

Preparation of Multivalent Inactivated Vaccine Against Some Bovine Respiratory Viruses Adjuvanted by *Nigella sativa* Oil and its Evaluation in Pregnant Buffaloes and Their Calves

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Abstract: A 15 pregnant buffalo-dam and their offspring were used to test the potency of the vaccine. This vaccine was prepared from bovine viral diarrhoea virus BVDV genotype I and II, Bovine herpes virus type 1 (BHV-1), Parainfluenza type 3 virus (PI3V) and Bovine respiratory syncytial virus (BRSV). The vaccine was adjuvanted with *Nigella Sativa* oil. In dams, the vaccine gave a high protective antibodies titer for all viruses from the 3rd week of injection. In pregnant buffalo cows, the antibody titer was remained high till the time of delivery. Also, the buffalo-calves responded speedily to the vaccine. They showed a protective antibody titer from the 1st week of delivery, especially that group delivered just within the 1st month after second vaccination for their dams. Buffalo-calves remained with a protective antibody level till the 28th week post delivery which means that the vaccine gave a long lasting duration of coverage to the animals. The vaccine is proved to be effective and safe in buffalo-cows and their offspring.

Key words: BRDC % Pregnant buffalo % *Nigella Sativa* Oil % Inactivated vaccines % Maternal immunity

INTRODUCTION

The respiratory viruses are commonly responsible for bovine respiratory disease complex (BRDC) include BHV-1 or Infectious Bovine Rhinotracheitis; IBR virus, BRS virus, BVD virus and PI3 virus [1-5]. BRDC has a complex and multifactorial etiology that usually is divided into three major categories: environmental factors, host factors and infectious factors [6-8].

All of these viruses are known to be involved in BRD solely or in synergism with each other and bacteria. Some of these viruses affect the lung parenchyma directly (BRSV and PI3V), while others act on the immune system (BVDV and BRSV) or local defenses (BHV-1), like the ciliated epithelium [6, 7]. Economic losses result from death, decreased performance of diseased animals, lowered weight gain, increased cost of gain, reduced carcass value and treatment costs [9]. Infection of pregnant cattle with non-cytopathic BVDV strain during

the first four months of gestation may result in a persistently infected (PI) calf [10].

In Egypt, since 1960, an attention was drawn to these viruses and their infections as the most significant threatens that causing economic losses [11-15].

BVD virus is a pestivirus of the family *Flaviviridae* [16]. This virus is of particular interest in studies of virus-induced apoptosis because of the existence of closely related 'pairs' of cytopathic (cp) and non-cytopathic (ncp) biotypes [17, 18]. The cp biotype of BVD virus detected in animals with mucosal disease may be the result of a mutation of the ncp biotype (hence the term 'virus pair'), or may be due to super infection. The cp biotype is believed to kill these immunotolerant animals because their immune systems fail to control virus multiplication [19,20].

The vaccines with *Nigella sativa* oil adjuvant are of good quality and immuno-stimulant [21-23]. The extract of *Nigella sativa* seeds showed antibacterial, antioxidant,

analgesic, anti-inflammatory and antifungal effects [24-26]. Boostering the cattle annually with certain vaccines might result in transfer of high levels of antibodies to their calves [27].

The present work aimed to prepare a multivalent vaccine of BVDV genotype I and II, BHV-1, PI3V and BRSV, adjuvanted with the *Nigella Sativa* oil. This vaccine was evaluated in field pregnant buffalo cows and their calves after delivery.

MATERIALS AND METHODS

Animals: Fifteen apparently healthy pregnant buffalo-cows were used for field evaluation of the prepared polyvalent inactivated vaccine adjuvanted with *Nigella sativa* oil. Animals were at late stage of pregnancy (third trimester). Also, their offspring (15 calves) were used to detect the antibodies titers of the five viruses. The experiment was kindly conducted in the farm of Dair Beramous, Wadi Elnatroun; Alexandria, Egypt.

Viruses

BVD:

Genotype I, Egyptian strain [Iman strain] of 10^7 TCID₅₀/ml was isolated from at Tahrir province [12].

Genotype II, strain 125 C of 10^7 TCID₅₀/ml which kindly obtained from the National Animal Disease Center, Ames, Iowa, USA.

BHV-1: Virus, a reference Egyptian strain [Abou Hammad strain] of 10^8 TCID₅₀/ml was isolated and identified [13].

PI3: Virus, a reference strain of Parainfluenza type 3 (strain 45) of 10^8 TCID₅₀/ml which isolated and identified [11].

BRS: virus, strain 375L (10^6 TCID₅₀/ml) was kindly supplied by SmithKline Beecham Animal Health, Norden Laboratories, USA.

