

EVALUATION OF INACTIVATED INFECTIOUS BOVINE RHINOTRACHEITIS (IBR) AND PARAINFLUENZA-3 (PI-3) VACCINE IN SHEEP

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Abstract

The locally prepared inactivated respiratory virus vaccine (Pneumo-3) which contains IBR, PI-3 and BVD viruses was used to immunize 18 lambs free of IBR and PI-3 virus antibodies. These were divided into 2 groups, the first was immunized with 2 doses of the vaccine 2 weeks apart, and the second was kept as control.

Kinetics of immune response were elicited by ELISA and SNT against IBR and PI-3 constituent of the vaccine and HI against PI-3 virus only.

The protective capacity of each constituent was studied by challenge exposure which was performed on one month post vaccination (PV). The unvaccinated control lambs became affected by each challenge dose, they were shedding the virus and showed clinical symptoms post-infection.

The immunized lambs and contact control lambs did not exhibit clinical symptoms and did not shed the viruses. Adverse reactions were not observed in lambs following vaccination. The results indicated that vaccination with Pneumo-3 can provide protection against IBR and PI-3 virus infection in sheep.

INTRODUCTION

Sheep and goats represent important domestic livestock species in the world. In Egypt, it was found that population of sheep and goats were 3,491,000 and 3,250,000, respectively (FAO, 1996). The disease in sheep and goats may be caused by a combination of infectious agents in conjunction with stress (Robinson, 1983). Viral and mycoplasma infections may predispose the lower respiratory tract to colonization by bacteria such as pasteurilla which is the most frequent isolate from the lungs of sheep and goats with pneumonia (Baskerville, 1981).

The estimated sheep population in USA decreased from 40, 129, 61 in 1940 to 11,411,000 in 1984. One of the contributory factors to this decrease was found to be due to the prevalence of infectious diseases especially respiratory tract infections

(FAO, WHO, OIE, 1984).

In Egypt, ovine respiratory affection as infectious bovine rhinotracheitis (IBR) and parainfluenza-3 (PI-3) causes heavy economic losses through mortalities, reduced meat, milk and wool production besides cost of disease prevention program.

The picture of the disease in cattle and buffaloes in Egypt had been studied, but much less information is available concerning the disease in sheep. No proper attention was given for vaccination of these animals against the viral disease in Egypt, and accordingly, they may constitute an incidious source of infection to other animals.

Parainfluenza-3 virus (PI-3) is a pneumovirus in the family Paramyxoviridae and infectious bovine rhinotracheitis virus (IBRV) is a bovine herpes virus characterized by being an important cause of respiratory tract infection of sheep and goats Robinson (1983), Whetstone and Evermann (1988). Serological survey of antibodies against PI-3, IBR and Border disease in sheep flocks was studied by Tayler *et al.* (1975), Nawathe and Lamorde (1982), Lamontagne *et al.* (1985), Honger *et al.* (1989), Giangaspero *et al.* (1997).

Therefore, vaccines containing IBR and PI-3 antigens alone and in combination with other viral and bacterial antigens are commonly used in prophylaxis against IBR and PI-3 viruses (Woods *et al.*, 1975, Wells *et al.*, 1978, Salsbury, 1984a,b, Straub *et al.*, 1984, Lehmkuhl and Cutlip, 1985, Whetstone and Evermann, 1988).

This study gives information concerning efficacy and safety of an inactivated IBR and PI-3 virus vaccine used in combination with other agent as BVD virus (Pneumo-3) for protection of sheep against IBR and PI-3 infection and lessening the number of carrier in such animals.

MATERIALS AND METHODS

1. Vaccine: It is local combined inactivated respiratory virus vaccine pneumo-3 containing PI-3 (strain 45) $8 \log_{10}$ TCID₅₀/ml, IBR (Abou Hammad strain) $8 \log_{10}$ TCID₅₀/ml and BVD-MD (Iman strain) $6 \log_{10}$ TCID₅₀/ml. The antigens were inactivated by binary ethyleneimine and adsorbed by 30% alhydrogel in 50ml bottle. The vaccine was produced in the department of Rinderpest like disease in Veterinary Serum and Vaccine Research Institute.

