COMPARATIVE STUDIES ON THE IMMUNOGENECITY OF INACTIVATED BOVINE EPHEMERAL FEVER VACCINE USING OIL AND AL. HYDROXIDE GEL AS ADJUVANTS

Amani, A. Saleh.
Veterinary Serum and Vaccine Research Institute, Abbassia, Cairo

SUMMARY

A stable oil emulsion bovine ephemeral fever (BEF) vaccine with a low viscosity, composed by two different formulas, and aluminum hydroxide gel adjuvant vaccine were prepared. Twenty healthy cattle of 12-18 months old were divided into four groups, each of two groups was vaccinated either with bovine ephemeral fever (BEF) water-in-oil emulsion adjuvant vaccine (W/O) or water-in-oil-in-water emulsion vaccine (W/O/W) while the third group was vaccinated with aluminum hydroxide gel adjuvant vaccine. All of the three groups received a booster vaccination two weeks post-preliminary vaccination. The fourth group represented a non-vaccinated control group. The results of SNT and ELISA revealed that the using of aluminum hydroxide gel as adjuvant elicited BEF antibodies titer prior to the groups vaccinated with oil emulsion vaccines either in the form W/O or in W/O/W. On the other hand, the oil emulsion vaccines induced higher and longer antibody titers than al. hydroxide gel vaccine and this was attributed to the slow release of antigen from the oil formulation induced. There was no alteration in antibody titre in the groups either vaccinated with each type of oil emulsion vaccine but W/O/W emulsion vaccine was more safe because it induced little inflammation at the site of injection.

INTRODUCTION

Bovine ephemeral fever (BEF) remains a viral disease of a considerable importance to many countries including Egypt. Although BEF virus was first thought to contain 6 structural proteins, there is increasing evidence to suggest that it contains the 5 proteins characteristic of the Rhabdoviridae *(Uren, 1989)*. Although BEF is thought to be arthropod borne, the vector has yet to be identified but it is clear from the distribution of BEF that more than one vector is capable of transmitting the disease. *Vanselow et. al., (1995)* tested various BEF vaccines and found an apparent relationship between neutralizing antibody response and the level of protection. So, vaccination against the disease is the main measure for controlling the infection. The vaccines currently available are prepared from either live

attenuated or killed virus and may be less than reliable. It appears to be a need for a reliable, inexpensive vaccine *(Uren,1989)*. Water-in-oil (W/O) emulsions are known as the most effective adjuvant to generate high and durable antibody responses to vaccine antigens following a single immunization *(Jansen et. al., 2005)*. While, *Herbert, (1968)* reported that oil adjuvanted vaccines formulated as W/O emulsions would exert immune activity in mice by the slow release of antigen at the site of injection. *Gupta et. al., (1993)* noted that W/O/W emulsion vaccines were as potent as W/O emulsion and more safe because they induced little inflammation at the site of injection. However, *Blackall et. al., (1992)* reported that the improved W/O/W emulsion vaccine (vaccine containing a double-emulsion adjuvant system) against infectious coryza were effective on the immune responses in chickens. The present experiment was designed to investigate the difference between the antibody responses of vaccinated cattle to BEF vaccines with different adjuvants.

MATERIALS AND METHODS

1. Virus:

The BEF-AVS strain was propagated on BHK-21 cell culture *(Azab et. al., 2002)* and inactivated by binary ethyleneimine. The virus had a titer of $10^{7.5}$ $TCID_{50}$ /ml and separate lots of this inactivated virus suspension was used for each of the three vaccines.

2. Vaccines:

2.1. Water- in- oil emulsion adjuvant vaccine (W/O):

The vaccine was prepared according to *Stone et. al., (1983)* by adding aqueous-phase emulsifier (Tween 80) to the inactivated cell culture virus suspension and drop wise this aqueous antigen into the oil phase, composed of liquid paraffin with 10% v/v of Span 80. The ratio of the aqueous phase to the oil phase was 1:1.

