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PREPARATION OF INACTIVATED BOVINE EPHEMERAL FEVER VACCINE (BEF) IN EGYPT

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ABSTRACT

Inactivated tissue culture BEF vaccine was prepared from the low passage levels (3^{rd} – 5^{th}) for a locally isolated viral strain during the epidemic occurred in Egypt 2000. The virus was inactivated with ethyleneimine and in order to reduce the toxicity of ethylenimine [it was prepared in dilute form, from 2-bromoethylamine in sodium hydroxide solution which was called binary ethyleneimine (BEI)]. Inactivation studies showed that the virus completely loses its infectivity after 6 hours incubation at 37^{O}C. At the same time antigenicity of BEF virus was not affected by inactivation and produced an immune response in vaccinated cattle over 6 months with one or two successive doses at 4 weeks intervals. After challenge, no clinical manifestations appeared on vaccinated cattle, while control non-vaccinated groups developed typical BEFV manifested by fever, inappetence, salivation, nasal discharge, enlargement of lymph nodes and general stiffness. The blood picture of groups vaccinated either with one or two doses showed no abnormal changes, while the control non – vaccinated challenged groups showed neutrophilia with band form (immature lymphocytes) > 35%, and intracytoplasmic inclusion bodies in monocytes.

INTRODUCTION

Bovine Ephemeral Fever was first recognized in Rhodesia (**Bevan, 1907**) and the disease reappeared and spread over a wide area. The cause of the disease remained unknown until **Van der Wasthuizen, (1967)** succeeded in isolating the virus by intracerebral inoculation of suckling mice, and he could adapt the isolate in BHK_{21}, clone 13 T. C. cell line. The disease is found every year in northern Australia usually during the wet season. However sporadic outbreaks can occur during the dry season.

An outbreak with severe economic losses occurred in Egypt during 1999 – 2000. The disease is most sever in bulls, fed cattle, pregnant and lactating cow. **Van der Westhuizen, (1967) and Inaba *et al.*, (1969)** found that the virus strains used in vaccine trials lost its antigenicity with successive passages. The aim of the present study lies in production of an inactivated BEF vaccine using binary ethylenimine (BEI) as an inactivant according to **Bahnemann *et al.*, (1974)**, followed by vaccination of susceptible animals. The immunogenicity of BEF prepared vaccine was serologically measured in sera of vaccinated cattle for testing the quality of this newly prepared vaccine.

MATERIAL AND METHODS

Seed Virus:

An outbreak of BEF in Egypt during 2000 – 2001 occurred, the virus was originally isolated from the blood of naturally infected lactating cows at Menofyia Governorate by I / C inoculation of suckling mice (***Soad et al.*, 2001**) according to the procedure of **Van der Westhuizen, (1967)**.

Working virus:

The adapted virus was passaged once in Vero cell line and designed as (working virus).

Inoculum Virus:

The final working virus was passaged again (P2) in Vero cell. This inoculum virus (not exceeding three passages) was used for vaccine preparation.

Vaccine preparation and evaluation:

BEI was formed through the cyclization of 2-bromoethylamine (BEA) under alkaline conditions by maintaining 0.1 M BEA in 0.2 N NaoH for 2 hours at 37 ^{O}C in a water bath. The resultant 0.1 M BEI inactivant was used for inactivation of virus suspension. Inactivation rates were determined by regression of infectivity titers of aliquots drawn at different interval (1-7 hours).

The vaccine with aluminum hydroxide as adjuvant was prepared in the following manner, suspensions of BEFV was inactivated with BEI at a concentration of 0.01 M for 6 hours at 37^{O}C, the virus was titrated before inactivation and infectivity tests were performed in VERO cell culture as described by **Bahnemann, (1973)**. Safety test was conducted by inoculation of inactivated virus I / C into suckling mice and VERO cell line culture after removal of residual BEI by Sod. thiosulphate, the vaccine was prepared by

addition of an equal amount of aluminum hydroxide gel (with about 2.5% Al_2O_2) which was adjusted to pH 8.3 with glycine buffer.

