Journal of Applied Veterinary Sciences, 4(2): 35-40 (2019.)
ISSN: online; 2090-3308, print: 1687-4072
Journal homepage : https://javs.journals.ekb.eg

ENHANCEMENT OF PNEUMO-4 VACCINE EFFICACY BY USING OF CARBOMER AS AN ADJUVANT

Maha Raafat Abed EL-Fadil*; Rasha, I. EL-Hawary; Walaa S. Shabana ; Ekbal M. Farok and Naglaa I. Ali

Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo, Postal No: 11381 P.O. Box 131, Egypt.
* Corresponding author: Maha Raafat Abed EL-Fadil; email: drmaharaafat198015@gmail.com

ABSTRACT

The present study aimed to prepare a combined inactivated pneumo-4 vaccine containing bovine viral diarrhoea, infectious bovine rhinotracheitis (IBR), parainfluenza-3(PI-3) and bovine respiratory syncytial virus (BRSV) adjuvanted with carbomer and compare the animal protection scope with its product mate Pneumo-4 adjuvanted alhydragel . Carbomer adjuvant stimulated higher levels and longer lasting antibody to pneumo-4 vaccine than vaccines of equivalent antigenic content containing aluminium hydroxide gel adjuvant. Quality control results proved that the pneumo-4 vaccine adjuvanted either by aluminium hydroxide gel adjuvant or carbomer (different concentration 0.2,0.5 and0.8 %) was pure and completely safe to be used in calves without any abnomalities.Potency test was performed on five groups of five calves for each group,Where the first group was vaccinated with pneumo-4 vaccine adjuvant with carbomer 0.2(formula 2), second group was vaccinated with pneumo-4 vaccine adjuvant with carbomer 0.5 (formula 3), third group was vaccinated with pneumo-4 vaccine adjuvant with carbomer 0.8(formula 4),,The fourth group vaccinated with pneumo-4 vaccine with aluminum hydroxide gel(formula 1), and the fifth last group was left as non vaccinated control group.compared humoral immune response using serum neutralization and ELISA expressed by neutralization index of different vaccine formulation was revealed that The prepared pneumo-4 vaccine adjuvant with carbomer 0.5 proved to be highly potent formmula along 6 month after second booster dose applying. Also pneumo-4 vaccine adjuvant with carbomer was showed cellular immunity reaction higer than pneumo-4 vaccine with aluminum hydroxide gel using Estimation of nitric oxide activity test.

Original Article:
https://dx.doi.org/10.21608/javs.2019.62653

Received 03 Aug., 2019.
Accepted 18 Sept,.2019.
Published in October 2019.

Keywords: Adjuvant, aluminium hydroxide gel, BRSV, carbomer, pneumo-4 vaccine.

J. Appl. Vet. Sci., 4(2): 35-40.

INTRODUCTION

Pneumoentritis viral (Bovine viral diarrhea virus(BVDV) , infectious bovine rhinotracheitis virus(IBRV) , Parainflunza -3 virus(PI-3) and Bovine respiratory synctial virus (BRSV)) are important syndromal evidence which criminated in many economical losses and immunological drawbacks ((**Durham and Hassard, 1990**). Control of bovine respiratory disease complex (BRD) is a major focus of veterinary health programs (**Stokka and Edwards, 1990**). Inactivated pneumo-4 vacccine is locally prepared and widely used in Egypt for control of viral pneuoentritis syndrome (**Samira , *et al.*, 2001**). Carbomers have been evaluated as adjuvants in veterinary vaccines. These reports suggest that carbomers are not harmful in mammals and are more effective than antigen alone**(Mumford, *et al.*, 1994)**.

Carbopol or cabomer in animal models results in systemic adjuvant activity including strong pro-inflammatory type-1 T-cell (Th1) polarization. **(Schwabe et al., 1977).** Carbomer adjuvant has been shown to enhance the strength and duration of antibody responses stimulated by inactivated vaccine (**Zhang et al" 2018**).

The aim of this work was performed to prepare a safe potent polyvalent inactivated vaccine containing BVD-1, BVD-2, IBR, PI-3 and BRS viruses by using carbomer as an adjuvant and apply comparative study between its result and result of vaccine adjuvanted with alhydragel.

