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TRACKING OF THE HUMERAL IMMUNE RESPONSE AGAINST PNEUMOGEN-5 VACCINE IN PREGNANT COWS AND AFTER CALVING.

El-Bagoury, G.F. [a], El-Habbaa, A.S.[a], Maha, R. Abd El-Fadil [b] and Ghaly, H.M. [b]

[a] *Department of Virology, Faculty of Veterinary Medicine, Benha University,* [b] *Rinderpest like Diseases Dept., Veterinary Serum and Vaccine Research Institute, Abbassia , Cairo.*

A B S T R A C T

Pneumogen-5 vaccine, an inactivated polyvalent vaccine (BVD- genotypes 1 and 2, IBR, PI-3, and BRS viruses), was prepared for control of respiratory diseases producing great losses to calves. It was tested for quality and then applied in pregnant cattle 8 weeks before calving for evaluation of their humeral immune response under field conditions. The prepared vaccine was able to induce detectable protective levels of specific antibodies for all reference viruses contained in the vaccine by the 2nd week post vaccination (6 weeks pre-calving period) and continued till 4 month post-calving as measured by SNT and 6 month post- calving by ELISA. Protective levels of specific antibodies were detected in colostrum persist at their higher level till the 3rd day post calving for all reference viruses contained in the vaccine as measured by SNT and confirmed by ELISA. It is concluded that the prepared vaccine was highly potent and provided good colostrum immunity that can protect newborn calves.

Key Words: BRSV, BVDV, Cows, IBRV, ELISA, PI-3V, SNT, Vaccine.

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1. INTRODUCTION

Bovine respiratory diseases (BRD) due to Infectious bovine rhinotracheitis virus (IBRV), parainfluenza type-3 virus (PI-3V), bovine viral-diarrhoea virus type-1 and 2 (BVDV) and bovine respiratory syncytial virus (BRSV) is the major cause of mortality and economic loss in calves [11, 12, 21]. In Egypt, Pneumoenteritis problem in cattle and calves is caused by viral agents include BVDV, IBRV, PI-3V and BRSV [4, 5, 18, 19].Prevention and control of BRD syndrome should include active immunization with either inactivated or live vaccines against BRSV, IBRV, PI3V and BVDV [4, 14, 18]. Modified live vaccines [MLV] is characterized by virus replication in the host tissues, stimulate the immune response of the host over a period of several days [10] but the virus may shed from the host, activate concurrent infections and infect susceptible cattle [16] and attenuated viruses may revert to virulence causing disease in vaccinated animals [6]. The use of inactivated vaccine produces good results for protection of calves from pneumo-enteritis and death with the advantage of being largely safe [13]. Vaccination of pregnant cows with combined inactivated respiratory viruses' vaccine is usually recommended at last stage of pregnancy in cow because of abortion, cerebellar hypoplasia, still birth, weakness and diarrhea occurred particularly with infection in the period of gestation [3].

So the aim of this work to prepare a polyvalent vaccine of BVDV genotype I and II, IBRV PI3V and BRSV and its evaluation in pregnant cows before and

after calving under field conditions and estimation of the colostrum immunity produced.

2. MATERIALS AND METHODS

2.1. *Virus strains:*
2.1.1. *BVDV Genotype -1:*
Egyptian BVDV cytopathic strain (Iman strain) of a titer $10^{6.5}$ $TCID_{50}$/ml.
2.1.2. *BVDV Genotype -2:*
Egyptian BVDV 125 cytopathic strain of a titer $10^{6.5}$ $TCID_{50}$/ml.
2.1.3. *IBR virus:*
A local Abou Hammad strain of a titer $10^{7.5}$ $TCID_{50}$/ml.
2.1.4. *PI-3 virus:*
Reference Egyptian strain "strain 45" of a titer 10^{8} $TCID_{50}$/ml.
2.1.5. *BRS virus:*
Reference strain "375L" of a titer $10^{6.5}$ $TCID_{50}$/ml.
All reference viruses were adapted on MDBK cell line and kindly obtained from the department of the Rinderpest like diseases, Veterinary serum and vaccines research institute (VSVRI), Abbasia, Cairo. They were used in preparation of polyvalent inactivated respiratory virus vaccine (pneumogen-5) and as reference viruses for SNT and ELISA.

