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## **IMMUNOLOGICAL STUDIES ON SIMULTANEOUS VACCINATION WITH PNEUMO-3 AND PPR INACTIVATED VACCINE IN SHEEP**

(With 3 Tables)

By

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**دراسة مناعية للتحصين المتزامن لكلاً من لقاح النيمو-٣ و لقاح طاعون  
المجترات الصغيرة المثبط في الأغنام**

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في هذه الدراسة تم استخدام اثني عشر من الغنم قابلة للتأثير للعدوى بفيروسات مرض التهاب القصبة الهوائية الرغامى المعدى ومرض الأسهال البقري الفيروسي ومرض البارأنفلونزا-٣ فيروس وايضاً فيروس مرض طاعون المجترات الصغيرة فتم تقسيمهم عشوائياً الى اربعة مجموعات تتكون كل مجموعة من ثلاثة أغنام. تم تحصين المجموعة الأولى بطريقة التحصين المتزامن بلقاحي (النيمو-٣ و لقاح طاعون المجترات الصغيرة المثبط). بينما تم تحصين المجموعة الثانية بلقاح النيمو-٣ ثم بعد ١٥ يوم تم تحصينها بلقاح الأخر. وفي المجموعة الثالثة تم تحصينها اولاً بلقاح طاعون المجترات الصغيرة ثم بلقاح النيمو-٣ بعد ١٥ يوم. وتركت المجموعة الرابعة بدون تحصين كمجموعة ضابطة للتجربة. وقد أظهرت النتائج أن كل الحيوانات المستخدمة في التجربة استمرت سليمة اكلينيكيًا طوال فترة التجربة. كما أظهرت النتائج السيرولوجية وجود استجابة مناعية كافية لحماية الحيوانات من العدوى بهذه الفيروسات في المجموعات الثلاثة المحصنة وقد أظهرت النتائج بوضوح سلامة وكفاءة اللقاح مناعياً في هذه التجربة كما أظهرت النتائج عدم وجود أى منافسة بين مكونات اللقاحين سواء بالتزايد او بالتضاد بين كل من لقاح النيمو-٣ و لقاح طاعون المجترات الصغيرة المثبط.

### **SUMMARY**

A total of twelve seronegative sheep, against infectious bovine rhinotracheitis (IBR), bovine viral diarrhoea (BVD) and parainfluenza-3 (PI-3) and peste des petits ruminants (PPR) viruses were randomly divided into four groups (each group consists of 3 sheep). The first group, received simultaneous vaccination with pneumo-3 and inactivated PPR vaccines, while the second group received pneumo-3 vaccine and after 2 weeks that received inactivated PPR vaccine. The third group

received firstly the inactivated PPR vaccine, then received inactivated pneumo-3 vaccine after 2 weeks, while, the fourth group was kept as non-vaccinated control group. All tested animals remained clinically normal throughout the whole experimental period. Very satisfactory serological responses were detected in animals, simultaneously vaccinated with both vaccines. No differences were detected in serum neutralizing antibody titres against IBR, BVD, PI-3 and PPR viruses, in sheep which received inactivated PPR vaccine, neither before nor after vaccination with pneumo-3 vaccine. The results clearly indicated the safety and efficacy of simultaneous vaccination of sheep with pneumo-3 and inactivated PPR vaccine without any competition for the response to individual vaccine. Also there is neither synergistic nor antagonistic effect for the two vaccines in sheep.

**Key words:** *Pneumo-3vaccine, inactivated PPR vaccine, sheep*

## INTRODUCTION

Respiratory virus affections as bovine viral diarrhoea (BVD), infectious bovine rhinotracheitis (IBR) and parainfluenza-3 (PI-3) represent the most important and drastic upset among fattening sheep and are widely spread throughout the world. These affections constitute major causes of morbidity and mortality rates in feed lot sheep despite the high prevalence and economic importance of respiratory virus affection (St. George, 1971, Liess *et al.*, 1983).

On the other hand, peste des petits ruminants (PPR) is an acute highly contagious viral disease of goats and sheep, caused by Morbillivirus (paramyxoviridae family) and is closely related to but quite distinct from rinderpest virus (Gibbs *et al.*, 1979), PPR has been reported to occur in Egypt in limited outbreaks (El-Sanousi *et al.*, 1989; Ismail *et al.*, 1990 and El-Alloway *et al.*, 1993 and Mouaz *et al.*, 1995). Hence, the prepared inactivated PPR vaccine to be used in this investigation was undertaken by Mirchamsy *et al.* (1974), who succeeded in preparation of killed vaccine of cell-cultured rinderpest virus, also, Scot and Witcomb (1958) who compared vaccines produced by inactivation of a suspension of rinderpest infected bovine spleen using of 1% formalin and Provost *et al.* (1969) who tested cell cultured rinderpest virus inactivated with B. propiolactone in oil adjuvant. Although, vaccination against PPR is not practiced in Egypt.