MATERIALS AND METHODS

1.Viruses and related antisera

Bovine viral diarrhoea virus genotype (BVD)

A local Egyptian strain (Iman strain) with a titre of $10^{6.5}$ $TCID_{50}$/ml, Infectious Bovine Rhinotracheitis virus (IBRV): A local isolate of "Abou Hammad strain" with a titre of $10^{7.5}$ $TCID_{50}$/ml,and Parainfluenza-3 virus **(PI-3V**): Reference Egyptian strain of PI-3 (Strain 45) with a titre of 10^{8} $TCID_{50}$/ml in addition to Bovine respiratory syncytial virus: Reference strain of BRSV (375L) with a titre of $10^{6.5}$ $TCID_{50}$/ml**)** All viral strains were propagated and tittered on Madin Darby Bovine Kidney (MDBK) cell culture , which has been proved free of any infectious agents especially non cytopathic strain of BVD virus . These viruses were supplied by Rinderpest Like Diseases Research Dept, Veterinary Serum and Vaccine Research Institute Abbasia, Cairo (VSVRI). And used in vaccine preparation and SNT.

2. Inactivated viral suspension preparation

according to **Bahnemann, (1975).**

3. Vaccine preparation

3.1.Carbomer adjuvant

Carbomer is a term used for a series of polymers primarily made from acrylic acid. The Carbomers are white fluffy powders, was disolved in d.d.w then sterilized by outoclaving that in different conc. (0.2,0.5 and 0.8 %) in final dose . They adsorbe Iral partile on surface and also used to keep emulsions from separating into their gel and liquid components. **(Zhang J *et al.,* 2018).**

3.2.Preparation of vaccine formulas

Part of inactivated virus fluids were mixed together with alhydragel to make formula (1) according to **(Samira *et al.,* 2001)** and other parts mixed with carpabol with diferent concentrations (0.2,0.5and 0.8) according to make other formuls (2,3,4) respectively .The PH was adjusted to 7.5. Thiomersal was added as a vaccine preservative at final concentration of 0.0001% and distributed in sterile bottles of 50 ml capacity.

4. Quality control of the prepared vaccine

It were performed in accordance to **USA Code of Federal Regulations, CFR (1987)**.

4.1. Expirmental desgien *:*

Fifteen male calves were used in this study and divided into 5 groups, three calves for each group as follow:

Group (1): Each calf was intramuscularly immunized with 5 ml of formula (1) of pneumo-4 vaccine by two injections, with 2 weeks interval.

Group (2): Each calf was intramuscularly immunized with 5 ml of formula (2) of pneumo-4by two injections, with 2 weeks interval.

Group (3): Each calf was intramuscularly immunized with 5 ml formula (3) of pneumo-4by two injections, with 2 weeks interval.

Group (4): Each calf was intramuscularly immunized with 5 ml formula (4) of pneumo-4by two injections, with 2 weeks interval.

Group (5): Consists also of three calves and this group was left as non vaccinated contact control group.

4.2. Evaluation of humoral immune response

Serum neutralization test

It was performed on MDBK cell lineacccording to (**Fulton *et al.,*1995**) and calculated by **Reed and Muench (1938). Enzyme linked immunosorbent assay (ELISA):** It is carried out according to **Voller *et al.*, (1976)** .

4.3. Evaluation of cellular immune response

Estimation of nitric oxide activity

It was carried out According to **Rajaraan, *et al* ,. (1980)**

RESULTS

Table 1: Serum neutralizing titres against BVD, IBR, PI-3 and BRS viruses expressed in NI in calves vaccinated with Aluminium hydroxide gel adjuvanted pneumo-4 vaccine

Time	G1				G5
	BVDV	IBRV	PI3	BRSV	Respond negatively lower than protection a ranged between NI (0.20-0.40) .
0 DPV	0.05	0.02	0.00	0.03	
14 DPV	0.20	0.35	0.30	0.25	
21 DPV	1.22	1.33	1.28	1.19	
29 DPV	1.9	2.1	2.05	1.91	
60 DPV	1.72	1.93	1.98	1.7	
90 DPV	1.50	1.72	1.79	1.49	
120 DPV	1.31	1.56	1.60	1.3	
150 DPV	1.09	1.3	1.3	1.1	
180 DPV	0.91	1.12	1.15	0.95	

DPV Days Post Vaccination.