2. Animals: Eighteen Baladi breed lambs of approximately 4-6 months old, were used in this study. These lambs were kept at Veterinary Serum and Vaccine Research

Institute, Abbasia, Cairo, Egypt, Rinderpest Like Diseases Dept. These lambs were screened prior to vaccination for their IBR and PI-3 serologic status.

3. Tissue culture: This is Madin Darby bovine kidney (MDBK) cell line culture tested to be free from the non cytopathic (NCP) BVD-MD virus by fluorescent antibody technique or by interference phenomenon by using cytopathic strain of BVD (homologous interference).

4. Media: Eagle's minimal essential medium (MEM) was obtained from Egyptian Organization of Biological Products and Vaccines, Giza.

Safety of inactivated PI-3 vaccine in lambs

Safety in lambs was conducted following the method of Tomoglia (1968). Four susceptible lambs were used. They were kept under observation for 7 days before the test for general clinical examination and the rectal temperature was recorded daily. Then, two lambs were inoculated intramuscularly (I/M) with 10 fold of the vaccinal dose of the pneumo-3. The other 2 lambs were inoculated intramuscularly with 20ml of physiological saline and were left as control. All lambs were kept under observation for 21 days post-inoculation for the development of any clinical abnormalities.

Evaluation of the IBR and PI-3 virus constituent of pneumo-3 vaccine

Experiments were conducted on two groups of Baladi lambs each group consisted of 9 lambs which were divided into 3 subgroups:

Group (1): Each lamb was immunized with pneumo-3 vaccine I/M by two doses, 2 weeks apart each dose was 3 ml (Koves *et al.*, 1982 and Hansen *et al.*, 1995). At one month post-vaccination, three of them were challenged intranasally and intratracheally, each with 2ml (2×10^8 TCID₅₀) of the virulent PI-3 virus strain 45. The challenge infection was repeated 24 hours later according to Koves *et al.* (1982). Another 3 lambs were exposed to challenge of immunity with virulent IBR virus as the same routes and dose (2ml) (2×10^7 TCID₅₀) as Lehmkuhl and Cutlip (1985) and the remaining 3 lambs were used for studying the humoral immune response for 6 months post-vaccination.

Group (2): The other 9 lambs were left as normal controls. Three of them were experimentally infected with 4ml of virulent PI-3 virus strain 45 (2×10^8 TCID₅₀), 2ml instilled intranasally and 2ml intratracheally. The infection was repeated 24 hours later and the other 3 lambs were experimentally infected with virulent IBR virus as the same

route and dose. The rest 3 lambs were used as uninfected controls. Reisolation of viruses was attempted from nasal, conjunctival swabs, and buffy coat taken daily for 14 days post-infection in MDBK cell cultures.

A. Virological studies

Trials of viral reisolation and identification of isolated agent using mono-specific hyperimmune serum against PI-3 and IBR virus were carried out according to Whetstone and Evermann (1988).

B. Serological studies

- i. Serum neutralization test: The test was performed for the measurement of IBR and PI-3 serum neutralizing antibodies in vaccinated lambs according to Salsbury (1984a, b).
- ii. Enzyme Linked Immunosorbent Assay (ELISA): It was carried out according to Voller *et al.* (1976).
- iii. Micro-haemagglutination inhibition test: for measuring PI-3 antibodies. It was carried out according to the technique of Cho *et al.* (1985).

RESULTS

Safety test in lambs

Neither elevation of body temperature nor development of clinical illness were recorded in four groups post-vaccination. No virus detection was recorded in the immunized lambs or in the contact control lambs post-vaccination and post-challenge. The vaccinated challenged lambs showed a slight increase in body temperature on the second day post-challenge (PCh) and a serous nasal discharge.