At the same time, probable future outbreaks of PPR virus might cause great losses in sheep and goat population, also, vaccination is considered a basic tool for control of infectious viral diseases (Hansen *et al.*, 1995) other than eradication nor medication.

The increasing number of vaccines which administered to the animals at any age and at any time without accurate programs of vaccination makes it necessary to search the effect of the inactivated PPR vaccine on safety and efficacy of combined inactivated respiratory virus vaccine (pneumo-3 vaccine) which containing BVD, IBR and PI-3 viruses and the potential for simultaneous vaccination is being greater because of higher quality of vaccines produced.

Efforts, funds and time would be saved at launching vaccination campaign for more than one disease, provided the protective response to each vaccine is not impaired.

The aim of this work is to study the effect of inactivated PPR vaccine on BVD, IBR and PI-3 antibody titres by vaccination of sheep with the prepared inactivated PPR vaccine, before, simultaneously and/or after vaccination with combined inactivated respiratory viruses vaccine (pneumo-3).

## **MATERIALS and METHODS**

### **1. Viral strains:**

#### **1.1. BVD virus:**

Egyptian bovine viral diarrhoea virus (Iman strain) with a titre of  $7 \log_{10}$  TCID<sub>50</sub>/ml, firstly, isolated and identified by Baz (1975).

#### **1.2. IBR virus:**

A reference Egyptian strain of infectious bovine rhinotracheitis virus (Abou Hammad strain) with a titre of  $8 \log_{10}$  TCID<sub>50</sub>/ml, was firstly isolated by Hafez *et al.* (1976).

#### **1.3. PI-3 virus:**

A reference Egyptian strain of parainfluenza-3 virus (strain 45) with a titre of  $8 \log_{10}$  TCID<sub>50</sub>/ml which was isolated and identified by Singh and Baz (1966).

All virus entities were propagated and titrated on Madin Darby bovine kidney (MDBK) cell culture, which has been proved free of any adventitious agents. These viruses were supplied by Rinderpest-Like Disease Research Dept., Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo.

#### **1.4. PPR Virus:**

A local cell culture, live attenuated PPR virus (Samia *et al.*, 2000) was supplied by the Department of Rinderpest Research, Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo. It was used for preparation of inactivated PPR vaccine which used for experimental animal vaccination and SNT.

All live virus strains were used to estimate the induced antibodies in vaccinated sheep using SNT.

**2. Animals:** A total of twelve (12) IBR, BVD, PI-3 and PPRV-susceptible, clinically healthy sheep of local breed of about 4-6 months old, were used in this study. These sheep were housed in the same experimental facilities at Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo.

Blood samples were collected for serum preparation and tested to be free from antibodies against BVD, IBR, PI-3 and PPR viruses prior to vaccination.

**3. Inactivants:** 2-bromomethyl ammonium bromide ( $C_2N_7Br_2$  N, Panneac, Qumica, SA Barcelona, Spain) was used for preparation binary ethyleneimine (BEI).

**4. Sodium thiosulphate:** It was obtained from Difco Laboratories and it was prepared as 20% solution. It was used to stop the action of BEI.

**5. Alhydrogel solution:** Aluminium hydroxide 2% gel (Honil, limited, London, UK). It was used as stabilizing adjuvant in vaccine preparation. It was sterilized by autoclaving for 30 minutes at 121°C, then aliquoted in bottles and stored at 4°C.

**6. Thiomersal (Merthiolate):** It was used as a vaccine preservative. It was added to the vaccine at final concentration of 0.001%.

#### **7. Vaccines:**

##### **7.1. Combined inactivated vaccine (Pneumo-3):**

- The vaccine formulation comprised three viruses, BVD, IBR and PI-3, was already produced and registered with a patent name (Pneumo-3 vaccine).
- This vaccine was prepared and used for sheep vaccination in the dose and route according to the method of El-Sabbagh *et al.* (1995) and Samira *et al.* (2001).
- Pneumo-3 vaccine used in this experiment was gently supplied from Rinderpest Like Disease Dept., Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo. It was taken from batch 002/2006.

##### **7.2. Inactivated PPR vaccine:**

The preparation of inactivated PPR vaccine was under taken according to Fayed *et al.* (2000) and Hussein (2001).