The immunogenicity of the vaccine was determined in groups each of 5 cattle, 15 to 28 months old and of about 350 kg weight each, found to be free from antibodies against BEFV and were kept in an isolated stable, group (1) of these animals were each inoculated with 2ml of the vaccine S/C, while group (2) was re-inoculated with another dose of the vaccine 4 weeks later, and group (3) kept as non inoculated group. Blood samples were collected before and at 7, 14, 21, 35, 60, 90, 120, 150, 180 and 215 days post-vaccination. The collected sera were used for determination of serum neutralizing index. Two animals of each group were challenged with the virulent isolated virus by a dose of $10^{4.5}$ $MICID_{50}$/ml. Blood samples were collected to study the blood picture and level of neutralizing antibodies after challenge.

Virus Assay:

Virus was assayed for infectivity in either in suckling mice or in Vero cell. The mice and cell culture were observed for seven days and the virus titer calculated according to **Reed and Muench, (1938).**

Virus Neutralization Test:

Serum samples were collected from all experimental cattle before vaccination, and at various intervals post-vaccination and challenge. The NI was carried out according to **Theodoridis *et al.*, (1973).**

Challenge Virus:

Virulent BEF virus obtained at peak of the febrile reaction from an experimentally infected cattle inoculated by blood from naturally infected cases (**Soad, 2001**) and stored in sealed ampoules at $-70^{O}C$ in the form of 10% suspension of leukocytes in Buffer Lactose Phosphate (BLP).

RESULTS

Table (1) show the inactivation of BEF virus by using different concentrations of BEI at 37 ^{O}C. The titers of the original harvest and for 5 hours exposure to BEI indicated that after 4 hours exposure, titers would be already below the level of detection at the concentration of (0.01), and could be detected by a titer of 1.2 log $TCID_{50}$ / ml in concentration of 0.001. After 5 hours the titer of the virus was below the level of detection in both concentrations and this was confirmed by the results in 6^{th} and 7^{th} hours samples.

Result of safety test in cattle indicated that the vaccine is safe for cattle after S / C inoculation of ten doses of the vaccine or repeated inoculation of one dose. No systemic reactions were observed, and a slight acceptable local reaction may be observed at the site of injection, and recovered 3 weeks later.

The results of potency test of the prepared vaccine in vaccinated cattle, (Table, 2) indicate that the vaccinated challenged groups G1 & G2 showed no rise in body temperature compared with non-vaccinated challenged group (G3) and at the same time no change in blood picture which showed the characteristic form (Band form of lymphocytes). There is an increase in the neutralizing indices in all groups being over 4 index at the 35 days post-challenge.

The mean neutralization indices (protective indices) obtained in sera of cattle vaccinated with the prepared vaccine are given in table (3) which showed that the rise of antibodies against BEF started 7 days post-vaccination in both groups (G1 & G2) reaching (2.2 – 2.4) indices after 21 days. Group2 showed about one log $_{10}$ differences increase in indices, which extended over 7 months post-vaccination.

Table (1): Inactivation of BEFV by BEI at 37°C.

Inactivant	Precaution	Concentration	Infectivity titers log_{10} $TCID_{50}$/ ml / hours							
			0	1	2	3	4	5	6	7
BEI	BEI in NaoH (Cyclization)	0.01	7.9	6.1	1.2	0.7	0	0	0	0
		0.001	7.9	6.3	1.2	1.2	1.2	0	0	0

Preparation of 0.1M in 0.2N NaoH for 7 hours at 37°C

DISCUSSION

The use of BEI for inactivation of BEF virus is very efficient after cyclization of 2-Bromoethylamine hydrochloride with NaoH according to the report and the technique applied by **Bahnemann, (1975)** for inactivation of FMD virus. From the results recorded in table (1) it is clear that 1 hour after exposure of BEF virus to BEI, the original titer lost about 1.8 – 1.6 log $_{10}$ at 0.01 and 0.001 concentrations, more time (1hour) is required for complete inactivation when using 0.001 concentration at 37 °C, the results of these studies show that BEI prepared with 0.01 or 0.001 final concentration is a good inactivator for BEF virus.

