

EVALUATION OF COMBINED INACTIVATED RESPIRATORY VIRUS VACCINE PNEUMO-4 IN PREGNANT COW DAMS

El-Sabbagh*, M. M. A.; El-Sawalhy**, A. A. ;

Samira Said* and Ghaly*, H. M.

Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo.

**Department of Infectious Diseases, Fac. Vet. Med. , Mansoura University.

ABSTRACT

Evaluation of combined inactivated respiratory virus vaccine (Pneumo-4) against IBR, BVD, PI-3 and BRSV in cattle was achieved in private farm located at Dommiatta Governorate (El-Basateen Farm). Twenty apparently healthy pregnant cows were vaccinated 2 doses by intramuscular injection of 5 ml of the vaccine, 2 weeks apart at late stage of pregnancy (1-3 months before delivery) and ten pregnant cows were left as unvaccinated control. The antibody levels were estimated quantitatively using SN and ELISA techniques for both cows and their off-springs.

Immunized Dams gave off-springs IBR, BVD, PI-3 and BRSV antibody within the protective level up to 4 and 6 months of age according to the time of delivery post vaccination

INTRODUCTION

Respiratory diseases and the bovine mucosal disease complex are of considerable economic importance to the cattle industry. Routine inoculation of cattle with either individual or combined live attenuated viral vaccines prepared with IBR, BVD, PI-3 and BRSV viruses are used widely for prophylaxis against these diseases. However, inoculation of pregnant cattle with attenuated vaccines is discouraged since this practice may lead to abortion (Rosner, 1968). Furthermore, BVD virus can pass the placenta and infect the fetus at all stages of gestation (Radostitis et al., 2000).

Abortion, cerebellar hypoplasia, ocular lesions still births, weakness and diarrhoea occurred particularly with infection in the first half of gestation. For these reasons, vaccination of susceptible pregnant cows with inactivated virus vaccines is usually recommended at any stage of pregnancy in cow calf operations of both the beef and dairy industries where cow might be in all

stages of pregnancy (Ellis et al., 1990 and Donkersgoed et al., 1991).

Pneumoenteritis problem in young calves is no longer be regarded as occurring sporadically with the increase in intensive systems of management in Egypt. The viral agent causing respiratory diseases in Egypt include infectious bovine rhinotracheitis (IBR), bovine viral diarrhoea (BVD), parainfluenza-3 (PI-3) and bovine respiratory syncytial (BRS). Occurrence of pneumoenteritis syndrome is most frequent and sever within the calves younger than 3 months and inversely related to the level of maternal antibodies (Kimman, et al., 1988).

It was recorded that effective lactogenic immunity against pneumoenteritis of viruses could be achieved by ingestion of colostrum containing adequate levels of protective antibodies (Joseph et al., 1973 and Ellis et al., 1996).

The aim of this study was to evaluate the immune response in pregnant cows (last stage) as well as to follow up the acquired colostrum immunity of their calves for 6 months after parturition under field conditions.

MATERIAL AND METHODS

1. Cows : Thirty apparently healthy pregnant cows aged 4-5 years and of about 500 Kg B.Wt. Proved to be negative to IBR BVD , PI-3 and BRSV antibodies, were used for evaluation of combined inactivated respiratory virus vaccine (Pneumo-4) at Basateen Farm in Dommiatta Governorate. These animals were divided into two groups.

Group (1) : Consists of 20 cows vaccinated with pneumo-4 vaccine intramuscularly (I/M) with 2 doses 2 weeks apart.

Group (2) : Consists of 10 cows were left as unvaccinated control.

2. Viruses:

- a. IBR virus: Abou-Hammad strain ($10^{-7.5}$ TCID₅₀/ml).
- b. PI-3 virus: Strain 45 (10^{-8} TCID₅₀/ml).
- c. BVD virus: Iman strain ($10^{-6.5}$ TCID₅₀/ml).
- d. BRS virus: Strain 375L ($10^{-6.5}$ TCID₅₀/ml).

These viruses strains were kindly supplied from Rinderpest like diseases Vaccine Production Department Serum and Vaccine Research Institute, Abbassia, Cairo.

