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IMPROVING OF COMBINED INACTIVATED RESPIRATORY VIRAL VACCINE PNEUMO-3 BY USING OF MONTANIDE OIL ISA 206 AS AN ADJUVANT

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A B S T R A C T

The present study aimed for improving of newly improved combined inactivated vaccine containing bovine viral diarrhoea virus, infectious bovine rhinotracheitis and parainfluenza-3 viruses using Montanide oil ISA 206. Potency test was performed on three groups of calves four for each group, first group was vaccinated with newly developed vaccine adjuvant with Montanide oil ISA 206 and the second group vaccinated with pneumo-3 vaccine adjuvant with Aluminum hydroxide gel, while the third group was left as non-vaccinated control group. Explaining of humoral immune response by using of serum neutralization test in group 1 revealed that the serum neutralizing antibody titres were developed more higher than the minimal acceptable titre of protective level at one month post second vaccination in newly improved vaccine adjuvant with Montanide oil ISA 206 and lasts for 12 months post vaccination to IBRV, for 9 months to BVDV and lasts for 10 months to PI-3V. Also the results of calves vaccinated with aluminum hydroxide gel adjuvanted vaccine in group 2 showed the highest antibody titers at 7 months for IBRV and PI-3V while 6 months for BVDV post vaccination.

In conclusion, the prepared newly developed combined inactivated vaccine being pure, completely safe and perfectly potent and effective control of pneumo-enteritis disease complex syndrome

Key Words: Montanide oil ISA 206, Aluminum hydroxide gel, Pneumo-3, SNT

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1. INTRODUCTION

Bovine respiratory diseases (BRD) have an important and serious impact on the beef and dairy cattle industry, both for stocker and feedlot entities. Economic losses result from death, decreased performance of diseased cattle, lowered weight gain, increased cost of gain, reduced carcass value and treatment costs [1].

Pathogens associated with BRD include bovine viral diarrhea virus (BVDV), bovine herpes virus type 1 (BHV-I), and parainfluenza virus type 3 (PIV-3) [2].

Abortion, cerebellar hypoplasia, ocular lesions, stillbirth, weakness and diarrhea occurred particularly with infection in the period of gestation. For these reasons, vaccination of pregnant cows with combined inactivated respiratory viruses' vaccine is usually recommended at last stage of pregnancy in cow calf operations of both the beef and dairy industries [3]. Antibodies help protect against clinical disease caused by BHV-l [4], BVDV [5], and PI-3 [6].

Nowadays, the use of inactivated vaccines against these respiratory diseases produces good results for protection of calves from pneumo-enteritis and death [7]. Inactivated virus vaccines have an advantage in that vaccine virus dose not replicate in the host tissues. Therefore, there has been interest in replacing MLV with inactivated ones, largely because of safety issues.

The present study was designed to develop combined inactivated vaccine containing BVD, IBR, PI-3 virus and using Montanide oil ISA 206 as an adjuvant. This may covered the valid requirement of vaccine evaluation to be pure, safe and fully potent to develop maximum and satisfactory protection for control of pneumo-enteritis disease complex syndrome in calves.

2. MATERIALS AND METHODS

2.1. *Vaccines:*

2.1.1. *Tissue culture inactivated pnumo-3 vaccine adjuvanted with montanide ISA 206:*

It was prepared from BVDV genotype-1 (Egyptian BVDV cytopathic, Iman strain of a titer $10^{6.5}$ $TCID_{50}$/ml), BHV-I (A local Abou Hammad strain of a titer $10^{7.5}$ $TCID_{50}$/ml) and PIV-3(Reference Egyptian strain "strain 45" of a titer $10^{8.0}$ $TCID_{50}$/ml). Viruses were propagated in MDBK cell line and inactivated by 0.001 M of BEI according to [8], then pooled according to [2] and thoroughly mixing with montanide ISA 206 at ratio 1:1 vol/vol according to [9]. The pH was adjusted to 7.5. The vaccine was produced and provided by department of the Rinderpest like diseases, Veterinary serum and vaccines research institute (VSVRI).

2.1.2. *Tissue culture inactivated pnumo-3 vaccine adjuvanted with aluminum hydroxide:*

It was prepared according to [10] and provided by department of the Rinderpest like diseases, Veterinary serum and vaccines research institute (VSVRI)

2.2. *Experimental design:*

Potency evaluation of the newly improved vaccine adjuvant with Montanide oil ISA 206 vaccine was carried out according to [11].

Potency evaluation was determined by immune response post vaccination over the permissible limit of protective level against each viral component of tested vaccine, as well as the duration of immunity.

