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PREPARATION OF ATTENUATED BOVINE EPHEMERAL FEVER (BEF) VACCINE

By

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ABSTRACT

Bovine Ephemeral Fever Webster's 919 V_{10} strain, originated from a virulent virus by a series of passages through susceptible calves until its virulence was reduced and then adapted to tissue culture system Vero cell line. The lyophilized virus was reconstituted in saponin buffer (1mg/ml) which used as vaccine. The prepared vaccine developed soft, painless swelling within 24 hours after S / C inoculation and produced a good serological response with a protective dose at $10^{4.5}$ $TCID_{50}$ / ml. Two successive doses of the vaccine with 3 – 4 weeks intervals produced a good immunological response and could protect cattle against challenge when conducted 14 days after the last vaccinal dose and the protective neutralizing antibodies continued elevated about 8 –9 months post vaccination.

INTRODUCTION

Several outbreaks among cattle and buffaloes with clinical manifestation of BEF were recorded at different Governorates in Egypt, Summer (1991) by **Hassan *et al.,*** and Summer (1994) by **Soheir** and also in summer (2000).

Van Der Wasthuizen, (1967) succeeded in isolating and maintaining BEF virus by intracerebral inoculation of suckling mice, and also adapted the virus to grow in cell culture and found that passages in either tissue cultures or mouse brain resulted in rapid loss of Pathogenicity for cattle.

Tzipori and Spradbrow, (1973) produced a vaccine from the third generation mouse brain passage virus was effective in protecting 12 cattle, they were given one dose from an adjuvant vaccine followed by a second dose without adjuvant.

Tzipori and Spradbrow, (1978) they also in 1978 produced another strain of vaccine which had a great potential as an immunizing agent in Australia.

Gard *et al.*, (1983) and Gard *et al.*, (1984) mentioned that there were four viruses and seemingly there was no cross immunity between them and the virus can be grown in chick embryos or by intracerebral inoculation of suckling mice or in cell culture.

In **(1988), Arthur Webster** has produced a bovine ephemeral fever living vaccine as freeze-dried product with diluent containing Quil A for vaccination of cattle.

The aim of this study is to produce a living attenuated freeze- dried vaccine against BEFV using Webster strain with modified diluent.

MATERIAL AND METHODS

Viruses:

1. Vaccinal strain "Webster's strain of BEF (919 V_{10})":

The original virus was isolated in Veterinary Research Institute, Yecrongpilly Qid. from 1967 - 1968 natural field outbreak of BEF. This virus was treated by series of passages through susceptible calves until its virulence was reduced (attenuated), then adapted to tissue culture system (designated 919 V_{10}) and became the master seed culture. This virus is capable of inducing immunity without any clinical symptoms of the disease and considered as master seed virus, then passaged twice in BHK_{21} (working virus).

2. Challenge virus:

Virulent BEF virus obtained at the highest of the febrile reaction from a cow infected by inoculation of blood from a naturally infected cow during 2000-Egyptian BEF outbreak (**Soad *et al.*, 2001**), the virus was stored at -70^{O}C in the form of 10% suspension of leukocytes in Buffer Lactose Phosphate (BLP).

3. Virus assay:

Master seed and working viruses as well as the lot batch were assayed by preparing ten fold dilutions of the virus in MEM medium and seeding 0.1 ml of each dilution onto at least 5 wells of Vero cell culture plate, the culture were observed for CPE for 7 days and titers were calculated according to the method of **Reed and Muench, (1938)** expressed as log $_{10}$ TCID $_{50}$ / ml.

Experimental animals:

Susceptible Frezian cattle about 14 - 18 months in age were used in all the experiments, the animals were kept in insect free stable. Rectal temperatures were recorded and the animals were daily observed for clinical symptoms and changes in blood picture after vaccination and challenge.

Samples:

Blood samples were collected from all experimental cattle, samples blood smears were done for detection of changes in blood picture and at the same time serum was collected for application of serum virus neutralization test which was carried out in microplates containing Vero cells, (**Soad *et al.*, 2001**) using constant serum virus dilution procedure. Field isolate tissue culture adapted BEF virus. (**Soad *et al.*, 2001**) was employed throughout the study, serial ten fold dilutions of the virus were prepared in MEM, a fixed volume of each virus dilution was mixed with an equal volume of undiluted serum sample and incubated at 37 ^{O}C for I hour, each serum virus mixture was inoculated into five wells of a T.C. plate each with 0.02ml. The plate incubated at 37 ^{O}C and observed for CPE for 7 days, The neutralizing indices are expressed as Log $_{10}$ TCID $_{50}$ of neutralized virus.