2.2. *Pregnant cows under field condition*
Fifty Friesian apparently healthy unvaccinated pregnant cows aged 4-5 years and of about 350-450 Kg body weight at last 2 month of gestation were vaccinated 8 weeks before calving with one dose of inactivated polyvalent Pneumogen-5 vaccine. They were belonged to private farm located in El-Dear, Wadi El-Natroon, Behera governorate. Forty cows were kept for evaluation of the vaccine and ten pregnant cows were kept as non-vaccinated control

2.3. *Preparation of the inactivated polyvalent pneumogen-5 vaccine:*
2.3.1. *Virus Inactivate:*
Two-bromoethyleneimine hydrobromide (BEI) obtained from Aldrich Chemical Company, Melwaukeewis, USA. It has a molecular weight of 204.90. 0.1 M stock solution of BEI was used for inactivation of the viruses of the prepared vaccines.

2.3.2. *Sodium thiosulphate:*
It was obtained from Difco laboratories. It was prepared as 20 % solution in double distilled water and was sterilized by autoclaving. It was used to stop the action of BEI.

2.3.3. *Thiomersal powder:*
It was used by addition of 1 g for each liter of the prepared vaccine after inactivation as preservative with final concentration of 1:100,000. It has anti-bacterial and anti-fungal effect. It obtained from Koch-Light laboratories LTD, Colnbrook, Bucks, England with code number of P282109.

2.3.4. *Aluminum hydroxide gel:*
It was obtained from Honil Limited, London, United Kingdom, Lot No. 3238402. It was used as adjuvant for the inactivated vaccine.

2.4. *Evaluation of the vaccine:*
2.4.1. *Purity test:*
Equal volumes of serial dilution of each of BVDV-1, BVDV-2, IBRV, PI3V and BRSV used in the vaccines were mixed with 1:10 dilution of their specific reference antiserum then inoculated into 4 washed MDBK cell culture well. There was no cytopathic effect 'CPE' of the inoculated cell line with mixture of specific viruses with its hyperimmune sera, which indicates that the vaccines were free from other viruses.

2.4.2. *Sterility for bacterial and fungal contamination:*
The prepared inactivated vaccine was found to be sterile free from any bacterial and fungal contaminants upon cultivation on different synthetic media for bacterial and fungal growth.

2.4.3. *Safety test:*
It was done in mice and guinea pigs and it was observed that there are no clinical abnormalities observed throughout 10 days of observation. In calves, neither elevation of body temperature nor development of any clinical signs or illness was recorded on all calves during 21 days of observation, which indicates safety of the prepared vaccine.

2.5. Samples:
2.5. 1. *Serum samples:*
They were collected post-vaccination form pregnant cows (before and after calving). The sera were inactivated at 56°C for 30 minutes, and then stored at -20°C until used in the serological tests.

2.5. 2. *Colostrums:*
Milk samples from cow dams in the first three days after calving then centrifuged at 3000 rpm for 15 min several time till obtained clear whey for detection of specific antibodies for BVDV-1, BVDV-2, IBRV, PI3V and BRSV using SNT and ELISA.

2.6. *Reference hyper-immune sera:*
Reference hyper-immune sera against BVDV-1, BVDV-2, BHV-1, PI3V and BRSV were obtained from Department of the Rinderpest like diseases, VSVRI, Abbassia, Cairo. It was used in SNT and ELISA .

2.7. *Serum neutralization test (SNT):*
It was carried out on sera from vaccinated pregnant dams before and after calving and on colostrums from them for evaluation of the prepared vaccine [17].

2.8. *Enzyme linked immunosrobent assay (ELISA):*
Sera collected from pregnant dam and colostrums from them after calving were tested for antibodies against BVDV-1, BVDV-2, IBRV, PI3V and BRSV using ELISA [22].