2.2. Water-in-oil-in-water adjuvant vaccine (W/O/W):

This vaccine prepared with internal aqueous phase: oil phase: external aqueous phase where the oil-to-aqueous ratio was 1:2 according to *Shin et. al., (2000)*.

2.3. Aluminum hydroxide gel adjuvant vaccine:

Inactivated cell culture antigen was adjuvanted by aluminum hydroxide gel according to *(Daoud, et. Al., 2001)*.

The three vaccines were used in a dose of 2ml / cattle inoculated (S/C), containing 10^{7} $TCID_{50}$ /ml.

3. Animals:

Twenty healthy, free from BEF antibodies cattle of 12-18 months old were divided into 4 groups (5 cattle/group) as follows:

Group I: Vaccinated with (W/O) emulsion vaccine.

Group II: Vaccinated with (W/O/W) emulsion vaccine.

Group III: Vaccinated with aluminum hydroxide gel BEF vaccine.
The three previous groups were received a booster dose of the corresponding vaccine two weeks post first vaccination.
Group IV: Kept as a non-vaccinated control.
Serum samples were collected from all cattle, pre-vaccination and at intervals of 1, 2, 3, 4 weeks and monthly till the 12th month post vaccination.

4. Serum neutralization test (SNT):

Microtitre SNT was carried out according to *Burgess (1974)* to detect the developed BEF antibody titers in the sera of vaccinated cattle. The antibody titer was calculated as the reciprocal of the final serum dilution, which neutralized 100-200 $TCID_{50}$ of BEF virus according to *Singh et. al., (1967)* .

5. Preparation of BEF antigen:

The BEF antigen was prepared by using polyethyelene glycol (MW6000) according to *Brian and Hiller (1996).*

6. Enzyme linked immunosorbent assay (ELISA) :

ELISA was applied for detection of antibody titers induced by each kind of vaccine as described by *Zakrzewski et. al., (1992).*

RESULTS AND DISCUSSION

The present study revealed the absence of side effects or symptoms of illness in all vaccinated animals, either with aluminum hydroxide gel or oil emulsion adjuvant BEF vaccines allover the experimental period except severe local reactions which were observed following the injection of the booster dose of W/O vaccine.

The results of the serum neutralization test carried out on the sera of vaccinated animals either with W/O or W/O/W emulsion vaccines as shown in (Table1) revealed no differences between the antibody responses elicited by the two formulations. In both cases, BEF antibodies were not detected prior to as well as 2 weeks after vaccination. Two weeks post the booster dose; the antibody titers began to increase. These results were agreed with *Cameron et. al., (1987)* who recorded that the antibody responses in cattle to oil emulsion ephemeral fever vaccines were not satisfactory after a single injection.

The antibody titers were reaching the maximal level 8 weeks post vaccination and these levels were tended to maintain up to 8th month post vaccination. Afterwards, the antibody titers were decreased successively and it was detected till 12th after vaccination and at the end of the experiment.

On the other hand, the induced BEF antibodies were found to be detectable by the 2nd week post vaccination with the gel vaccine then increased by booster dose, showing maximal values 8 weeks post vaccination. Similar findings were recorded by *Daoud et. al., (2001) and Eman et. al., (2003).* This high level of BEF neutralizing antibodies was

detected up to the 6th month post initial vaccination then decreased successively. These results were agreed with *Vanselow et. al., (1995)* who stated that antibody responses were highest for the vaccine incorporating adjuvant (Quil A) when it was given as two consecutive injections and provided excellent protection against BEF for at least 12 months, whereas single dose with the Quil A vaccine or two doses with vaccine containing the adjuvant aluminium hydroxide gel did not provide significant protection.

The differences between the antibody levels elicited by different adjuvanted vaccines were probably related to antigen release from the formulation. Consequently, the strong and maintained immune responses of oil emulsion vaccines appeared to be achieved by slow release of antigen. Also, the nonionic detergent Tween 80, which added as aqueous-phase emulsifier in oil vaccines enhances the immune response and prolongs the duration of immunity as, suggested by *Stone et. al., (1983); Cajavec et. al., (1996); and Shin, et. al. (2000)*.