Twelve adult male cross-breed calves were used in this study and divided into 3 groups, four calves for each group:

Group I: each calf was intramuscularly (I/M) immunized with 5 ml of the locally produced oily prepared vaccine (BVD, IBR and PI-3) by two injections. This group was used for studying the duration of immunity. Potency evaluation at one month post boostering.

Group II: Calves of this group were vaccinated with Pneumo-3 vaccine by I/M of 5 ml in two doses, two weeks apart.

Group III: Consists also of four calves and this group was left as non-vaccinated contact control group.

2.3. *Serum samples:*

Serum samples were collected from calves in three groups periodically examined for 12 months. The sera were inactivated at 56°C for 30 minutes, and then stored at -20°C till used in detection of specific antibodies for BVDV, BHV-I and PIV-3 using SNT

2.4. *Serum neutralization test (SNT):*

It was performed on MDBK cell line using the micro technique as described by [12].

3. RESULTS

Fig(1): Mean sera neutralizing antibody titer in serum of animal vaccinated with newly improved vaccine adjuvant with Montanide oil ISA 206 and Aluminum hydroxide gel

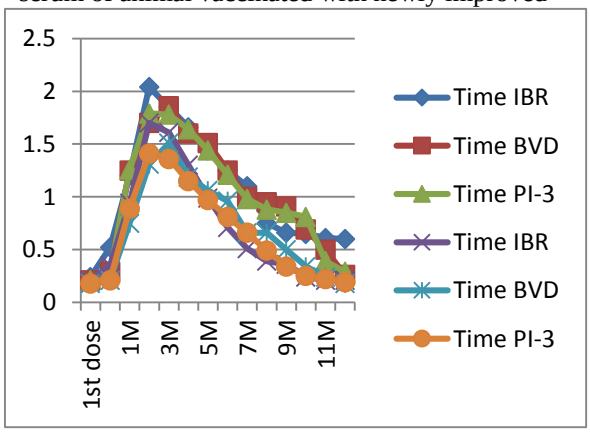

Table 1. Mean Neutralizing antibody titer of BVD, IBR, and PI-3 viruses in sera of calves vaccinated with inactivated pneumo-3 vaccine adjuvanted with either Montanide ISA 206 or aluminum hydroxide.

Time	*Group I*			*Group II*		
	IBR	BVD	PI-3	IBR	BVD	PI-3
1st dose	0.24	0.21	0.21	0.21	0.18	0.18
2nd dose	0.52	0.29	0.28	0.35	0.21	0.21
1M	1.20	1.25	1.26	0.94	0.75	0.89
2M	2.04	1.70	1.79	1.70	1.31	1.41
3M	1.85	1.86	1.78	1.61	1.51	1.36
4M	1.66	1.60	1.64	1.30	1.19	1.15
5M	1.44	1.51	1.44	0.99	1.06	0.97
6M	1.25	1.25	1.21	0.71	0.96	0.81
7M	1.10	1.00	0.98	0.51	0.66	0.66
8M	0.75	0.95	0.88	0.39	0.66	0.49
9M	0.66	0.91	0.85	0.36	0.50	0.34
10M	0.65	0.69	0.81	0.24	0.34	0.25
11M	0.61	0.50	0.39	0.21	0.24	0.22
12M	0.60	0.26	0.29	0.21	0.18	0.19

First dose: 0 day of vaccination, Second dose: 14 days post vaccination for gel and 21 days for oil, Protective serum neutralizing (SN) antibody titre against BVD is 0.90 [13], IBR is 0.60 [14], PI-3 is 0.60 [15].

4. DISCUSSION

Vaccination programs for breeding herds are integral parts of preventive health programs designed to lessen the effects of infectious respiratory diseases in cattle [7]. In Egypt, since fifty and sixty years of the last century, a much attention was drawn to the pneumo-enteritis disease complex syndrome. The control and preventive measures of these infections are based on mainly hygienic management and effective prophylactic vaccination [16]. There is a great need for improve and upgrade of this vaccine product to reach newly developed vaccine by using another adjuvant to maximize the effectiveness of the prepared vaccine and greatly enhance the body responses to the vaccine. Hence the selection of Montanide oil ISA 206 to be used as an adjuvant, due to it has many advantages, as the Montanide oil ISA 206 has a great safety margin to be inoculated without any local or systemic allergic reaction, also its mode of action to be used as an adjuvant was trap antigen and release it over a larger period producing a more increase in the immune response 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 [17]. Therefore, the key of this study was the preparation and evaluation of newly developed, combined inactivated vaccine containing BVD, IBR and PI-3 viruses adjuvanted with Montanide oil ISA 206 for using in calves for controlling of such infections and the newly developed vaccine was prepared and evaluated in our laboratory. The potency evaluation of the prepared newly developed vaccine in calves in table (1) and Fig. (1) results revealed that all vaccinated animals developed serum neutralizing antibody titres (SN antibody) when reached their peak at one month and remained stable higher than the minimal acceptable titre of protective level which lasts for 12 months post vaccination in IBRV, 9 months to BVDV and 10 months to PI-3V . Such data are similar to that obtained by (13) who recorded that the BVD antibody level of 1:8