BEFV is one of the many antigens which are not sufficiently immunogenic to produce adequate quantities of protective antibodies in the vaccinated animal (**Theodoridis, *et al.*, 1973 and Tzipori and Spradbrow, 1978**) moreover aluminium hydroxide gel are added to the BEF antigen to increase their ability to make vaccinated animals producing large quantities of protective antibodies, (**Taha, 1982**) who used these adjuvant to increase

the stimulating effect of RVF inactivated vaccine. The temporary swelling causes by the prepared vaccine at the site of the injection subsides and recovered over a period of 2 – 3 weeks.

Inoculations of BEF inactivated vaccine succeeded in inducing satisfactory level of neutralizing antibodies either with one or two successive doses (3.5 and 4.2 NI). The results presented in Table (2), shows that non of the vaccinated challenged animals possessed any clinical symptoms or rise of temperature, while the control non-vaccinated group (G3) developed fever, salivation, nasal discharge, swollen lymph nodes, stiffness with changes in blood picture.

Sera obtained from vaccinated and non–vaccinated animals were tested for the presence of neutralizing antibodies (NA) as indicated in Table (3). From the results it is evident that all vaccinated animals (once or twice) developed protective antibody titers against BEFV, and the interval of 4 weeks that elapsed between the first and second inoculation increased the neutralizing indices by about one log differences and influence on the eventual titers (3NI) of NA at the end of examined period.

In conclusion the dose of the locally prepared vaccine is 2 ml which has to be injected subcutaneously on the side of the neck over the shoulders of the cattle, two doses of the vaccine, separated by an interval of four weeks are recommended to produce a duration of protective immunity not less than 7 months.

REFERENCES

Bahnemman, H. G. (1973): The inactivation of Foot and Mouth Disease virus by ethylenimine and propylenimine
ZbL. Vet. Med. B., 20: 356 – 360

Bahnemman, H. G. (1975): Binary ethylenimine as an inactivant for Foot and Mouth Disease virus and its application for vaccine production
Arch. Virol., 47, 47 – 56

Bahnemman, H. G.; Auge, D. P.; Abaracon, D. and Gomes, I. (1974): Immunogeniçity in cattle of Foot and Mouth Disease vaccine inactivated with Binary ethyleneimine
Bull. Off. Int. Epiz., 81 (11 – 12): 1335 – 1343

Bevan, L. E. W. (1907): Preliminary report on the so called "stiff sickness or three day – sickness of cattle in Rhodesia
J. Comp. Path. Ther., 20 : 104 – 113

Inaba, Y.; Tanaka, Y.; Sato, K.; Ito, H.; Omori, T. and Matumoto, M. (1969): Bovine epizootic fever. Loss of virus pathogenicity and immunogenicity for the calf during serial passage in various host systems
Jap. J. Microbiol., 13 : 181 – 186

Reed, L. J. and Muench, H. (1938): A simple method for estimating 50 percent end points.

Am. J. Hyg. , 27 : 493 - 497

Soad, M. Soliman; Taha, M. M.; Samir, S. S. and Daoud, A. M. (2001): Isolation and identification of BEF virus in Egypt Under press

Taha, M. M. (1982): Studies on inactivated vaccine of Rift Valley Fever V. Ph. D. Thesis (Microbiology), Fac. Vet. Med., Cairo Univ.

Theodoridis, A.; Boshoff S. E. T. and M. J. Botha (1973): Studies on vaccine against bovine ephemeral fever Onderstepoort. J. Vet. Res. 40 (3): 77 – 82

Tzipori and Spradbrow (1978): Development of experimental infectivment BEF vaccine using aluminium hydroxide gel as an adjuvant. Cited by Arthur Webster Pty Limited 226 Windsor Road, Northmead NSW 2152, Australia.

