Preliminary findings for an inactivated African horsesickness vaccine using binary ethyleneimine

M.M. Hassanain [1]

HASSANAIN (M.M.). Emploi de l'éthyléneimine binaire pour la production d'un vaccin inactivé contre la peste équine. Résultats préliminaires. *Revue Élev. Méd. vét. Pays trop.*, 1992, **45** (3-4) : 231-234

Des recherches ont été effectuées pour mettre au point un vaccin inactivé contre la peste équine au moyen de l'éthyléneimine binaire. Le processus d'inactivation de la souche virulente type 9 en utilisant ce produit, montre une inactivation complète du virus au bout de 18, 48 et 84 h avec des concentrations de 0,004, 0,003 et 0,002M, respectivement, sans détection virale résiduelle. Une concentration de 0,003M en inactivateur est recommandée et aucun changement dans les propriétés antigéniques virales n'est constaté dans le test de fixation du complément. Les paramètres physiques propres au vaccin avec l'adjuvant de Freund ont été étudiés. Une durée d'émulsification de 25 secondes est suffisante pour obtenir un produit émulsifié à 100 p. 100, de consistance crémeuse, avec un temps d'écoulement de 2,2 secondes/0,1 ml. Le vaccin est stable pendant six mois à la température de conservation de 4 °C et pendant 15 jours à 37 °C. Expérimenté sur deux chevaux, avec un rappel au bout de deux mois en employant le même vaccin, il a conféré une immunité acceptable pendant une période d'observation de six mois, au cours de laquelle la baisse maximale du taux d'anticorps a été de 0,2 $\log_{10}$ à la fin de cette période. Deux mois après la vaccination, l'inoculation des animaux avec la souche virulente de référence n'a provoqué l'apparition d'aucun signe clinique. *Mots clés* : Peste équine - Vaccin inactivé - Ethyléneimine binaire - Immunité - Égypte.

INTRODUCTION

African horsesickness (AHS), an endemic disease in Africa affecting solipeds has recently been reported in Saudi Arabia (1, 9) and Qatar (7). Its expansion has directed the attention to the need for adequate and effective methods for control and eradication.

Vaccination is one of the major steps in controlling AHS, especially in the endemic areas. Although the attenuated AHS virus vaccines are widely used in endemic countries, use of inactivated vaccines is still preferred in non-endemic countries.

Formalin-inactivated vaccine belongs to the latter category, but it is of poor quality (5). Consequently, inactivants which act directly on the viral nucleic acids leaving antigenic viral proteins unimpaired, are now preferred. Such inactivants are aziridine derivatives, a group with some disadvantages including instability at room temperature, a high toxicity and special precautions in handling to avoid skin contact (3). For these reasons a binary-ethyleneimine inactivant using 2-bromoethylamine hydrobromide has been developed in foot-and-mouth disease vaccine (2, 5). This paper describes the application of inactivated African horsesickness vaccine using binary ethyleneimine.

1. Serum and Vaccine Veterinary Research Institute, POBox 131, Abbasia, Cairo, Egypte.

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MATERIALS AND METHODS

Virus

Lyophilized virulent strain of AHS type 9 (T9) was obtained from Plum Island Lab, Ministry of Agriculture, USA. The virus was propagated in the brain of suckling mice and then adaptated to Vero cells for 2 to 4 passages until a virus titer of Log $10^{6.8}$ $TCID_{50}$/ml was reached.

Binary ethyleneimine inactivant (BEI)

This was formed through cycling 0.1M 2-bromoethylamine hydrobromide in previously warmed 0.15N sodium hydroxide (NaOH) at 37 °C. This gave 0.5 % ethyleneimine which was kept at room temperature (25 °C) until used (5).

Virus inactivation

Concentrations of 0.002, 0.003 and 0.004 M were obtained from 0.1M BEI. Equal volumes of virus suspension were added separately onto each BEI concentration. The pH of the mixtures was adjusted to 7.4 and the flasks were then incubated in a water bath at 37 °C. Volumes of 3.6 ml of the mixtures were collected at intervals in vials containing 0.4 ml of 20 % sodium thiosulfate. A control virus without inactivant was collected at the same intervals. Samples were then assayed for virus infectivity in Vero cell cultures and virus inactivation rates were calculated. A safety test was performed according to STELLMANN *et al* (10) in tissue cultures and mice for detection of residual virus activity. The antigenic properties of the inactivated virus were checked against type-9 reference AHS serum using the complement fixation test (CFT). Briefly, 2-fold dilutions (up to 1/64) of both inactivated and

uninactivated viruses were tested against reference type-9 antiserum. Complement fixing reactions between the antigens and the serum were read.