3. Vaccine : Combined inactivated respiratory virus vaccine containing bovine virus diarrhoea (BVD), infectious bovine rhinotracheitis (IBR), parainfluenza-3 (PI-3) and bovine respiratory

ry syncytial virus (BRSV) was prepared locally in Rinderpest Like Disease Dept., Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo.

4. Cell culture : Madin Darby Bovine Kidney (MDBK) cell line culture which tested to be free from non-cytopathic (NCP) of BVD. MD virus by fluorescent test (Marcus and Moll, 1968) was used in this study.

5. Serological test :

- a. The Micro-serum Neutralization Test (MSNT): It was performed according to Rossi and Kiesel (1971).
- b. Indirect ELISA technique: It was carried out according to Edwards and Newman (1985) for detection of antibodies against BVD, IBR, PI-3 and BRSV viruses.

6. Sampling :

Blood samples were taken from cows before inoculation of the first dose of the vaccine (0 time) then prior to administration of the second dose of the vaccine (2, 3, 4, 6, 8 weeks post vaccination) and before parturition. Also blood samples were similarly collected from unvaccinated cows at the same intervals. Also blood samples were collected from the new born calves at 1 week post delivery then at 1, 2, 3, 4, 5, 6 month post delivery. Sera were separated by centrifugation and kept at -20°C for antibody assays.

7. Clinical examination :

The vaccinated cows and unvaccinated control group as well as their off-springs were observed clinically during the experiment for any respiratory manifestation or diarrhea or any clinical abnormalities during the experiment. The body temperature of the pregnant cows was recorded before and daily after vaccination for one week.

RESULTS AND DISCUSSION

Bovine respiratory disease complex is one of the serious infectious multifactorial problems which cause high economic losses due to mortality, therefore it is important to vaccinate all susceptible animals with a safe, potent and highly immunogenic vaccine. In Egypt, recently "Pneumo 4" was introduced to vaccination program of animals. So, this field experiment was performed to evaluate of a combined inactivated respiratory virus vaccine "Pneumo 4" in pregnant cows and their off-springs.

All cows in group I vaccinated with pneumo 4 in the late stage of gestation period and their offspring were normal during the experimental period (6 Months) and did not exhibit any rise in

body temperature or showed any signs of respiratory disease. This might be contributed to the safety of the locally produced vaccine "Pneumo 4".

Studies on the immunogenicity and duration of the immune response following immunization of pregnant cows with locally produced "Pneumo 4" under field condition as demonstrated in Tables (1&2).

The neutralizing antibodies started to increase following vaccination at 3 weeks post vaccination (The protective level) and increased to maximal level at 8 weeks post vaccination as shown in Table (1).

The results of maternal immunity of calves born to vaccinated pregnant cows and their in-contact born to unvaccinated control cows are shown in Tables (3&4). The titer of the off-springs born to vaccinated cows are matched with the titer of their dams at the time of parturition, these results are in agreement with that obtained by **Baz (1975)** who reported that calves get the same titer of antibodies or higher than their dams.

The antibodies gradually decreased toward the six months post parturition and reached to the minimal protective level of immunity. These results agreed with that obtained by **Joseph et al. (1973)**, **Ukka and Edward Alvin (1990)**, **Donkersgoed et al. (1991)** who concluded that the maternal antibodies of cattle calves that were developed as a result of vaccination of their dams with killed vaccine containing BVD, IBR, PI-3 and BRSV may persist for 6 months.

Cattle calves that were delivered at 3 months of vaccination of their dams remained protected till the fourth month as obtained by **Brar et al. (1978)** who concluded that passive colostral antibodies lost one half of their remaining antibody titre every 21 days and serologically responded to vaccine at a time when maternal antibody titre was between 1:96 and 1:20 (about 3-7 months). Thus, the present studies indicated the maternal antibodies in calves remained within the protective level from 3-6 months when it comes from dams vaccinated with BVD inactivated vaccine at the last gestation period of pregnancy.

Ellis et al. (1996) found that after inoculation of pregnant cows with Cattle Master-4 discovered that immunization of young calves with Cattle Master-4 one shot on day 10 of life did not result in the production of increased serum level of antibodies and it may be attributed to a blocking effect of maternal antibody or an age effect on the response to the vaccine.

