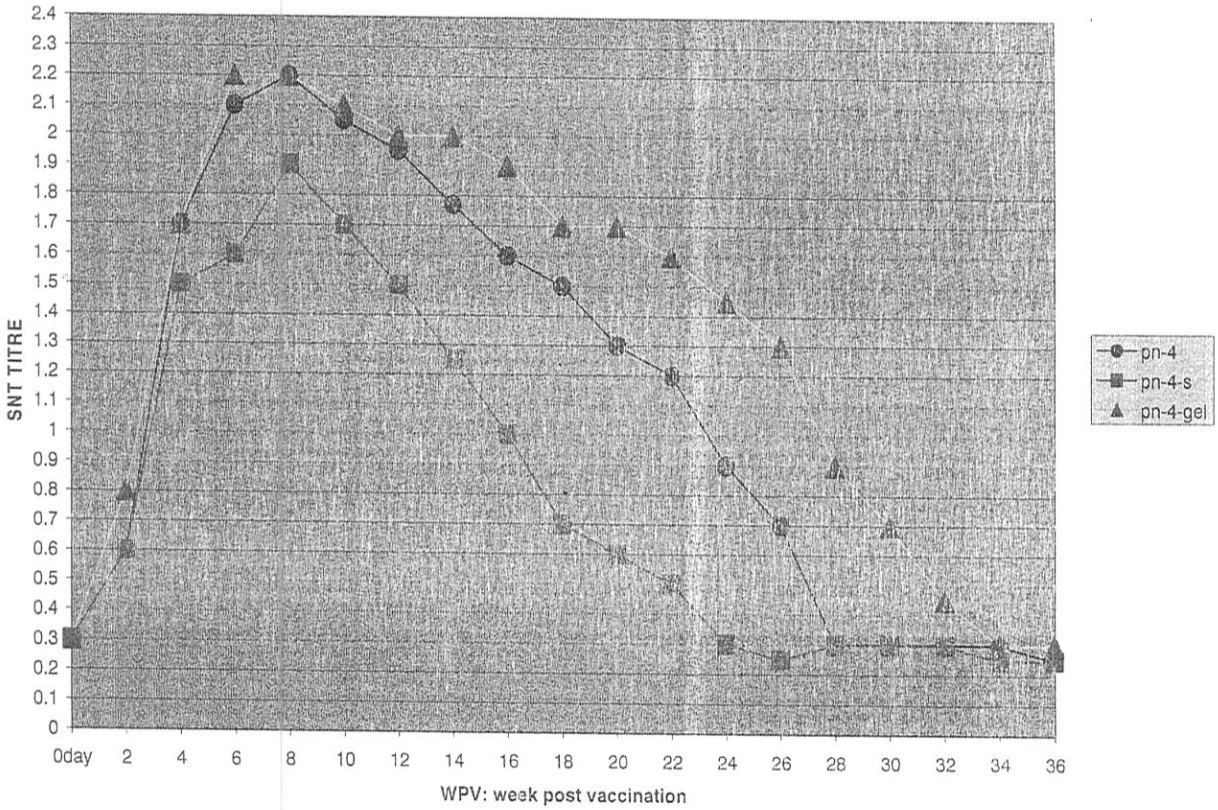

Fig. 2. Mean SNT against IBR in sera of Calves

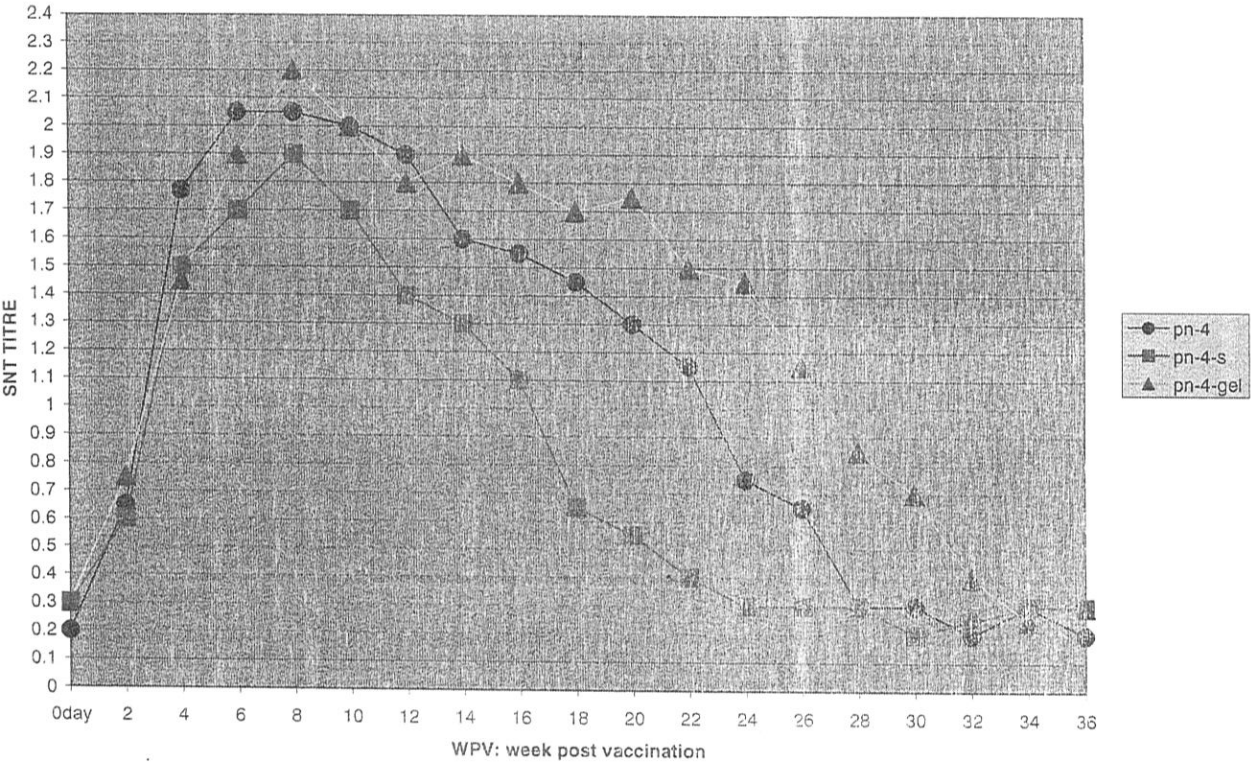

Fig. 3. Mean SNT against PI-3 in sera of Calves

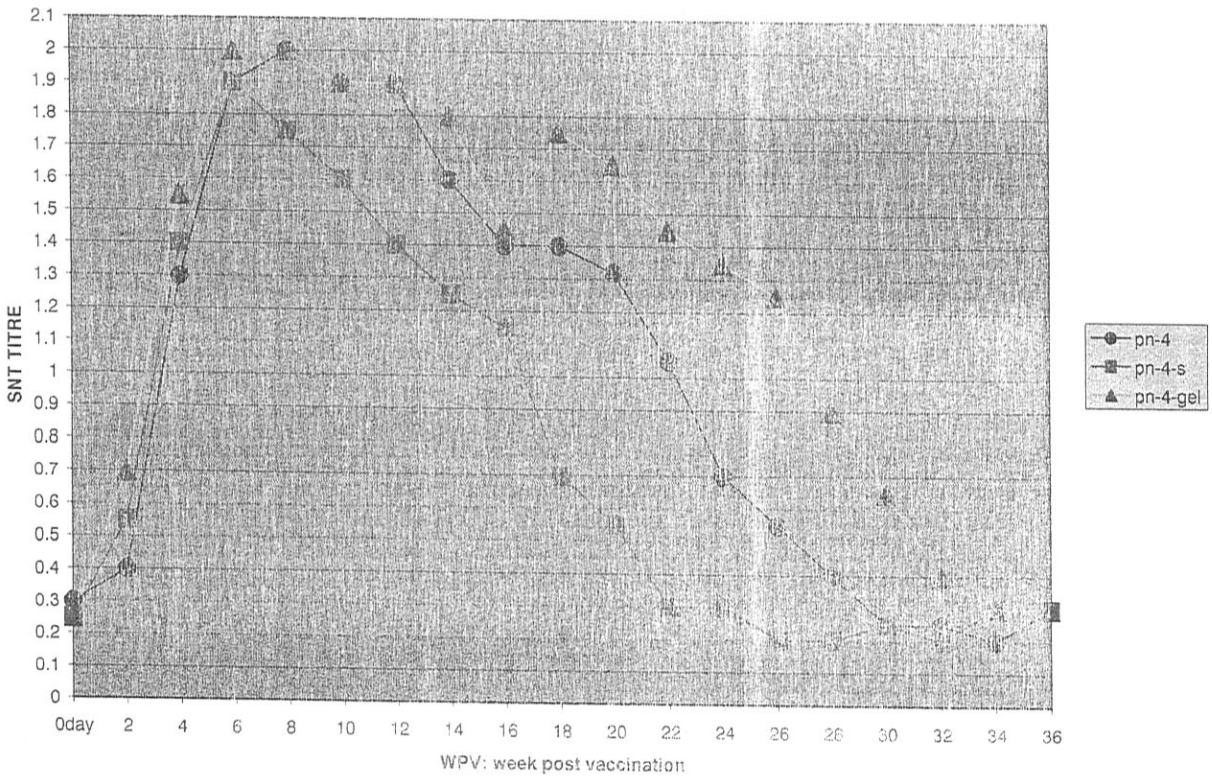

Fig. 4. Mean SNT against BRS in sera of Calves

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الملخص العربى

محاولة لتطوير لقاح الأمراض التنفسية الفيروسية المثبط (نيمو-٤) باستخدام مركب التحفيز المناعى "ISCOM"

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تمت هذه الدراسه لتطوير لقاح منتج يحمل الاسم التجارى نيمو-٤ وهو لقاح مثبط جامع للفيروسات التنفسيه ومحمل على الالمونيوم هيدروكسيد جيل كعامل مساعد وذلك باستخدام عامل مساعد مركب للتحفيز المناعى يحتوى على مادة الكويل السابونيني (ISCOM) بالإضافه الي الجيل.