3. RESULTS AND DISCUSSION

Polyvalent vaccines are used in the protection of animals against BRD with reduction of mortality and decrease incidence of the respiratory disease. Field efficacy of these vaccines is variable depending on many factors including animals' age and immune status, virus pathogenicity and its dose and the presence of multiple viral and bacterial infections [8]. This work was carried out to prepare and evaluate Pneumogen-5 (a polyvalent inactivated alhydra gel adjuvant vaccine of BVDV genotype I and 2, BHV-1, PI3V and BRSV) under field condition in a private farm located in El-dear farm in Wadi El natroon, Beheira governorate. Clinically healthy pregnant cows at late stage of pregnancy (third trimester) were used for evaluation of the vaccine and three cows were kept as non-vaccinated control animals.
Evaluation of humeral immune response to inactivated pneumogen-5 vaccine in pregnant cows at 8, 6 and 2 weeks pre-calving showed that mean antibodies titers were gradually increased from 8 weeks pre-calving till reached higher level at 2 weeks pre- calving for all reference viruses contained in the vaccine compared to the negative antibody response showed by the non-vaccinated group, as measured by SNT and ELISA (Tables 1&2; Fig. 1&2).

Table 1 Mean neutralizing antibody titers in sera of pregnant cows following vaccination with inactivated pneumogen-5 vaccine.

Reference virus	Mean SNA titers		
	8*WPreC	6 WPreC	2 WPreC
BVDV -1	**0.2	1.2	1.5
BVDV -2	0.2	1.25	1.6
IBRV	0.0	1.4	1.8
PI-3V	0.3	1.5	1.8
BRSV	0.2	1.2	1.5
Control	0.0	0.0	0.0

* WPreC: Week Pre-Calving. **Mean serum neutralizing antibody (SNA) titers expressed as $\log_{10}TCID_{50}$ on MDBK cell line.

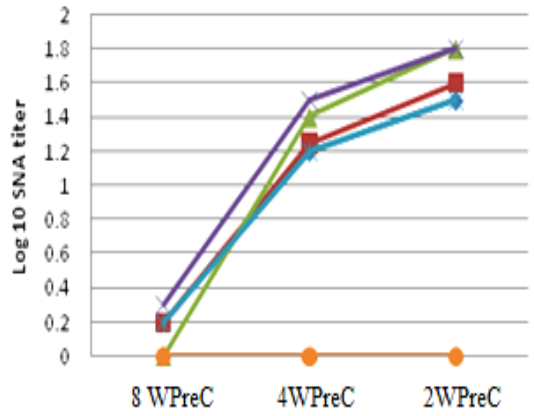

Fig. 1 Mean neutralizing antibody titers of BVD (Genotype 1(◆) &2 (■)), IBR (▲), PI-3 (×) and BRS (✳) versus control (●) in sera of pregnant cows following vaccination with inactivated pneumogen-5 vaccine.

Pneumogen-5 vaccine induced high titers of serum neutralizing antibody in vaccinated pregnant cows at both pre-calving and post-calving periods which is confirmed with ELISA (Tables 1, 2, 3 and 4). This result indicated high potency of the prepared vaccine which is adequate to protect susceptible animals from infection.

This agreed with the studies which reported that the minimum accepted neutralizing antibody titers were 0.9 $\log^{10}$, 0.6 $\log^{10}$ and 0.6 $\log^{10}$ for (BVDV), (PI-3V and IBRV) and (BRSV), respectively[2, 9, 10].

Humeral immune response to inactivated pneumogen-5 vaccine in cows at 1 day, 3 days, 1 week, 2 weeks, 1 month, 2 months, 3 months and 4 months post- calving showed that mean antibodies titers were gradually decreased from 1st day post-calving till reached lower level at 4 month post- calving for all reference viruses contained in the vaccine as measured by SNT and ELISA, but the antibody remain within the protective level (Tables 3&4; Fig. 3&4). The control non-vaccinated group showed no antibody response as measured by SNT and ELISA.