**8. Experimental Design:** All sheep (twelve sheep) were randomly divided into 4 groups each of 3 sheep, as follows:

**Group I:** This group was simultaneously vaccinated with the combined inactivated pneumo-3 and inactivated PPR vaccines. The combined

inactivated pneumo-3 vaccine was inoculated in each animal at the dose of 3 ml, by deep I/M according to Hansen *et al.* (1995). While the inactivated PPR vaccine was inoculated at the dose of 2 ml S/C route for each animal after the procedure applied by Fayed *et al.* (2000) and Hussein (2001) then followed by booster dose after 2 weeks by the same vaccinal dose and the same route for each animal of this group.

**Group II:** This group was vaccinated with inactivated PPR vaccine before vaccination with combined inactivated pneumo-3 vaccine by 2 weeks. Two prepared vaccines (inactivated PPR and combined inactivated pneumo-3 vaccines) were used at the same vaccinal dose, same routes (S/C for PPR vaccine and deep I/M for pneumo-3 vaccine) and the same interval for booster dose as in animal grouping I).

**Group III:** This group was vaccinated with the prepared inactivated PPR vaccine after the vaccination with combined inactivated pneumo-3 vaccine by 2 weeks with same vaccinal doses, inoculation routes, and same interval time for boosting as in group (I).

**Group IV:** This group was kept without vaccination as non-vaccinated contact control group.

**9. Serological Investigation:** All the collected serum samples were assayed for presence of specific antibodies against all vaccinal viruses (BVD, IBR, PI-3 and PPR vaccine) using serum neutralization test (SNT) in microtitre plates, following the procedure described by Dannacher and Martel (1978) and Rossi and Kiessel (1971). The serum neutralizing antibody titres (SN-Ab) titres were expressed as log<sub>10</sub> TCID<sub>50</sub> of serum dilution that protected 50% of the wells of microtitre plate following the calculation procedure of Reed and Muench (1938).

## RESULTS

The results of the tests were illustrated in Tables 1, 2, and 3.

**Table 1:** Mean serum neutralizing antibody titres of vaccinated sheep with inactivated PPR vaccine before vaccination with combined inactivated pneumo-3 vaccine

	Inactivated PPR	Combined Inactivated Pneumo-3 vaccine		
		BVD	IBR	PI-3
1 WPV	0.00 *			
2 WPV	1.15 **			
4 WPV	1.30	0.00 *	0.30 *	0.30 *
6 WPV	1.60	0.90 **	0.90 **	1.00 **
8 WPV	1.82	1.35	1.40	1.50
10 WPV	1.60	1.70	1.70	1.77
12 WPV	1.25	1.60	1.65	1.77
16 WPV	1.20	1.45	1.50	1.60
20 WPV	1.90	1.25	1.30	1.40
24 WPV	0.9	1.20	1.20	1.30

**Table 2:** Mean serum neutralizing antibody titres expressed in log<sub>10</sub> of simultaneous vaccination of combined inactivated pneumo-3 and inactivated PPR vaccine

	Inactivated PPR	Combined Inactivated Pneumo-3 vaccine		
		BVD	IBR	PI-3
0 WPV	0.00 *	0.00 *	0.20 *	0.3 *
2 WPV	1.00 **	0.90 **	0.90 **	1.10 **
4 WPV	1.20	1.45	1.55	1.65
6 WPV	1.75	1.80	1.90	2.10
8 WPV	1.90	1.85	2.00	2.10
10 WPV	1.85	1.75	1.75	1.90
12 WPV	1.68	1.65	1.70	1.80
16 WPV	1.30	1.33	1.35	1.35
20 WPV	1.20	1.20	1.25	1.30
24 WPV	1.00	0.90	1.00	1.10

WPV: Weeks Post Vaccination \* 1<sup>st</sup> vaccination  
 \*\* Booster dose } For each vaccination

**Table 3:** Mean serum neutralizing antibody titres expressed in log<sub>10</sub> of vaccinated sheep with inactivated PPR vaccine after vaccination with combined inactivated pneumo-3 vaccine

	Inactivated PPR	Combined Inactivated Pneumo-3 vaccine		
		BVD	IBR	PI-3
0 WPV		0.00 *	0.30 *	0.30 *
2 WPV		0.90 **	1.10 **	1.20 **
4 WPV	0.00 *	1.55	1.65	1.65
6 WPV	1.00 **	1.77	1.85	1.90
8 WPV	1.20	1.77	1.80	1.95
10 WPV	1.75	1.75	1.75	1.90
12 WPV	1.95	1.60	1.70	1.75
16 WPV	1.85	1.30	1.35	1.35
20 WPV	1.65	1.15	1.15	1.20
24 WPV	1.35	0.90	0.90	0.90

WPV: Weeks Post Vaccination \* 1<sup>st</sup> vaccination  
 \*\* Booster dose } For each vaccination

## DISCUSSION

The use of simultaneous vaccination has found much attention recently and could greatly simplify prophylactic effect induced by vaccination and so control of such diseases with reducing time, effort and cost of the campaign which make it economically more acceptable (Joseph and Hedger, 1984 and Gugium *et al.*, 1989).