PI-3 parainflunza 3.

BVD Bovine viral diarrhea

IBR infecticous bovine rhino tracheitis .

BRS Bovine respiratory synthial.

Table 2: Serum neutralizing titres against BVD, IBR, PI-3 and BRS viruses expressed in NI in calves vaccinated with carbomer (0.2%) adjuvanted pneumo-4 vaccine

Time	G2				G5
	BVDV	IBRV	PI3	BRSV	Respond negatively lower than protection a ranged between NI(0.20-0.40)
0 DPV	0.05	0.02	0.00	0.03	
14 DPV	0.19	0.33	0.28	0.24	
2 DPV	1.20	1.32	1.26	1.17	
29 DPV	1.85	2.09	2.04	1.9	
60 DPV	1.61	1.91	1.97	1.6	
90 DPV	1.48	1.71	1.78	1.47	
120 DPV	1.30	1.54	1.58	1.1	
150 DPV	1.08	1.2	1.1	1.00	
180 DPV	0.85	1.11	1.13	0.9	

DPV Days Post Vaccination.

PI-3 parainflunza 3.

BVD Bovine viral diarrhea

IBR infecticous bovine rhino tracheitis

BRS Bovine respiratory synthial .

Table 3: Serum neutralizing antibody titres against BVD, IBR, PI-3 and BRS viruses expressed in NI in calves vaccinated with carbomer (0.5%) adjuvanted pneumo-4 vaccine

Time	G3				G5
	BVDV	IBRV	PI3	BRSV	Respond negatively lower than protection a ranged between NI(0.20-0.40)
0 DPV	0.05	0.02	0.00	0.03	
14 DPV	0.25	0.40	0.35	0.30	
21 DPV	1.27	1.38	1.33	1.24	
29 DPV	1.95	2.15	2.1	1.96	
60 DPV	1.77	1.98	2.03	1.75	
90 DPV	1.55	1.77	1.84	1.54	
120 DPV	1.26	1.61	1.65	1.8	
150 DPV	1.14	1.8	1.8	1.6	
180 DPV	0.96	1.17	1.2	1.1	

DPV: Days Post Vaccination.

PI-3 :parainflunza 3.

BVD: Bovine viral diarrhea

IBR :infecticous bovine rhino tracheitis .

BRS : Bovine respiratory synthial .

Table 4: Serum neutralizing antibody titres against BVD, IBR, PI-3 and BRS viruses expressed in ND in calves vaccinated with carbomer (0.8%) adjuvanted pneumo-4 vaccine

Time	G4				G5
	BVDV	IBRV	PI3	BRSV	Respond negatively lower than protection a ranged between NI(0.20-0.40)
0 DPV	0.05	0.02	0.00	0.03	
14 DPV	0.25	0.40	0.35	0.30	
21 DPV	1.27	1.38	1.33	1.24	
29 DPV	1.95	2.15	2.1	1.96	
60 DPV	1.77	1.98	2.03	1.75	
90 DPV	1.55	1.77	1.84	1.54	
120 DPV	1.26	1.61	1.65	1.8	
150 DPV	1.14	1.8	1.8	1.6	
180 DPV	0.96	1.17	1.2	1.1	

DPV Days Post Vaccination.

PI-3 parainflunza 3.

BVD Bovine viral diarrhea

IBR infecticous bovine rhino tracheitis .

BRS Bovine respiratory synthial

Table 5: Nitric oxide mean reading(m/ml) of different sera samples from different groups calf inoculated by different pneumo-4 vaccine formulations.

Time	Nitric oxide reading(m/ml)				
	G1	G2	G3	G4	G5
0 DPV	2.25	2.1	2.4	2.42	0.073
3 DPV	4.23	3.77	5.67	5.68	0.061
7 DPV	5.28	4.48	6.32	6.34	0.06
10 DPV	5.78	3.09	6.28	6.27	0.058
14 DPV	4.02	2.65	5.25	5.26	0.057
21 DPV	2.27	1.26	3.79	3.8	0.054
28 DPV	1.18	0.98	2.34	2.35	0.049

DPV: Days Post Vaccination.
G1 group1inoculated by formula 2.
G2 group1inoculated by formula3.
G3 group1inoculated by formula4.
G4 group1inoculated by formula1.
G5 control diarrhea.