Generally, ELISA results showed higher values of antibody titers than that of SNT and its duration extended for 6th month post calving (table 4). This may be attributed to ELISA considered sensitive serological test, used for determination both the neutralizing and non-neutralizing antibodies.

Table 2 Mean ELISA antibody titers in sera of pregnant cows following vaccination with inactivated pneumogen-5 vaccine.

Reference virus	Mean ELISA antibody titers		
	8*WPreC	6 WPreC	2 WPreC
BVDV -1	0.25	1.4	1.65
BVDV -2	0.5	1.45	1.76
IBRV	0.6	1.65	1.9
PI-3V	0.4	1.7	1.96
BRSV	0.2	1.35	1.7
Control	0.0	0.01	0.0

* WPreC : Week Pre-Calving.

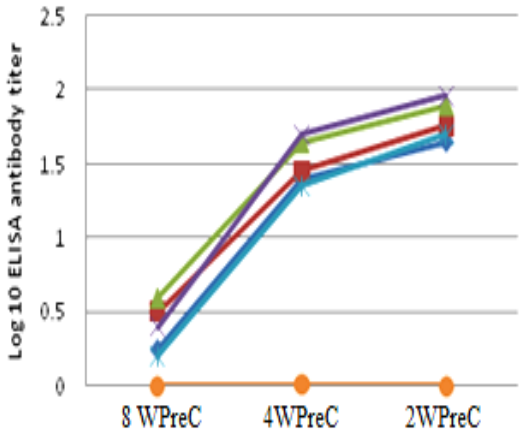

Fig. 2 Mean serum ELISA antibody titer of BVD (Genotype 1(◆) &2 (■)), IBR (▲), PI-3 (×) and BRS (✳) versus control (●) in sera of pregnant cows following vaccination with inactivated pneumogen-5 vaccine.

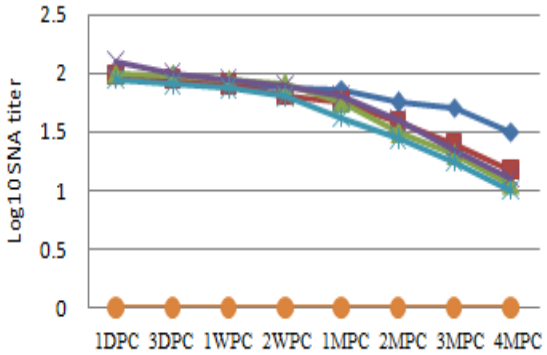

Fig. 3 Mean serum neutralizing antibody titer of BVD (Genotype 1(◆) &2 (■)), IBR (▲), PI-3 (×) and BRS (✳) versus control (●) in sera of cows following vaccination with inactivated pneumogen-5 vaccine after calving.

Table 3 Mean neutralizing antibody titer of in sera of cow dam following vaccination with inactivated pneumogen-5 vaccine after calving.

Reference virus	Mean serum neutralizing antibody titers							
	1* DPC	3DPC	1*WPC	2WPC	1*MPC	2MPC	3MPC	4MPC
BVDV-1	**1.8	1.76	1.75	1.7	1.6	1.45	1.2	0.9
BVDV-2	1.85	1.82	1.8	1.75	1.65	1.5	1.2	0.94
IBRV	2.00	1.98	1.95	1.9	1.8	1.5	1.2	0.9
PI-3V	2.1	2.08	2.06	2.01	1.8	1.6	1.4	1.2
BRSV	1.8	1.76	1.75	1.7	1.6	1.4	1.15	0.9
Control	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

* DPC: day post-calving, WPC: week post-calving, MPC: month post-calving.**Mean serum neutralizing antibody (SNA) titers expressed as $\log_{10}TCID_{50}$ on MDBK cell line.

Table 4 Mean ELISA antibody titer in sera of cow dam following vaccination with inactivated pneumogen-5 vaccine after calving.