Samples: Blood samples were collected from all calves and pregnant buffalo-cows before and after vaccination and serum samples were separated. Calves' serum samples were collected until antibodies titre became inprotective. Offsprings' serum samples were collected after ingestion of colostrums from calves [post-colostral samples]. Then, calves were periodically examined post delivery for detection of antibodies for the vaccine.

Inactivant: Binary ethyleneimine (BEI) is used as inactivant by 0.01% of whole virus solution for each virus.

Sodium Thiosulphate: It was used to stop the action of BEI.

Adjuvant: *Nigella Sativa* oil was obtained from Farco pharmaceutical company, Cairo, Egypt.

Merthiolate (Thiomersal): It was used as a preservative.

Methods: *Serum neutralization test*, serum samples were tested by SNT [28]. The serum neutralizing antibody titres were calculated following the formula of Reed and Muench [29].

Evaluation of the Vaccine in Pregnant Dams:

1-Purity test: It was performed in accordance with the USA Code of Federal Regulation (CFR 1987), product testing code numbers 113-26, 27 and 30 to be free from any extraneous contaminations as bacteria, mycoplasma, fungi and viruses.

Safety Test: Test was achieved using 4 male adult healthy buffalo-calves. Two of them were inoculated intramuscularly with 10 times of the vaccination dose (50 ml) of the prepared vaccine according to the USA CFR (1987), product testing code number 113-41. The other two calves were inoculated with the physiological saline solution by same dose and kept under observation as non-vaccinated control group. All animals were kept under observation for 2 weeks post vaccination for detection of any clinical changes and recording the rectal temperature.

Potency Evaluation: It was applied on the antibodies titres response of all viruses as well as the duration of protective antibodies titres level in the serum of offspring post vaccination of the dams by the prepared vaccine. Fifteen apparently healthy pregnant buffalo-cows were used for field evaluation of the prepared polyvalent inactivated vaccine adjuvanted with *Nigella sativa* oil. They were at late stage of pregnancy (third trimester). Serum samples were obtained from buffaloes-cows on three occasions; before inoculation of the first dose of the vaccine, prior to administration of the second dose of the vaccine and during the time of parturition. Also, serum samples were obtained from unvaccinated (control animals) buffalo-cows at the same intervals. Serum samples were obtained from the newborn calves till 7 months post delivery.

Statistical Analysis: Data were analyzed using an analytical software program Microsoft® Office Excel 2003 by using ANOVA test to assess the significance between the serum neutralizing titers of the viruses for calves delivered on the 1st, 2nd and 3rd month post 2nd dose of vaccination.

RESULTS

As shown in Table 1, the mean neutralizing antibodies of the five viruses expressed in log₁₀ for the dams. It was observed that a protective level of antibodies titres from the 3rd week post vaccination for all viruses is clear. BHV-1, PI3V and BRSV were of the fastest response (0.60, 0.55 and 0.65, respectively). They showed a protective response from the 2nd week. Then, BVDV with its both types I and II starts to show a protective level (1.55 and 0.85, respectively) from the 3rd week post vaccination. This protective level of the antibodies for all viruses lasts till the last serum samples which were at 12th week post vaccination in pregnant buffalo cows. Also, the high protective level of all viruses till the 12th week post vaccination (BVDV I, 1.85; BVDV II, 1.70; BHV-1, 2.00; PI3V, 2.00 and BRSV, 1.95) is evident.

The antibodies titer expressed in log₁₀ for the offspring post delivery time and receiving colostrums are shown in Table 2. We had 3 groups depending on the time of delivery in relation to the second dose of the vaccine given to the dams. All calves in all three groups show a high protective level for all viruses from the 1st week post delivery. In groups A (delivery within 1st month post vaccination) and B (delivery after 2nd months post vaccination), calves still have a high protective level of the antibodies till the end of the experiment (28th weeks). While, the calves in the third group (delivery after 3rd month post vaccination) showed a protective level for the 4 viruses till the 20th week post delivery. A very high significance (P> 0.001) difference was found between the SN titers for each same virus in calves delivered during the 1st and the 2nd and 3rd months post 2nd vaccination. Except in the group of the BVDII, there was a high significance difference (P>0.01) between the SN titers.

Control groups for both dams and offspring showed no or lower antibody titers than the protective level of the vaccine.