dilution (log10 0.9) was protective, [14, 15]. Those authors reported that the minimal acceptable titre of neutralizing antibodies was 1:4 dilutions or 0.6 log_{10} was protective against PI-3 and IBR viruses. Serum neutralizing antibody titers in group 1, which vaccinated with the vaccine adjuvanted with Montanide oil ISA 206 showed the highest level among all viruses at one month post second vaccination .The titers remained stable in the protective level at 12 months for IBRV, 9 months for BVDV and 10 months for PI-3V. Now it is obvious that the oil vaccine is good and the enhanced action observed with this vaccine was said to be due to a gradual and continuous release of antigen to stimulate antibody production. Oil is a material for transport of the antigen throughout the lymphatic system and finally a stimulus for accumulation of immunologically important cells [18]. The results of calves vaccinated with aluminum hydroxide gel adjuvanted vaccine in group 2 showed the highest antibody titers at 7 months for IBRV and PI-3V also at 6 months for BVDV post vaccination and the titers began to decrease gradually until reach to the minimal protective level, which agreed with the results obtained by [3]. In conclusion, the prepared newly developed combined inactivated vaccine containing of BVD, IBR and PI-3 viruses and adjuvanted with Montanide oil ISA 206 gives a considerable and highly immunogenic in vaccinated calves.

5. REFERENCES

1. Fulton, R.W., Ridpath, J.F., Saliki, J.T., Briggs, R.E., Confer, A.W., Burge, L.J., Purdy, C.W., Loan, R.W., Duff, G.C. and Payton, M. E. 2002. Bovine viral diarrhea virus (BVDV) 1b: predominant BVDV subtype in calves with respiratory disease. *Can J Vet Res*. 66: 181-190.
2. Samira, S.T., El-Sabbagh, M.M.A. and Ghaly, H.M. 2001. Preparation of combined inactivated BVD, IBR, PI3 and respiratory syncytial virus (BRSV). J. Egypt. Vet. Med. Ass. 61: 251-263.
3. Donkersgoed, J. van, Hurk, J.V. van Den., McCarteny, D. Harland, R.J., Van, Donkersgoed, J. and Van den Hurk, J.V. 1991. Comparative serological responses in calves to eight commercial vaccines against infectious bovine rhinotracheitis, parainfluenza-3, bovine respiratory syncytial and bovine viral diarrhea viruses. Can. Vet. J. 32: 727-733.
4. Lemaire M, Weynants V, Godfroid J, Schynts, G. Meyer, J.J. Letesson, and Thiry, E. 2000. Effects of bovine herpesvirus type 1 infection in calves with maternal antibodies on immune responses and virus latency. J. Clin. Microb. 38: 1885-1894.
5. O'Connor, A., Martin, S., and Nagy. E. 2001. The relationship between the occurrence of undifferentiated bovine respiratory disease and titer changes to bovine Coronavirus and bovine viral diarrhea virus in 3 Ontario feedlots. Can. J. Vet. Res. 65: 137-142.
6. Bryson, D.G., Adair, B.M., McNulty, M.S., Mc Aliskey, M., Bradford, H.E.L., Allan, G.M., Evans, R.T., Forster, F. 1999. Studies on the efficacy of intranasal vaccination for the prevention of experimentally induced parainfluenza type 3 virus pneumonia in calves. Vet. Rec. 145: 33-39.
7. 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 PI3. Vet. Glas. 44: 503-512.
8. El-Sabbagh, M.M., Samira Said, T., Ghally, H.M. and Saad, M.S. 1993. Binary ethyleneimine as an inactivant for infectious bovine rhinotracheitis (IBR), bovine viral diarrhea (BVD) and parainfluenza-3 (PI-3) viruses and its application for vaccine production. Beni-Suef Vet. Med. Res. 5: 29-57.