ELISA was done on the same serum samples of vaccinated cattle and showed that the obtained results were in parallel to those of SNT as indicated in (Table2) which showed recorded protective titer of BEF antibodies as mentioned by *Voller et. al.,(1976 and Zakrzewsk et. al., (1992)*.

It could be concluded from this experiment that the W/O and W/O/W emulsion vaccines with inactivated BEF virus induced good immune responses in terms of maintained high antibody titers of BEF considering the W/O/W emulsion vaccine more safe due to little local reaction at the site of injection.

REFERENCES

Azab,A.M.; Khodeir,M.H.; Attyat,M.Kotb and El-Gallad,S.B.KH. (2002): Susceptibility of different cell cultures to bovine ephemeral fever virus.6th Sci. Cong.,Fac. Vet. Med. Zag. Uni. P 41-56.

Blackall, P. J., L.E. Eaves, D. G. Rogers, and G. Firth. (1992): An evaluation of inactivated infectious coryza vaccines containing a double-emulsion adjuvant system. Avian Dis. 36: 632-636 .

Brian, W.J. and Hiller, O.K. (1996): virology methods manual. Academic Press, New York, USA. Pp. 108-110.

Burgess, G.W. (1974): A micro-titre serum neutralization test for bovine ephemeral fever virus. Aust. J. Exp. Bio. Med. Sci., Oct., 52 (5): 851-855.

Cajavec, S., Z. Bidin, D. Sladic, and B. Pokric (1996): Tween 80-solubilized Newcastle disease virus prepared as a water-in-oil-in-water vaccine. Avian Dis. 40:193-201.

Cameron CM, Barnard BJ, Erasmus E, Botha WJ. (1987): Antibody response in cattle to oil emulsion rabies and ephemeral fever vaccines. Onderstepoort J. Vet Res. Jun; 54 (2) : 157 - 8.

Daoud, A.M. ; Soad, M.S. ; Azab, A. ; and Taha, M.M. (2001): Preparation of inactivated bovine ephemeral fever vaccine (BEF) in Egypt. Beni-Suef. Vet. Med. J. 11 (2). 619-626.

Eman, M.El-Rawy; Amina,A.El-Bayomy; Azab,A.M.; Eman, M.Abd El-Rahman and Daoud, A.M. (2003): Trials for preparation and evaluation of a combined vaccine against haemorrhagic septicemia and bovine ephemeral fever in cattle. Kafr El-Sheikh. Vet. Med. J. Vol.1 149-163.

Gupta, R. K. E. H. Relyveld, E. B. Lindblad, B. Bizzini, S. Ben-Efrain, and C. K. Gupta (1993): Adjuvants-a balance between toxicity and adjuvanticity. Vaccine 11 : 293-306.

Herbert, W.J. (1968): The mode of action of mineral oil emulsion adjuvants on antibody production in mice. Immunology 14:301-318.

Jansen, T., Hofmans, M. P., Theelen, M. J. and Schijns, V.E. (2005):
Structure-activity relations of water-in-oil vaccine formulations and induced antigen-specific antibody responses. Vaccine. 2005 Jan 11; 23 (8): 1053-60.

Shin, I.F.; Kohei, M.; Hajime, M. and Reiji, T. (2000) : relationship between antigen release and antibody response of infectious coryza water in oil in water emulsion vaccines. Avian Dis. 44 :869-873.

Singh,K.V.; Osman,O.A.; Ivon,F.E. and Thanaa,T. (1967) : Cloistral transfer of rinderpest neutralizing antibody to offspring of vaccinated dams. J.Comp. Med. Vet. Sci., 31: 295-298.

Stone, H.D., M. Brugh, and C.W. Beard. (1983): Influence of formulation of the efficacy of experimental oil-emulsion Newcastle disease vaccines. Avian Dis. 27: 688-697.

Uren, M.F(1989): Bovine ephemeral fever. Aust Vet J. Aug; 66 (8) : 233-6.

Vanselow, B. A.; Walthall, J.C. and Abetz, I. (1995): Field trials of ephemeral fever vaccines. Vet. Microb. 46 (1-3) : 117-30 .