Under natural field conditions in this experiment, the obtained results showed that there was only double fold increase between the mean titres of calves born from vaccinated and non-vaccinated dams. This may be attributed to the time of delivery in some dams. Also, some calves may not received the colostrum from their dams at the optimum time post parturition and it

should be mentioned this farm was suffering from bad hygienic measures.

The results of clinical examination after vaccination and seroconversion indicate that the locally produced pneumo 4 can be safely used to immunize pregnant cows to control the infections in new born calves results from BVD, IBR, PI-3 and BRSV viruses.

Table 1 : Serum neutralizing antibody titre in pregnant cows post vaccination with combined inactivated respiratory virus vaccine (Pneumo-4).

Animal groups	Log 10 serum neutralizing antibody titre in pregnancy																							
	BVD						IBR						P1-3						BRS					
	Weeks Post Vaccination						Weeks Post Vaccination						Weeks Post Vaccination						Weeks Post Vaccination					
	0	2	3	4	6	8	0	2	3	4	6	8	0	2	3	4	6	8	0	2	3	4	6	8
vaccinated goup	0.4	0.4	0.9	1.15	1.6	1.7	0.5	0.55	1.0	1.25	1.7	1.85	0.6	0.7	1.1	1.45	1.85	1.95	0.6	0.65	0.9	1.3	1.8	1.8
Control group	0.65	0.95	0.55	0.3	-	-	0.9	0.95	0.85	0.4	-	-	0.85	0.85	0.9	0.3	-	-	0.6	0.8	0.65	0.45	-	-

Table 2 : ELISA mean antibody titres against BVD, IBR, PI-3 and BRS antigen in sera of vaccinated cows

Animal groups	Log 10 serum neutralizing antibody titre in pregnancy											
	BVD			IBR			P1-3			BRS		
	Weeks Post Vaccination			Weeks Post Vaccination			Weeks Post Vaccination			Weeks Post Vaccination		
	0	2	4	0	2	4	0	2	4	0	2	4
vaccinated goup	0.4	1.25	1.8	0.65	1.4	1.85	0.9	1.5	1.80	0.6	1.2	1.6
Control group	0.55	0.4	0.35	0.4	0.6	0.5	0.75	0.5	0.4	0.65	0.6	0.35

Table 3 : Log₁₀ serum neutralizing antibody titre in vaccinated, control cows and their offsprings.

Animal groups	Delivery time	SN antibody titre for BVD						SN antibody titre for IBR						SN antibody titre for PI-3						SN antibody titre for BRS													
		Cows		off-spring				Cows		off-spring				Cows		off-spring				Cows		off-spring											
		Cows at birth	1 WPD	1 MPD	2 MPD	3 MPD	4 MPD	5 MPD	6 MPD	Cows at birth	1 WPD	1 MPD	2 MPD	3 MPD	4 MPD	5 MPD	6 MPD	Cows at birth	1 WPD	1 MPD	2 MPD	3 MPD	4 MPD	5 MPD	6 MPD	Cows at birth	1 WPD	1 MPD	2 MPD	3 MPD	4 MPD	5 MPD	6 MPD
vaccinated group	1-1.5 months	1.95	2.00	1.95	1.90	1.7	1.6	1.2	1.0	2.0	2.05	2.1	2.0	1.9	1.8	1.5	1.2	2.1	2.1	2.2	2.0	1.95	1.75	1.6	1.4	2.0	2.0	1.9	1.8	1.75	1.6	1.4	1.25
	2-3 months	1.7	1.7	1.5	1.25	1.1	1.0	0.9	0.6	1.8	1.8	1.7	1.5	1.1	0.85	0.8	0.6	1.9	1.85	1.75	1.6	1.2	1.1	0.9	0.7	1.65	1.7	1.4	1.3	1.0	0.75	0.6	0.6
Control group		0.55	0.45	0.8	0.55	0.5	-	-	-	0.6	0.7	0.6	0.6	0.65	-	-	0.9	0.85	0.9	0.75	0.5	-	-	-	0.8	0.6	0.8	0.4	0.3	-	-	-	

WPD : Weeks Post Delivery

MPD : Months Post Delivery.