The monitoring of humoral immune response post vaccination is a mean for evaluation of the protective capacity of vaccines, and the commonly used serological test is the neutralization test which is considered as a queen test for determination of serum neutralizing antibody titres (SN Ab). SNT has been extensively used as a measure of immune response against combined inactivated respiratory virus vaccine (Pneumo-3) and inactivated PPR vaccine (Rossi and Kiesel, 1971).

The results obtained in Tables (1, 2 and 3) revealed that the mean SN-Ab titres of vaccinated sheep at 4 weeks post first vaccination, were fully sufficient to protect susceptible animals from infection with respiratory viruses (IBR, BVD and PI-3) as reported by Zuffa and Feketova (1980), Mihylovic *et al.* (1979) and Bittle *et al.* (1968) who reported that the minimum acceptable titre of protective level against IBR, PI-3 and BVD viruses are 0.6, 0.6 and 0.90 respectively and there is no significant differences could be detected in the mean SN antibody titres against IBR, PI-3 and BVD viruses in the three groups post vaccination of sheep during the whole experimental periods, and these results were coincide with Matsuoka *et al.* (1966), who used PI-3 virus with *Pasteurella multocida* and *Pasteurella haemolytica*. Tribe *et al.* (1969) who applied vaccination of Guinea pigs and colostrum deprived calves with bovine Adeno-3 virus with parainfluenza virus vaccine.

Matsuoka *et al.* (1972) also applied vaccination of calves with inactivated PI-3 and IBR simultaneously with killed *Pasteurella multocida* and *Pasteurella haemolytica* vaccine. Sampson *et al.* (1972) applied trials of immunization to calves with an inactivated IBR and PI-3 vaccine. Simultaneously with *Pasteurella bacterin* and Ghally *et al.* (1996) who studied the effect of sheep pox vaccine and combined inactivated respiratory virus vaccine (with patent name "Triangle-4") in the immune response of calves against IBR, PI-3 and BVD viruses and El-Sabbagh *et al.* (1999), who applied immunological studies on simultaneous vaccination of combined inactivated respiratory virus vaccine with Rift valley fever vaccine in sheep and he concluded that there is no significant effect on the safety and efficacy of simultaneous inoculation of sheep with pneumo-3 with Rift valley fever vaccine.

Concerning the synchronous vaccination of sheep with PPR vaccine, with other vaccines were reported before as Afaf *et al.* (2003) for attenuated PPR, attenuated RVF and BCG, Nahed *et al.* (2003) for attenuated PPR and polyvalent clostridial vaccine, Nahed *et al.* (2002) for PPR vaccine and combined *Pasteurella multocida* vaccine and Samir *et al.* (1999) for PPR and sheep pox combined vaccine.

The increasing number of vaccines makes it necessary to design special schemes for their national use to save time, efforts and fund, other successful trials of simultaneous vaccination of animals with more than one vaccine were reported before (Macadam, 1964), for rinderpest and black quarter or anthrax spore vaccine, (Brown and Taylor, 1966) for rinderpest and contagious bovine pleuropneumonia, (Darie *et al.*, 1979) for foot and mouth disease and clostridia and anthrax (Polydora *et*

*al.*, 1980) for foot and mouth disease (FMD) and anthrax, enterotoxaemia and enzootic abortion, (Joseph and Hedger, 1984) for FMD and haemorrhagic septicaemia (Hedger *et al.*, 1986) for rinderpest and FMD (Osman *et al.*, 1987) for rinderpest and BCG, Osman *et al.* (1991) for rinderpest and haemorrhagic septicaemia, Osman *et al.* (1997) who applied synchronous vaccination of cattle with rinderpest and BCG vaccine Ghally *et al.* (1996) sheep pox and combined inactivated respiratory virus vaccine and finally El-Sabbagh *et al.* (1999), who applied simultaneous vaccination between combined inactivated respiratory virus vaccine (Pneumo-3) and Rift valley fever vaccine.

In this study, very satisfactory immune response was obtained, for both vaccines used, to pneumo-3 vaccine and inactivated PPR vaccine, such results in tables 1, 2 and 3, assure that the simultaneous vaccination had no effect on the immune response of sheep against both pneumo-3 vaccine and inactivated PPR vaccine. So on conclusion, our results could be indicated clearly the safety and efficacy of simultaneous vaccination of pneumo-3 vaccine with inactivated PPR vaccine without any completion neither synergistic nor antagonistic effect, and it could be recommended to use for sheep vaccination.

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