Table 6: Comparative ELISA mean BVD, IBR, PI-3 and BRS virus antibody and SNT index in calves vaccinated with carbomer (0.5%) and aluminium hydroxide gel adjuvanted pneumo-4 vaccine.

Time	Newly developed Pneumo-4 vaccine adjuvanted carbomer(0.5)				Pneumo-4 vaccine adjuvanted with Aluminium hydroxide gel			
	IBR	BVD	PI-3	BRS	IBR	BVD	PI-3	BRS
0 DPV	0.25	0.21	0.21	0.20	0.21	0.18	0.18	0.20
14 DPV	0.55	0.40	0.53	0.40	0.50	0.35	0.49	0.39
29 DPV	2.2	2.05	2.26	1.99	2.14	2.01	2.07	1.94
60 DPV	2.0	1.80	2.01	1.80	1.97	1.84	1.99	1.80
90 DPV	1.85	1.65	1.78	1.63	1.81	1.60	1.73	1.63
120 DPV	1.66	1.49	1.64	1.50	1.61	1.42	1.60	1.41
150 DPV	1.44	1.2	1.44	1.25	1.40	1.18	1.39	1.18
180 DPV	1.25	1.8	1.21	1.05	1.21	1.05	1.15	1.0

DPV Days Post Vaccination. PI-3 parainflunza 3. BVD Bovine viral diarrhea
IBR infecticous bovine rhino tracheitis . BRS Bovine respiratory synthial

DISCUSSION

Vaccination is the most efficient and cost effective method of controlling infectious diseases in animals .Also control of such infectious disease particularly viral infections would not have been possible without the use of safe and effective vaccines (**Yavru *et al.*, 2005**).

The appearance of respiratory disease may be due to stress factors as bad environment , transportation , accumulation of ammonia and excessively high humidity in closed areas which lower the resistance of animal which enhanced the multiplication of microorganisms **(Bickert and Herdt, 1985).** Infectious agents associated with bovine respiratory diseases include four viruses which are BVD, IBR, PI-3 and BRS was used in preparation of inactivated pneummo-4 vaccine in Egypt **(Samira *et al.*, 2001).** Due to its low reactivity, non virucidal nature and efficacy in one shot vaccination schedules carbopol, a lightly cross-linked polymer of acrylic acid, has become a widely used adjuvant in the veterinary field **(Diamantstein *et al.*, 1971**).

In spite of wide applications, there is little information available relating to the type and magnitude of adaptive immune response induced by carbopol compared to other well characterized adjuvants. Specifi-cally, the contribution of carbopol to enhance/modulate cellular immunity . Although the precise immune pathways mediated by carbopol remains to be understood, our data suggest that addition of carbopol t0 vaccine improves cellular responses by inducing early IFN-g-producing cells and by preferen-tially driving T cell differentiation to effector phenotypes. The bias towards a Th1 phenotype is supported by human preclinical studies where carbopol generated strong cellular responses to soluble HIV-1 envelope glycoprotein along with high IgG2a antibody titres **(Krashias *et al.*, 2010**). Th1 responses are associated to increased CD8T cell proliferation (**Ekkens *et al.*, 2007),** production of opsoniz-ing antibodies (**Lefeber *et al.*, 2003**) and maximizing macrophage killing activity (**Stout *et al.*, 2005**),

This study was carried out to compare inactivated pneumo-4 vaccines,containing Carbomer adjuvant with an inactivated vaccine containing aluminium Hydroxide gel as adjuvant and to assess the acceptability of Carbomer as an adjuvant For cattle. **(Mumford *et al.*, 1994).** Currently different prepared vaccine formulas pass succesivly through sterility and safety testes.