وقد تم تحضير ثلا ثة اشكال من اللقاح وهي :- الشكل المنتج حالياً وهو لقاح محمل على الهيدرا جيل (النيمو-٤) والشكل الثانى باستخدام مادة الكويل السابونيني كمحفز مناعى تحت اسم (النيمو-s) وايضا الشكل الثالث وذلك باستخدام مادة الكويل السابونيني بالاضافه الي الالمونيوم هيدروكسيد جيل عليه تحت اسم (النيمو-s جيل).

وقد تم معايره الثلاثه أشكال من اللقاح المحضر لإثبات نقاوة و سلامة وفاعلية اللقاحات الثلاثه وذلك باستخدام الحيوانات المعمليه والعجول الذكور السليمه ظاهريا، واثبتت هذه الدراسه أن الثلاثة اشكال من اللقاح المحضر هى نقيه وخاليه من الاجسام المناعيه للفيروسات المكون منها اللقاح وآمنه تماماً عند استخدامها للتحصين فى العجول بدون اى رد فعل للتحصين سواء موضعياً او كلياً. وبالفحص السيرولوجى باستخدام اختبار المصل المتعادل والإليزا للثلاثة اشكال من اللقاح المحضر، وجد انها قامت بتحفيز الجهاز المناعى للحيوان وانتجت اجساماً نوعية مضادة لكلا من الفيروسات المحتوى عليها اللقاح.

اما عن طول الفتره المناعيه لكل شكل من اشكال اللقاح المحضر فقد أثبتت هذه الدراسه أن اللقاح المحضر و المحمل على الهيدرا جيل مع السابونين قد أعطى فتره من المناعه تصل الى ٣٠ اسبوع بعد التحصين وهى فتره اطول من الفتره التى يعطيها اللقاح المنتج محليا عند استخدامه وهى لا تزيد عن ٢٤ اسبوع.

وعلي ذلك يمكن القول بتفضيل اللقاح المحضر باستخدام الهيدرا جيل والكويل السابونيني وينصح بإنتاجه واستخدامه فى الحقل لتحصين الماشية.