Voller, A.;Bidwell, D. E.; and Ann Bartlett (1976): Enzyme immunoassay in diagnostic medicine, theory and practice. Bull World Health Organization Vol., 35:55.

Zakrzewski, H., Cybinski, D. H.; and Walker, P. J. (1992) : Ablocking ELISA for the detection of specific antibodies to bovine ephemeral fever virus. J. Immunol. Methods, 151; 289-297.

Table (1): Mean BEF neutralizing antibody titers in sera of vaccinated cattle

Periods post vaccination	BEF antibody titers* /Animal groups			
	W/O BEF vaccine	W/O/W BEF vaccine	Al. gel BEF vaccine	Unvaccinated control
1W	0	0	<2	0
2W**	<2	<2	6.4	0
3W	11.2	11.2	14.4	0
4W	35.2	32	38.4	0
2M	102.4	108.8	70.4	0
3M	102.4	108.8	70.4	0
4M	89.6	89.6	64	0
5M	89.6	89.6	64	0
6M	89.6	89.6	44.8	0
7M	89.6	89.6	32	0
8M	64	64	9.6	0
9M	57.6	53.3	<2	0
10M	51.2	51.2	0	0
11M	32	32	0	0
12M	16	17.6	0	0

*** Serum neutralizing antibody titers are expressed as the reciprocal of last serum dilution inhibiting the CPE of 100- 200 $TCID_{50}$ of BEF virus.**

**** Booster dose.** W = week M = month

Table (2): Mean BEF ELISA antibody titers in sera of vaccinated cattle

Periods post vaccination	BEF antibody titers / Animal groups			
	W/O BEF vaccine	W/O/W BEF vaccine	Al. gel BEF vaccine	Unvaccinated control
1W	0.06	0.03	0.95	0.03
2W**	09	0.92	1.1	0.03
3W	1.35	1.2	1.44	0.06
4W	1.65	1.55	1.95	0.05
2M	2.1	2.2	1.8	0.03
3M	1.95	1.9	1.8	0.03
4M	1.9	1.9	1.8	0.06
5M	1.9	1.9	1.85	0.06
6M	1.9	1.9	1.55	0.05
7M	1.9	1.9	1.4	0.03
8M	1.8	1.8	1.1	0.03
9M	1.6	1.65	0.8	0.06
10M	1.35	1.25	0.02	0.05
11M	1.2	1.2	0.05	0.03
12M	1.1	0.95	0.03	0.03

** Booster dose. W = week M = month

الملخص العربي

دراسات مقارنة علي القوة المناعية للقاح الحمى العابرة المثبط باستخدام الزيت وهيدروكسيد الالومنيوم الجل كمساعدات

اماني علي صالح

معهد بحوث الامصال واللقاحات البيطرية – العباسية – القاهرة

تم تحضير لقاح مثبط ضد مرض حمى الثلاثة ايام باستخدام نوعين من المساعدات , زيت البارافين الابيض وهيدروكسيد الالومنيوم الجل , وقد تم تحضير اللقاح الزيتى بطريقتى (الماء في الزيت) و (الماء في الزيت في الماء) . وقد تم تحصين عدد من الابقار الخالية من الاجسام المناعية المضادة لمرض حمى الثلاثة ايام بكل من اللقاح الجل و اللقاح الزيتى بنوعية . وقد استدل من النتائج الاكلينيكية و السيرولوجية علي عدم حدوث اية اعراض مرضية في جميع الحيوانات المحصنة فيما عدا حدوث التهاب موضعي في مكان حقن اللقاح الزيتي ولكفه كان اقل في حالة اللقاح المحضر بطريقة (الماء في الزيت في الماء) . كما تبين حدوث ارتفاع في المستوى المناعي في الحيوانات المحصنة باللقاح الزيتي مع استمرارية وجود هذه الاجسام المناعية حتى الشهر الثانى عشر بعد التحصين مقارنة باللقاح المحضر مع هيدروكسيد الالومنيوم الجل الذي اعطى فترة مناعه ثمانية اشهر.