The unvaccinated infected lambs showed a febrile response (40-41.5°C) on days 3 and 4 post-infection. The lambs showed clinical symptoms including serous nasal discharge, sneezing and dullness from the 5th day post-infection. The condition was further aggravated by the 7 days when the lamb exhibited severe coughing and dyspnoea.

The results of reisolation post-challenge from 4 groups attempts from nasal swabs, conjunctival swabs and buffy coat are summarized in Table 1.

Humoral immune response

The post-vaccination immune responses SN and ELISA antibody against IBR and PI-3 virus were shown in Table 2 which revealed that IBR and PI-3 antibodies increased in vaccinated lambs sufficient to protect them from infection with the virulent IBR and PI-3 virus, respectively.

Mean haemagglutinating antibody titres against PI-3 virus were significantly increased as a result of vaccination (Table 3). In challenged lambs, challenge virus inoculation in vaccinated lambs induced high antibody titres (Table 4). It revealed detectable antibodies with the mean titres of (0.60 and 0.75) of 14 days post-infection in control infected lambs against IBR and PI-3, respectively (Table 4).

DISCUSSION

This work gives information concerning efficacy and safety in lambs of an inactivated IBR and PI-3 virus vaccine constituents used in combination with BVD virus (Pneumo-3 vaccine) in susceptible Baladi lambs. The results of safety of pneumo-3 vaccine in lambs revealed that the vaccine was safe. The vaccinated animals showed no clinical symptoms and neither IBR nor PI-3 were recovered from these animals throughout the experiment. This agreed with the findings of Koves *et al.* (1982) and Salsbury (1984a,b). They reported that vaccinated lambs with inactivated IBR and PI-3 vaccine did not become ill and did not transmit the virus to uninfected control group. This indicated the safety of the vaccine by preventing the shedding of the virus.

Vaccinated challenged lambs showed only slight increase of body temperature which indicated efficacy of the vaccine and protection of lambs against the challenge with the virulent viruses. The non-vaccinated control infected lambs looked dull and depressed from the 2nd day post-infection with the appearance of serous nasal discharge as Wood *et al.* (1975).

Picture of the disease in the control infected lambs was reported by Whetstone and Evermann (1988) against IBR virus. The lesions developed in the unvaccinated lambs in our challenge experiments were consistent with those described by Hore and Stevenson (1967) against PI-3 virus.

Results of humoral immune response showed that the first dose of vaccine just primed the vaccinated lambs to respond to the booster dose and no adverse effects of vaccination appeared (Straub *et al.*, 1984).

Serological studies on lambs vaccinated twice at 14 days intervals by 2 injections proved that the curves representing HI and SN antibodies did not run in parallel, while, HI antibody titres significantly increased after the first vaccination, the elevation of SN antibody titres was found only at 14 days after the first vaccination against PI-3 virus. Polno and Aleksandrowicz (1975) demonstrated HI antibodies of IgM nature in the early stage of immunity in rabbits inoculated with measles virus. However, in later stages and inconvalescent sera of HI antibodies and neutralizing antibodies of the IgG class were found.

Several authors have studied the efficiency of inactivated IBR and PI-3 vaccines and they have reported a definite protective effect of inactivated IBR and PI-3 vaccines in lambs with high serum antibody titres elicited by the vaccines as Davies (1985), Rodger (1989) and Hansen *et al.* (1995).

In our studies, the vaccinated challenged lambs failed to develop clinical symptoms, while, unvaccinated lambs were shedding the virus continuously and the virus was also isolated from buffy coat, nasal conjunctival swabs as reported by Lehmkuhl and Cutlip (1985). It was not possible to recover the IBR and PI-3 virus from the vaccinated lambs.

The correlation of challenge of immunity results with the serological findings indicates that lambs with a detectable IBR and PI-3 SN and ELISA antibody titres and PI-3 haemagglutination inhibition titre were protected after 6 months post-vaccination by using two injections of the vaccinated dose; adverse reactions to the vaccination were not observed. Additionally, none of contact control lambs developed SN nor ELISA titre to IBR or PI-3 virus and HI to PI-3 virus. The results indicated that vaccination with pneumo-3 can provide protection against IBR and PI-3 virus infection in sheep and lessening the number of carrier in such animals.