Reference Virus	Mean ELISA antibody titers									
	1* DPC	3DPC	1*WPC	2WPC	1*MPC	2MPC	3MPC	4MPC	5 MPC	6 MPC
BVDV-1	1.97	1.97	1.9	1.88	1.86	1.75	1.7	1.5	1.25	0.95
BVDV-2	1.98	1.95	1.9	1.81	1.76	1.59	1.39	1.17	1.03	0.85
IBRV	2.00	1.97	1.94	1.9	1.75	1.49	1.3	1.05	0.87	0.6
PI-3V	2.1	2	1.95	1.89	1.8	1.6	1.35	1.1	0.8	0.6
BRSV	1.95	1.9	1.87	1.8	1.62	1.44	1.24	1.0	0.77	0.51
Control	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

* DPC: day post-calving, WPC: week post-calving, MPC: month post-calving.

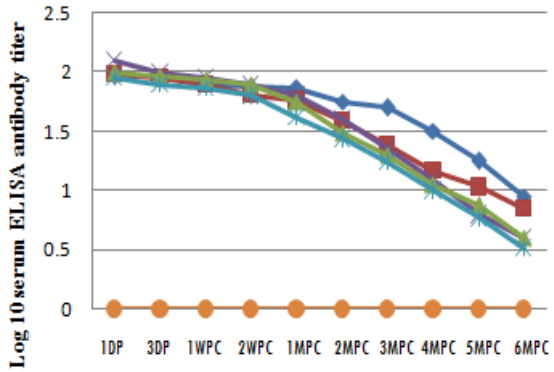

Fig. 4 Mean ELISA antibody titer of BVD (Genotype 1(◆) &2 (■)), IBR (▲), PI-3 (×) and BRS (✳) versus control (●) in sera of cows following vaccination with inactivated pneumogen-5 vaccine after calving.

These results agreed with the results obtained by the study which observed the characteristic decay of serum titers especially against for IBRV [20]. Duration of immunity elicited by aluminum hydroxide gel vaccine was short-lived and antibody concentration rapidly falls over periods of 4-6 months after administration [1, 7, 19]. Antibodies titers in colostrum persist at their higher level till the 3rd day post calving for all reference viruses contained in the vaccine as measured by SNT and confirmed by ELISA as shown in tables (5 & 6). The titer of antibodies detected in the colostrum of vaccinated cow is much increased than the titer of serum antibodies at the time of parturition. These results are agreed with the study which reported that the main colostrum neutralizing antibodies titer was almost double than that of the blood [15].

Table 5 Mean Neutralizing antibody titer in colostrum of cow dam following vaccination with inactivated pneumogen-5 vaccine.

Reference Virus	Mean neutralizing antibody titers	
	1*DPC	3DPC
BVDV-1	**2.1	2.0
BVDV-2	2.15	2.1
IBRV	2.3	2.25
PI-3V	2.4	2.34
BRSV	2.1	2.05
Control	0.0	0.0

* DPC: day post-calving. Mean neutralizing antibody titers expressed as $\log_{10}TCID_{50}$ on MDBK cell line.

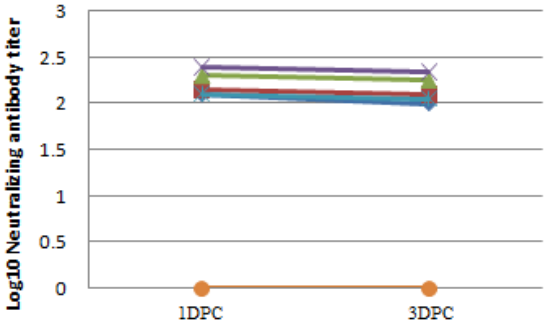

Fig. 5 Mean Neutralizing antibody titer of BVD (Genotype 1(◆) &2 (■)), IBR (▲), PI-3 (×) and BRS (✳) versus control (●) in colostrum of cow dam following vaccination with inactivated pneumogen-5 vaccine.