Vaccine preparation:

The Webster's strain of BEF virus was inoculated in VERO tissue culture Roller bottles with MOI 1 : 3. After 48 hours of incubation at 37 ^{O}C the virus was collected and subjected to two cycles of freezing and thawing, supernatant was collected after centrifugation at 3000 r.p.m, equal amount of stabilizer was added to the virus, then distributed in 1ml vial and lyophilized.

Dissolvent of the vaccine:

Saponin:

Saponin is a carbohydrate quillaic acid compound which can be supplied in a highly standardized form, and prepared by 1mg of saponin per dose.

Safety test:

Intravenous inoculation of 3 calves with 10 ml strain 919 V_{10} (titer $10^{6.5}$ $TCID_{50}$ / ml) equivalent to 100 vaccine doses. Clinical examination, Rectal temperature, daily blood samples were collected for 12 days post inoculation.

Experimental Design:

Exp. (1): <u>for detection of virus dose required for successful immunization</u>

10 susceptible calves were divided into 5 groups (G1 to G5), G1 was inoculated S/C with 2ml after immediately reconstituted vaccine with a dose of $10^{6.5}$ $TCIDS_{50}$ / ml.

G2 was inoculated with 2ml of the vaccine containing $10^{4.5}$ $TCID_{50}$ / ml. G3 was inoculated with 2ml of the vaccine containing $10^{3.5}$ $TCID_{50}$ / ml. G4 was inoculated with Saponin.(1mg / dose) and G5 non-vaccinated control groups. All groups were challenged with the virulent virus two weeks post-vaccination.

Exp. (2): <u>for choice of vaccination programme</u>

Two groups of susceptible calves were inoculated with the immediately reconstituted BEF vaccine, the first group inoculated with one dose ($10^{4.5}$ $TCID_{50}$ / ml), and the second group inoculated with two doses 4 weeks

interval, serum samples were collected for detection of neutralizing antibodies allover 7-8 weeks

RESULTS

Result of safety test of the prepared vaccine proved that the inoculated animals have no fever or clinical sickness, also no virus detected indicating that inoculation of large dose of BEF vaccine virus did not produce any detectable viraemia, but the inoculated vaccine induced a warm soft painless swelling developed within 24 hours and reached its maximum by about 48 hours, then gradually disappeared and accompanied by 0.7 - 1 ^{O}C rise in rectal temperature which returned to normal within 24 hours, the temperature raised after the first vaccination also occurred after the 2nd inoculation of the vaccine. In Table (1) group G4 which inoculated with saponin alone showed a rise in rectal temperature 1 ^{O}C.24 hours after inoculation, which indicated that the cause of rise in body temperature after vaccination is the presence of saponin as adjuvant. The results of the produced vaccine indicated that all animals vaccinated with one dose or two successive dose 3 weeks interval of the immediately reconstituted vaccine produced a temporary tissue reduction which subsided after 3 weeks. After challenged with virulent BEF virus intravenously 3 weeks after 2nd vaccination, clinical symptoms not developed and at the same time a high immunizing response expressed by NI was developed with a protective dose of $10^{4.5}$ $TCID_{50}$ / ml. The protective time response lasted over 8 weeks for twicelly vaccinated group. From table (1) the optimum virus dose is $10^{4.5}$ $TCID_{50}$.

From Table (2) it is clear that G2 gave higher neutralizing antibodies which was still protective over 9 months post 2nd vaccination.

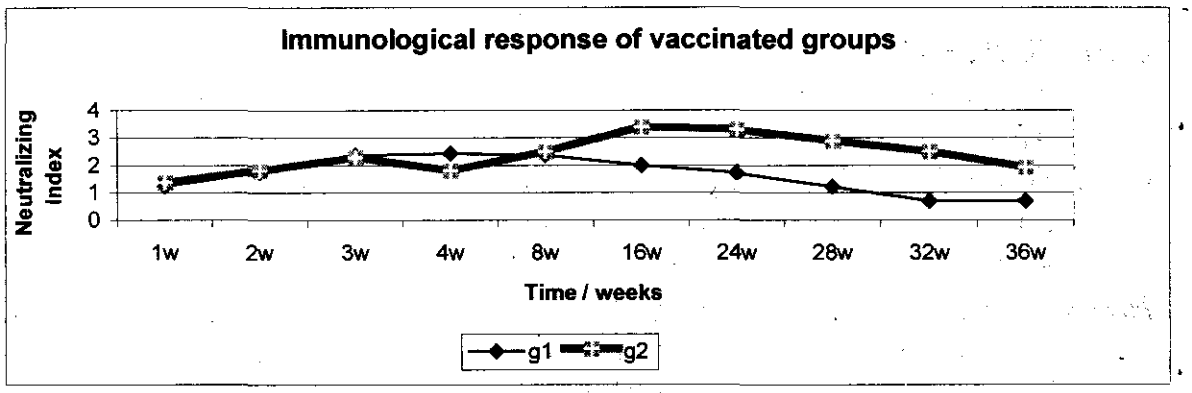
Table (1): The optimum virus dose required for successful vaccination.