The potency evaluation of the different prepared formulas of polyvalent viral vaccine in calves represented in table (1,2) revealed that all vaccinated animals developed serum neutralizing antibody titers (SN antibody) expressed as ND reached their peak at first 28 day post inoculation remained stable higher than the minimal acceptable titer of protective level until the end of experiment to the three formulation 1,3and 4. Such data are similar to those obtained by (**Bittle, 1968**) who recorded that the BVD antibody level 0f 1:8 diluation (ND 0.9) was protective (**Zuffa and Feketeova, 1980**) and Those authors reported that the minimal acceptable titer of neutralizing antibodies was 1:4 diluation (ND0.6) was protective against IBR, PI-3 and BRS viruses. compared with the control non vaccinated group (group 2) that showed no neutralizing antibody response.

Table 1,3 and 4 was clarified that effectiveness of formula 2 of carbomer posses the most superior humoral immunological reaction and nearly resempling result formula 3 although its highest concentration.so economical and biological we support formula 2. Table 6, the formula 2 with (0.5 % cabomere)vaccine was possed a good ELISA indeces than formula 4 with ordinary used while table 5 clarified that at cellular response level measured by Nitric oxide activity test mean reading , cabomere has a highest activity which reflect highest cellular immunity activity, safety margin to be inoculated without any local or systemic allergic reaction is sure as it was used widely in cosmetics issue due to its clear biodegradability that agreed with **Rajaraman *et al* ., (1980) ; Mumford *et al*., (1994) and Zhang (2018**). Also its mode of action to be used as an adjuvant was trap antigen and release it over period producing satisfied immune response ccomparing with aluminium hydroxid gel but with best economic view .

CONCLUSION

In conclusions, the prepared formula 3 (polyvalent inactivated respiratory viral Pneumo-4 vaccine containing of BVD,IBR, PI-3 and BRS viruses adjuvanted with carbomer 0.5%) was proved to be pure, fully safe and highly potent economic vaccine formula to be use .

Declaration of Competing interest

On behalf of all authors, I hereby declare that no conflict of interest may interfere with the publication of the manuscript.

REFERENCES

BAHNEMANN, H.G. 1975. Binary ethyleneimine as an inactivant for FMD and its application for vaccine production.Arch. Virol. (47): 46-56.

BARTELING, S. J.; VAN MAANEN, C.; YADIN H. AND ANEMAET, D. A. J.1990. A FMD vaccine bank. Purified inactivated antigen stored at ultra-low European commission for the Control of foot and Mouth disease. Session of the research group of the standing technical committee, Lindholm: 172-177.

BICKERT,E.A. AND HERDT, T.H. 1985. Environmental aspects of dairy calves housing comperdium on continuing education for the practicing veterinarians, (7): 5309.

BITTLE, J. L. 1968. Vaccination for bovine viral diarrhea mucosal disease.J. Amer. Vet. Med. Assoc., 192 (6): 861-865.

DIAMANTSTEIN, T., WAGNER, B., BEYSE, I., ODENWALD, M.V., SCHULZ, G. 1971. Stimulation of humoral antibody formation by polyanions. I. The effect of polyacrylic acid on the primary immune response in mice immunized with sheep red blood cells. Eur. J. Immunol. 1, 335–340.

DURHAM, P. J. K. AND HASSARD, L. E. 1990. Prevalence of antibodies to IBR, PI-3, BRSV and BVD in cattle in Saskatchewan and Alberta.Cand. Vet. J., 31: 815-820.

EKKENS, M.J., SHEDLOCK, D.J., JUNG, E.H., TROY, A., PEARCE, E.L., SHEN, H., PEARCE, E.J. 2007. Th1 and Th2 Cells Help CD8 T-Cell Responses. Infect. Immun. 75, 2291–2296.

FULTON, R.W.; CONFER, A.W.; BURGE, L.J.; PORINO, L.J.; D'OFFAY, J.M.; PAYTON, M.E. AND MOCK, R.E. 1995. Antibody viral vaccines containing BHV, BVD, PI-3 and BRSV immunogens and subsequent revaccination at day 140. Vaccines, 13: 725-733.

KNEZEVIC, N.; KOSANOVIC, P. AND ROGAN, D. 1990. Immunoprophylaxis of respiratory diseases of cattle with inactivated vaccine. III. Study of the immunogenicity of a bivalent inactivated oil vaccine against IBR and PI-3. Veterinarski Glesnik, 44 (7): 503-512.