Table 3. The immune response of vaccinated lambs with (pneumo-3) against PI-3 constituent using haemagglutination inhibition test.

Animal Group	Log ₁₀ haemagglutinating inhibiting titres post vaccination										
	Prevacc.	1 WPV	2 WPV	4 WPV	6 WPV	8 WPV	12 WPV	16 WPV	20 WPV	24 WPV	
Vaccinated Lambs	0.30	0.65	0.85	1.90	2.20	2.30	2.00	1.90	1.65	1.40	
Control Group	No haemagglutinating inhibiting antibody titre										

Table 4. The immune response of vaccinated lambs with (pneumo-3) against IBR and PI-3 constituent post-challenge with virulent viruses using serum neutralization test.

	Log ₁₀ serum neutralizing antibody titres post challenge																									
	0		7		14		21		28*		7		14		21		28		60		90		120			
	DPV	DPV	DPV	DPV	DPV	DPV	DPV	DPV	DPV	DPV	DPV	DPV	DPV	DPV	DPV	DPV	DPV	DPV	DPV	DPV	DPV	DPV	DPV	DPV	DPV	
Vaccinated challenged group	IBR	-	0.40	1.40	1.75	1.60	1.85	2.20	2.20	2.20	2.20	2.25	2.00	1.80	PI-3	-	0.65	1.55	1.90	1.75	2.10	2.35	2.30	2.20	2.10	1.95
Control infected group	IBR	-	-	-	-	-	0.60	1.70	2.50	2.40	2.20	2.40	2.00	PI-3	-	-	-	-	-	0.75	1.80	2.60	2.40	2.30	2.10	

- No antibody titres.

DPV: Days Post Vaccination.

DPCh: Days Post Challenge.

* First day of challenge.

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تقييم كفاءة لقاح التهاب القصبة الهوائية المعدى والبار أنفلونزا-٣ الميت فى الأغنام

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تم اختبار كفاءة لقاح التهاب القصبة الهوائية المعدى والبار أنفلونزا-٣ الميت الموجودين باللقاح الثلاثى (النيمو-٣) والذي يضم أيضاً الإسهال الفيروسي المعدى حيث تم اختباره سابقاً. وقد تمت هذه الدراسة على ثماني عشر من الحملان الصغيرة التي يتراوح عمرها من ٤-٦ شهور حيث تم تقسيمها إلى مجموعتين أساسيتين تتكون من ٩ حملان والتي بدورها انقسمت الى ثلاث مجموعات داخلية حيث تم حقن المجموعات الأولى بلقاح النيمو-٣ على جرعتين بينهما ١٤ يوماً وبعد شهر من التحصين تم إجراء اختبار التحدى باستخدام العترة الضارية من فيروس التهاب القصبة الهوائية على ثلاث حملان من الحقونة باللقاح وكذلك باستخدام العترة الضارية من فيروس البار أنفلونزا-٣ على ثلاث حملان أخرى مع ترك باقى المجموعة لدراسة مستوى الأجسام المناعية المتكونة من التحصين باللقاح التنفسي الفيروسي الميت.

أما المجموعة الأخرى التي لم يتم تحصينها فقد تم تقسيمها الى ثلاثة مجموعات تم عدوى المجموعة الأولى منها بفيروس التهاب القصبة الهوائية والمجموعة الثانية بفيروس البار أنفلونزا-٣ الضارى مع ترك باقى المجموعة كضابط للتجربة.

أثبتت الدراسة أن التحصين باللقاح أعطى مناعة واقية ضد الفيروسات الضارية مع استمرار هذه المناعة حتى ٦ شهور بعد التحصين، لذلك ننصح باستخدام اللقاح فى الأغنام ليقلل من حدوث النزلات المعوية الرئوية فى الحملان الصغيرة وكذلك تقليل نسبة الحيوانات الحاملة للفيروسات لمنع انتشار العدوى بهذه الفيروسات.