Groups	No of animals	Treatment	CMAV	CMAC	Neutralizing index / days			
					1	2	3	5
G1	2	$10^{6.0}TCID_{50}$/ml	RRT	No CR	1.5	2.4	2.6	3.0
G2	2	$10^{4.5}TCID_{50}$/ml	RRT	No CR	1.3	1.8	2.2	2.4
G3	2	$10^{3.5}TCID_{50}$/ml	RRT	+ve CR	1.2	1.4	1.6	1.6
G4	2	saponin alone control	+ve RRT	+ve CR	-	-	-	-
G5	2	non-vaccinated control	-	+ve CR	-	-	-	-

CMAV = clinical respect after vaccination./ CMAC = clinical respect after challenge
RRT = Rise of rectal temperature / No CR = no clinical reaction. / +ve CR= positive clinical reaction. The protective dose 50% PD_{50} was $10^{4.5}$ $TCID_{50}$ which consider as a vaccine potency standard.

Table (2): Immunological response of vaccinated groups.

Groups	1st vaccinal dose	NI post-vaccination/week			Booster dose	Mean NI post-vaccination/month						
		1	2	3		1	2	4	6	7	8	9
G1	$10^{4.5}TCID_{50}$ /ml	1.23	1.73	2.36	-	2.43	2.36	2.0	1.70	1.20	0.70	0.70
G2	$10^{4.5}TCID_{50}$ /ml	1.36	0.80	2.3	$10^{4.5}TCID_{50}$ /ml	1.8	2.5	3.4	3.3	2.9	2.5	1.93



DISCUSSION

From studies of **Theodoridis *et al.*, (1973); Tzipori and Spradbrow, (1978) and Vanselow, *et al.*, (1985) and the protocol of the development of Webster's BEF vaccine**, an attenuated vaccine was produced for the first time in Egypt from the vaccinal strain Webster's 919 V_{10} with some modification on the composition of the adjuvant (saponin instead of Quil A on aluminum hydroxide gel), which induce transitory temperature rise occur within 24 hours of each injection which quickly subside.

Inoculation of attenuated BEF virus strain of the in Vero cell by S/C route into susceptible cattle induce satisfactory level of neutralizing antibodies that can protected vaccinated animals against challenge with the locally isolated virulent strain, two inoculation within 3 weeks interval gave similar results. This indicated that BEF virus when incorporated with saponin

as adjuvant gave that protective titer (Table 1), this result agree with that of **Theodoridis *et al.*, (1973)** who found that BEF virus alone failed to induce satisfactory level of neutralizing antibodies but when BEFV incorporated with freund's incomplete adjuvant, the stimulation was very different and the vaccinated animals developed high antibody titer. From the result in table (2) annual booster doses of vaccine will be necessary to maintain effective protection as the level of neutralizing antibody was decreased after 10 months post-vaccination. The most satisfactory results were obtained when the animal vaccinated with two injections 3 weeks apart evoked higher antibody titers than those vaccinated once, and the immunity produced from two inoculation persisted long enough to protect animals through summer season.

Conclusion; subcutaneous injection of two vaccinal doses of BEF immediately reconstituted vaccine in saponin buffer adjuvant, did not produce viraemia, produced transitory rectal temperature rise for 24 hours and produced a good neutralizing antibody level and provided sound protection against virulent BEFV.

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الملخص العربي

تحضير لقاح حمى الثلاثة أيام المستضعف

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حمى الثلاثة أيام عترة ويستر ٩١٩ ف١١ والتي نشأت من الفيروس الضاري بعد العديد من التمريرات خلال عجول عالية الحساسية للفيروس حيث تم تقليل الضراوة ثم تعويد العترة على خلايا الزرع النسيجي النوع فيرو ٠

الفيروس المحضر بعد إذابته في محلول السابونين (١مجم / مل)٠ وبعد ٢٤ ساعة من حقن اللقاح يظهر تحت الجلد إنتفاخ ناعم غير مؤلم, وينتج عنه استجابة مناعية باستخدام الجرعة الوقائية ١٠ ^{٤,٥} $TCID_{50}$ / مل, جرعتين متتاليتين من اللقاح بينهما فترة ٣ – ٤ أسابيع, تعطى استجابة مناعية جيدة تحمى الأبقار عند استخدام إختبار التحدي بعد ١٤ يوم من آخر جرعة لقاح, وتستمر الأجسام المتعادلة الواقية لمدة ٨ – ٩ شهور بعد التحصين.