KRASHIAS, G., SIMON, A.K., WEGMANN, F., KOK, W.L., HO, L.P., STEVENS, D., SKEHEL, J., HEENEY, J.L., MOGHADDAM, A.E., SATTENTAU, Q.J. 2010. Potent adaptive immune responses induced against HIV-1 gp140 and influenza virus HA by a polyanioni carbomer. Vaccine 28, 2482–2489

LEFEBER, D.J., BENAISSA-TROUW, B., VLIEGENTHART, J.F.G., KAMERLING, J.P., JANSEN, W.T.M., KRAAIJEVELD, K., SNIPPE, H. 2003. Th1-directing adjuvants increase the immunogenicity of oligosaccharide-protein conjugate vaccines related to Streptococcus pneumoniae type 3. Infect. Immun. 71, 6915–6920.

MUMFORD JA, WILSON H, HANNANT D, JESSETT DM.1994. Antigenicity and immunogenicity of equine influenza vaccines containing a Carbomer adjuvant.Epidemiol Infect. Apr;112(2):421-37.

MIHAJLOVIC, B.; CVETOKOVIC, A.; KUZMANOVIC, E.; VUHOBRAT, D.; ASUUIN, R. AND LAZUREVIC, T.1979. Comparison of vaccines against respiratory diseases of cattle infectious bovine rhinotracheitis and parainfluenza-3. Veterinarski Glasnikyugoslavia, 33 (1): 33- 38.

RAJARAAN, V.;NONNECKE, B.J.;; FRANKLIN, S.T.;HAMELL, D.C.AND HORST, R.L.1980. Effect of vitaminA and E on nitric oxide prodution by blood mononuclear leuckoytes from neonatal calf. J. Immunol. 17, 342–349.

REED, L. J. AND MUENCH, A. 1938. A simple method of estimating fifty percent and points.Amer. J. Hyg., 27: 443-445

SAMIRA, S.T.; EL-SABBAGH, M.M.A. AND GHALY, H.M. 2001. Preparation of combined inactivated BVD, IBR, PI-3 and respiratory syncytial Virus (BRSV). J. Egypt. Vet. Med. Ass., 61 (4): 251-263.

SCHWABE, C.W.; RIEMANN, H. AND FRANTI, F. 1977. Epidemiology in veterinary practice. 1st Ed. Lee and Febiger, Philadelphia

STOKKA, G.L. AND EDWARDS ALVIN, L. 1990. Revaccination of stressed calves with a multiple polyvalent vaccine (IBR, BVD, BRSV). Agr. Pract 11 (5): 18-20.

STOUT, R.D., JIANG, C., MATTA, B., TIETZEL, I., WATKINS, S.K., SUTTLES, J.2005. Macrophages sequentially change their functional phenotype in response to changes in microenvironmental influences. J. Immunol. 175, 342–349.

USA CODE OF FEDERAL REGULATIONS 1987. Animal products. No. 0 Parts 1 to 199 Published by the Office of Federal Register National Archives and Records Administration

VOLLER, A., BIDWELL, D.E. AND ANNBARLETT, M. 1976. Enzyme immuno assays in diagnostic medicine, theory and practice.Bull.World. Health, organ, 63: 55-65.

YAVRU S, SIMSEK A, YAPKIC O, KALE M.2005. Serological evaluation of viral infections in bovine respiratory tract. Acta Vet. (Beograd), 55(2/3): 219–226.

ZHANG J1, WANG M1, ZHOU N1, SHEN Y1, LI Y2. 2018. Evaluation of carbopol as an adjuvant on the effectiveness of progressive atrophic rhinitis vaccine.Vaccine. Jul 16;36(30):4477-4484. doi: 10.1016/j.vaccine.06.023. Epub Jun 13.

ZUFFA, A. AND FEKETEOVA, N. 1980. Protective action of inactivated adjuvant IBR vaccine against experimental infection. Vet. Med., 25 (1): 51-61.

.

How to cite this article:

Maha Raafat Abed EL-Fadil; Rasha, I. EL-Hawary; Walaa S. Shabana ; Ekbal M. Farok and Naglaa I. Alie, 2019. nhancement of Pneumo-4 Vaccine Efficacy by Using of Carbomer as an Adjuvant**.** Journal of Applied Veterinary Sciences, 4(2): 35-40.

DOI : https://dx.doi.org/10.21608/javs.2019.62653