Table 1: Mean neutralizing antibodies of dams for BVD I and II, BHV-1, PI3V and BRSV expressed in log₁₀

Groups	Viruses	0 wpv (1 st vaccination)	1 wpv	2 wpv (2 nd vaccination)	3 wpv	4 wpv	6 wpv	8 wpv	12 wpv
Dams vaccinated with the vaccine	BVDV I	0.20	0.25	0.40	1.00	2.10	2.00	1.95	1.85
	BVDV II	0.00	0.12	0.25	0.85	1.80	1.90	1.85	1.70
	BHV-1	0.10	0.35	0.60	1.20	2.25	2.25	2.20	2.00
	PI3V	0.30	0.40	0.55	1.35	2.35	2.10	2.00	2.00
	BRSV	0.25	0.45	0.65	1.25	2.10	2.05	2.00	1.95

wpv weeks post vaccination

Table 2: Mean neutralizing antibodies of offspring for BVD I and II, BHV-1, PI3V and BRSV expressed in log₁₀

Groups	Delivery time	No. of delivered calves	Viruses	Weeks post delivery											
				1 wpd	2 wpd	3 wpd	4 wpd	8 wpd	12 wpd	16 wpd	20 wpd	24 wpd	28 wpd		
A	1 st month post 2 nd of vaccination	7	BVDV I	2.10	2.10	2.20	2.10	2.00	1.95	1.90	1.65	1.25	1.00	dose	
			BVDV II	1.80	1.85	1.80	1.75	1.75	1.70	1.70	1.50	1.20	1.00		
			BHV-1	2.20	2.00	1.95	1.90	1.85	1.85	1.80	1.70	1.30	1.10		
			PI3V	2.10	2.20	2.10	2.10	2.05	2.00	1.95	1.80	1.20	1.20		
			BRSV	2.00	2.00	2.00	1.95	1.85	1.85	1.80	1.70	1.50	1.10		
B	2 nd month post 2 nd dose of vaccination	5	BVDV I	2.00	2.20	2.10	2.20	2.10	2.00	1.95	1.85	1.55	1.10		
			BVDV II	1.95	2.00	2.10	1.95	1.85	1.75	1.75	1.60	1.40	1.00		
			BHV-1	2.10	2.20	2.25	2.20	1.95	1.85	1.80	1.75	1.55	1.20		
			PI3V	2.20	2.25	2.30	2.20	2.00	1.95	1.90	1.85	1.70	1.40		
			BRSV	2.00	2.10	2.00	2.00	1.90	1.90	1.85	1.75	1.55	1.10		
C	3 rd month post 2 nd dose of vaccination	3	BVDV I	2.00	1.90	1.95	1.85	1.65	1.55	1.25	0.90	0.45	0.25		
			BVDV II	1.75	1.65	1.80	1.70	1.25	1.10	1.00	0.55	0.25	0.30		
			BHV-1	1.90	1.95	1.90	1.80	1.65	1.35	1.10	0.90	0.65	0.35		
			PI3V	2.10	2.20	2.20	1.90	1.80	1.60	1.30	1.10	0.80	0.60		
			BRSV	1.80	1.95	2.00	1.85	1.60	1.40	1.10	0.90	0.60	0.45		

wpd weeks post delivery

DISCUSSION

Vaccination against respiratory viruses seems to be a sensible and prudent approach, especially when we can forecast moments of important immunity suppression associated with these viruses, such as during the weaning and grouping of young animals from different herds [6].

This study succeeded to produce a multivalent vaccine containing BHV-1, BRS, BVD I and II and PI3 viruses. This vaccine is adjuvanted by *Nigella Sativa* oil thus considered the first vaccine involves these viruses and containing a natural adjuvant.

Many studies approved the efficacy of the multivalent vaccine containing these viruses on the cows and their calves and this study for the first time was conducted on buffalo-cows and their offspring.

The produced multivalent vaccine gave a protective antibodies level in dams after the 3rd week of vaccination (1 week post 2nd inoculation of vaccine). These high titers and fast response are due to the use of *Nigella Sativa* oil as an adjuvant. These agree with many authors [15,22,23,30] who found that the vaccinated calves against these respiratory pathogens with vaccines adjuvanted by oil especially the *Nigella Sativa* oil gives a better neutralizing antibody response than to the aluminum gel vaccines.

Oil emulsions trap antigen and release it over a larger period producing a more pronounced increase in the immune response after one dose than do alum adjuvant. Oil emulsions increase the circulation and trap of lymphocytes in draining lymphoid tissue as well as oil adjuvant may affect the immune response by enhancing the physical presentation of the antigen to macrophages [24-26].

Buffalo-calves that were delivered after 3 months of vaccination of their dams remained protected to a short time (4-5 months) than that delivered after one month of second vaccination of their dams (6-7 months). These mean that passive colostral antibodies lost one-half of their remaining antibody titre every 21 days and serologically responded to vaccine. These results are similar to that obtained by Fulton and his colleagues 2004 who found the maternal antibodies in calves remained within the protective level from 3-6 months when it comes from vaccinated dams with inactivated vaccine at the last gestation period of pregnancy.

In conclusion, the vaccine containing BVDV, BHV-1, PI3V and BRSV and adjuvanted with *Nigella Sativa* oil shows a very pronounced protective antibody level for both buffalo cows and their offspring. Offspring who were delivered within 1 month of second vaccination to their

dams showed a protective antibody level from the first week of delivery. These results lead to minimize the doses of vaccination and cost of fattening.

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