Van Der Wasthuizen, B. (1967): Studies on bovine ephemeral fever. I. Isolation and preliminary characterization of a virus from natural and experimentally produced cases of bovine ephemeral fever. Onderstepoort. J. Vet. Res., 34: 29 – 40

Table (2): Potency test of the prepared vaccine in vaccinated challenged calves.

Treatment	Mean degree of recorded temp. Days post challenge							Change in blood picture Days post challenge				Mean neutralizing index Days post challenge					
	1	2	3	4	5	6	7	1	2	3	5	0**	3	7	12	21	35
Vaccinated once (G1)	38	38.5	38.5	38.2	38	38	38	N.C	N.C	N.C	N.C	3.5	2.1	3.6	4.0	4.0	4.1
Vaccinated twice (G2)	38.3	38.2	38.1	38	38	38.2	38.1	N.C	N.C	N.C	N.C	4.2	3.8	4.2	4.3	4.2	4.2
Non vaccinated (G3)	41.2	39.9	40	38.1	38.2	38.1	38.3	BF% 40	BF% 43	BF% 40	BF% 25	0.5	0.7	1.2	2.9	3.2	4.2

N.C = no change in blood picture. 0** = zero day before challenge
BF% = band form percent (characteristic for BEF virus) in blood examined slides
G1 challenged 4 weeks post vaccination. G2 challenged 2 weeks post 2nd dose (re-vaccination)

Table (3): Immunogenicity of the prepared inactivated BEF vaccine in vaccinated calves.

Treatment	Mean neutralizing index (NI) at different interval / days										
	0	7	14	21	35	60	90	120	150	180	215
Vaccinated once (G1)	0.3	1.2	2.1	2.4	3.5	3.1	3.0	2.1	2.0	2.1	1.9
Vaccinated twice (G2)	0.5	1.2	1.9	2.2	2.8*	4.2	4.0	4.3	4.1	3.7	3.0
Non vaccinated (G3)	0.3	0.3	0.3	0.5	03	0.5	0.5	0.5	0.3	0.3	0.7

* Re-vaccinated 4 weeks after the first dose

الملخص العربى

تحضير لقاح مثبط لفيروس حمى الثلاثة أيام فى مصر

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تم تحضير لقاح مثبط لفيروس حمى الثلاثة أيام من التمريرة الثالثة إلى الخامسة على خلايا الزرع النسيجى ومن العترة المعزولة محليا أثناء وباء ٢٠٠٠ فى مصر.

وقد استخدم الإثيلين أمين فى عملية التثبيط حومن اجل تقليل التأثير السام لمادة الإثيلين أمين تم تحضيره فى شكل مخفف من ٢بروم إثيلين فى الصوديوم هيدروكسيد وأطلق عليه بناري إثيلين أمين (BEI) وبدراسة عن التثبيط وتحضين الفيروس فى درجة٣٧ ٥م وجد أن الفيروس يفقد العدوي بعد ٦ ساعات وفى نفس الوقت لايفقد القدرة الأنتيجينية ويعطي استجابة مناعية فى الأبقار المحصنة عمر أكثر من ستة أشهر باستخدامه كجرعة واحدة أو جرعتين بينهما ٤ أسابيع.

بعد إجراء إختبار التحدى لم تظهر أى أعراض إكلينيكية على الأبقار المحصنة بينما أظهرت الضوابط إرتفاع فى درجة الحرارة وإفرازات من الفم والأنف وتضخم فى الغدد الليمفاوية وتيبس عام فى العضلات ولم تظهر اى تغيرات عند فحص صورة الدم فى الحيوانات المحصنة بينما اظهرت الضوابط زيادة فى عدد الخلايا المتعادلة ٣٥% وتجمعات صغيرة داخل سيتوبلازم الخلايا وحيدة النواة.