Table 4 : EISA mean antibody titers for BVD, IBR, PI-3 and BRS in sera of calves from vaccinated and control dams post vaccination.

Animal groups	BVD						IBR						PI-3						BRS									
	1 WPD	1 MPD	2 MPD	3 MPD	4 MPD	5 MPD	6 MPD	1 WPD	1 MPD	2 MPD	3 MPD	4 MPD	5 MPD	6 MPD	1 WPD	1 MPD	2 MPD	3 MPD	4 MPD	5 MPD	6 MPD	1 WPD	1 MPD	2 MPD	3 MPD	4 MPD	5 MPD	6 MPD
Calves from vaccinated cows	1.75	1.7	1.65	1.5	1.15	1.0	0.9	1.85	1.85	1.7	1.65	1.4	1.2	0.8	2.2	2.0	1.9	1.75	1.5	1.2	1.0	1.85	1.8	1.65	1.55	1.35	0.8	0.65
Calves from vaccinated cows	0.8	0.85	0.7	0.6	-	-	-	0.75	0.95	0.8	0.45	-	-	-	0.9	1.0	0.85	0.55	-	-	-	0.85	0.85	0.75	0.6	-	-	-

WPD : Weeks Post Delivery

MPD : Months Post Delivery.

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الملخص العربى

تقييم اللقاح التنفسى الفيروسى نيمو - ٤ فى الأبقار الحوامل

المشركون فى البحث

مجدى الصباغ أحمد الصوالحى

سميرة سعيد حسين متولى

معهد بحوث الأمصال واللقاحات البيطرية - العباسية

كلية الطب البيطرى - جامعة المنصورة

تعتبر الإصابة بفيروسات التهاب القصبة الهوائية ومرض الإسهال الفيروسى المعدى فى الأبقار والبارانفلونزا ٣ والفيروس السنسيتى التنفسى فى الأبقار من أهم الأمراض التى تؤثر تأثيراً مباشراً على العجول حديثة الولادة، ويعتبر تحصين الحيوانات التى تصاب بهذا المرض خاصة الأمهات المفتاح الرئيسى للتحكم والوقاية من هذا المرض. لذا تهدف هذه الدراسة فى الأبقار لتقييم لقاح النيمو - ٤ إكينيكاً ومناعياً فى الأمهات وكذلك فى العجول المولودة لأمهات محصنة.

حيث أجريت هذه الدراسة على عدد ٣٠ بقرة فى المرحلة الأخيرة من الحمل (٦-٩ شهور) حيث تم تحصين المجموعة الأولى (٢٠ بقرة) بلقاح النيمو - ٤ بالحقن العضلى ٥ سم مرتين يفصل بينهما إسبوعان بالإضافة إلى ١٠ أبقار غير محصنة وفى نفس مرحلة الحمل استعملت كمجموعة ضابطة (المجموعة الثانية) وتركت جميع الحيوانات تحت الظروف البيئية الطبيعية للمزرعة وتم تجميع عينات الدم من الأمهات والعجول على فترات مختلفة لمعايرة الأجسام المناعية ضد الفيروسات الأربعة الموجودة فى اللقاح وذلك باستعمال اختبار السيرم المتعادل والاليزا الغير مباشر، وقد أظهر الفحص الإكلينيكي للأبقار المحصنة عدم ظهور أية أعراض خاصة بالإصابة بهذه الفيروسات وقد أظهرت النتائج أن الأجسام المناعية التكوينية ضد الفيروسات الأربعة قد زاد بعد حقن الجرعة الثانية بإسبوعين إلى مستوى كافى لصد العدوى الطبيعية.

بدراسة المناعة المكتسبة للعجول أظهرت النتائج أن الأجسام المناعية المتعادلة الموجودة فى مصل العجول المولودة من أمهات محصنة كانت ذو قوة عيارية وظلت لفترة أطول تتراوح من (٤-٦ شهور)، وهذا ممكن أن يعزى إلى طول الفترة بين إعطاء اللقاح والولادة أو إلى سوء العوامل الصحية فى المزرعة.