Table 6 Mean ELISA antibody titer in colostrum of cow dam following vaccination with inactivated pneumogen-5 vaccine

Reference Virus	Mean ELISA antibody titers	
	1*DPC	3DPC
BVDV-1	2.3	2.1
BVDV-2	2.23	2.2
IBRV	2.29	2.25
PI-3V	2.35	2.32
BRSV	2.17	2.11
Control	0.0	0.0

* DPC: day post-calving.

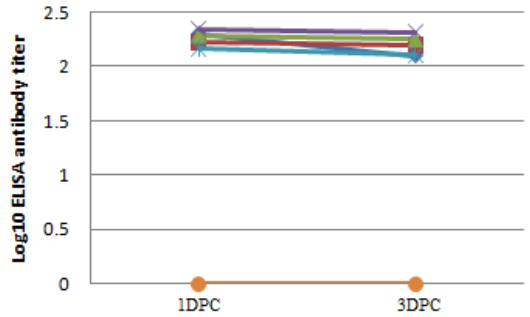

Fig. 6 ELISA antibody titer of BVD (Genotype 1(◆) &2 (■)), IBR (▲), PI-3 (×) and BRS (✳) versus control (●) in colostrum of cow dam following vaccination with inactivated pneumogen-5 vaccine

In conclusion, the prepared inactivated Pneumo-5 vaccine was found to be pure, sterile and safe. It was able to induce detectable levels of specific antibodies against BVDV (genotype 1 and 2), IBRV, PI-3V, and BRSV by the 2nd week post vaccination (6 weeks pre-delivery period) and continued till 4 month post-delivery as measured by SNT and 6 month post-delivery by ELISA. Antibodies titers in colostrum persist at their higher level till the 3rd day post calving for all reference viruses contained in the vaccine as measured by SNT and confirmed by ELISA that would probably protect newborn calves.

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تتبع الاستجابة المناعية الخلطية ضد لقاح نيموجين-5 فى الابقار العشائر وبعد الولادة

جبر فكرى الباجورى[1]، أيمن سعيد الهباء[1] ، مها رأفت عبدالفضيل[2] ، حسين متولى غالى[2]

[1] كلية الطب البيطرى- قسم الفيرولوجيا- جامعة بنها- القليوبية-مصر،

[2] معهد بحوث الامصال واللقاحات البيطرية بالعباسية-القاهرة-مصر

الملخص العربى

تم تحضير لقاح نيموجين-5 كلقاح مثبط متعدد العترات لفيروسات الاسهال البقرى الفيروسى النوع الجينى-1 والاسهال البقرى الفيروسى النوع الجينى-2 والتهاب الانف والقصبة المعدى والبارانفلونزا-3 والفيروس البقرى التنفسى المتضخم وذلك لمكافحة الامراض التنفسية المسببة لخسائر فادحة فى العجول. تم اختبار جودة اللقاح وتطبيقه فى الابقار العشائر قبل 8 أسابيع من الولادة وذلك لتقييم الاستجابة المناعية الخلطية لها تحت الظروف الحقلية. أعطى اللقاح مستوى واقى لعيارية الاجسام التعادلية المضادة لكل الفيروسات المستخدمة فى تحضيرة عند الاسبوع الثانى بعد التحصين أى قبل 6 أسابيع من الولادة وإستمرت حتى 4 شهور بعد الولادة بإستخدام اختبار المصل المتعادل وقد امتدت تلك الفترة الى 6 شهور بعد الولادة بإستخدام اختبار الإليزا. أعطى اللقاح مستوى واقى لعيارية الاجسام التعادلية المضادة لكل الفيروسات المستخدمة فى تحضيرة عند قياسه فى اللبن السرسوب حيث إستمر عند اعلى مستوى له حتى اليوم الثالث بعد الولادة بإستخدام اختبار المصل المتعادل حيث تاكدت تلك النتائج بإستخدام اختبار الإليزا. اكدت النتائج أن اللقاح المحضر عالى الفعالية و يعطى مناعة جيدة فى اللبن السرسوب قد تحمى العجول الوليدة المستهلكة لهذا السرسوب.

(مجلة بنها للعلوم الطبية البيطرية: عدد 23(2)، ديسمبر 2012:113-120)