9. 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 temperatures for the rapid preparation for double oil emulsion vaccine.
10. Zeidan, S.M., Samira S.T., El-Sabbagh, M.M., Ghally, H.M., Kassem, I. K.A. and El-Kholy, A.A. 2005. Trails for preparation of combined inactivated respiratory virus vaccine containing BVDV-1, BVDV-2, IBRV, PI3V, and BRSV. Fourth Int. Sci. Conf., Mansoura, 5-6 April.
11. USA Code of Federal Regulation 1987. Animal products. No. 9, Parts 1 to 199, Published by the Office of Federal Register. National Archives and Records Administration.
12. Rossi, C.R. and Kiessel, G.K. 1971. Microtitre tests for detecting antibody in bovine serum to PI3V, IBRV and BVDV. Microbiol. 22: 32-36.
13. Bittle, J.L. 1968. Vaccination for bovine viral diarrhea – mucosal disease. J. Amer. Vet. Med. Assoc., 152: 861-865.
14. Zuffa, A. and Fekeotova, N. (1980): Protective action of inactivated IBR vaccine against experimental infection. *Veterinaria, Med*., 25: 51-61.
15. Mihajilovic, B. Vetokovic, C.A. Kuzmanovic, M. Vuhobrat, D. Asauin, R. and Lazurevic, T. 1979: Comparison of vaccines against respiratory disease of cattle, rhinotracheitis and parainfluenza-3. Veterinarski Glansnik, Yugoslavia, 33: 33-38.
16. Durham, P.J.K. and Hassard, L.E. 1990. Prevalence of antibodies to IBR, PI-3, BRSV and BVD in cattle in Sakatchewan and Alberta, Canada. Can. Vet. J. 31: 815-820.
17. Vanselow, B.A. 1987.The application of adjuvants to veterinary medicine.
18. Bahnemann, H.G., Mesquita, J. Astudillo, V. and Dora, F. 1988. Production and application of an oil adjuvant vaccine against foot and mouth disease in cattle. In modern approaches to animal cell technology. Eds. Spier, R. E. and Griffith, J. B., Butterworth, Guildford 1: 628.

مجلة بنها للعلوم الطبية البيطرية
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تحسين لقاح الأمراض التنفسية الجماعى المثبط المحسن (النيمو-3) باستخدام زيت المانتونيد اى اس ايه

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الملخص العربى

استهدف البحث محاولة تحضير لقاح محسن جامع مخمد يحتوي على فيروسات الاسهال البقرى الفيروسى (بى فى دى فيروس) والتهاب القصبة الهوائية الرغامى المعدى (أى بى ر فيروس) وفيروس البارا–أنفلونزا–3 (بى أى – 3 فيروس) ومحمل على زيت المانتونيد اى اس ايه 206 كعامل مساعد للتحفيز المناعي. تم تحصين المجموعة الأولى بلقاح المحضر المحسن والمحمل على زيت المانتونيد اى اس ايه 206 والمجموعة الثانية بلقاح النيمو–3 المحمل على الالمونيوم هيدروكسيد جيل حيث تم تحصين العجول بنفس الجرعة وطريقة الحقن التى تم بها المجموعة الأولى. بينما تركت المجموعة الثالثة كمجموعة غير محصنة وضابطة للتجربة. وجاءت نتائج تتبع الاستجابة المناعية باستخدام اختبار المصل المتعادل لتثبت ان المستوى المناعى للاجسام المضادة المتعادلة قد تكونت فى العجول التى تم تحصينها باللقاح المحضر المحسن المحمل على زيت المانتونيد اى اس ايه 206 لتعطى مستوى مناعى استمرحتى 12 شهر فى فيروس التهاب القصبة الهوائية الرغامي المعدي واستمر حتى 9 أشهر فى فيروس الاسهال المعدى بينما استمر حتى 10 أشهر فى فيروس البارانفلونزا–3. المانتونيد. وجاءت نتائج تتبع الاستجابة المناعية باستخدام اختبار المصل المتعادل لتثبت ان المستوى المناعى للاجسام المضادة المتعادلة قد تكونت فى العجول التى تم تحصينها بلقاح النيمو–3 المحمل على الالمونيوم هيدروكسيد جيل لتعطى مستوى مناعى استمرحتى 7 اشهرفى فيروس التهاب القصبة الهوائية الرغامي المعدي واستمر ايضا حتى 7 أشهر فى فيروس البارانفلونزا–3 بينما استمر حتى 6 أشهر فى فيروس الاسهال المعدى. وفى الخلاصة يمكن القول أن اللقاح المحسن الجامع المثبط هو لقاح نقى وأمن وبفاعلية كاملة.

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