Preparation and standardization of African horsesickness inactivated vaccine

Three 50 ml samples of the inactivated virus were mixed with an equal volume of incomplete Freund's adjuvant (Difco Laboratories, Michigan, USA). The mixtures were then dispersed in a homogenizer at a high speed for three different periods. The physical properties of these mixtures were tested as follows : drop test according to WEIR (11), emulsion viscosity and stability according to CUNLIFE and GRAVES (4) and quality according to McKERCHER and GRAVES (8).

Experimental vaccination of susceptible horses

Two susceptible horses about 3 years old were subcutaneously inoculated with 5 ml/horse of the prepared vaccine at the base of the lower third of the neck and just anterior to the shoulder joint. Body temperature was recorded twice daily for one month post-vaccination, and inoculation sites were carefully observed. Each horse was given a booster of 5 ml/horse one month later on the other side of the neck.

Two months after the first dose, 2 ml of challenge virus containing 10^4 mouse LD_{50}/ml was inoculated into each horse. Immune responses of the vaccinated horses were traced using the serum neutralizing test (SNT), complement fixation (CFT) and passive haemagglutination test (PHT) at different intervals. Similarly, the challenge virus was inoculated into a third healthy susceptible non-vaccinated animal as a control.

RESULTS

The inactivation rates of the virus using BEI are demonstrated in figure 1. Complete viral inactivation using a BEI concentration of 0.002, 0.003 and 0.004M occurred at 84, 48 and 18 h respectively post-treatment. The coefficient of the virus inactivation rate of each BEI molarity were 0.09, 0.15 and 0.41 $\log_{10}$ $TCID_{50}$/h and the half-life values were 3.3, 2.0 and 0.7 h, respectively.

Residual virus infectivity test on the inactivated virus revealed neither a cytopathic effect (CPE) on tissue culture nor nervous signs in suckling mice, following intracerebral inoculation.

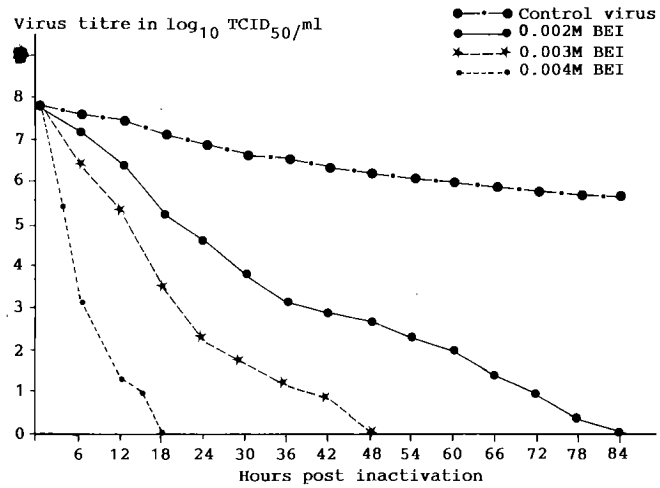

Fig. 1 : Inactivation curve of horsesickness T_9 virus with different concentration of 0.004, 0.003 and 0.002M BEI at 37 °C.

The results of CFT using reference type-9 antiserum against the inactivated and control uninactivated virus, showed no changes in the virus antigenic properties. The antigenic titer of the inactivated and uninactivated viruses were 1/16.

Table I illustrates the results of the parameters of different physical properties applied to the three samples of the prepared AHS oil adjuvant vaccine using 0.003M BEI.

The site of vaccine inoculation in horses showed soft walnut-sized swellings slightly painful to touch. These were later indurated, firmed and then disappeared completely within a few days post-inoculation. A slight rise in body temperature was also observed.

Antibodies were detected during the first week post-vaccination using the PHA test (fig. 2). However, complement fixing and neutralizing antibodies were first detected during the second week post-vaccination. In all three tests the antibodies produced reached their peak titres between 2 and 3 months post-vaccination.

The unvaccinated control horse showed typical AHS clinicopathological changes following virulent virus inoculation and died on day 13 post-inoculation.

DISCUSSION

Efficiency of killed virus vaccines depends both on the type of inactivating agent and on the adjuvant used (12).

The results concerning the influence of using varying molarities of BEI on AHS virus indicate that the concentration of 0.003M of BEI is the most suitable molarity for vaccine preparation. This is due to acceptable time requi-

TABLE I The physical properties of three samples of African horsesickness oil adjuvant vaccine using the choise concentration 0.003 M BEI.

Vaccine sample	Emulsification (in seconds) until stable drop forms	Stability test			Consistancy on solides	Relative viscosity (in seconds)	Keeping quality	
		% of aqueous phase	% of oil phase	% of emulsion phase			at + 4 °C	at 37 °C
1	15	1	15	84	Somewhat oily	1	Separation after 3 weeks	Separation within 3 days
2	25	–	–	100	Creamy	2.2	Stable for six months	Separation within 15 days
3	45	–	–	100	Thick oily	3.6	Stable for six months	Separation within 45 days

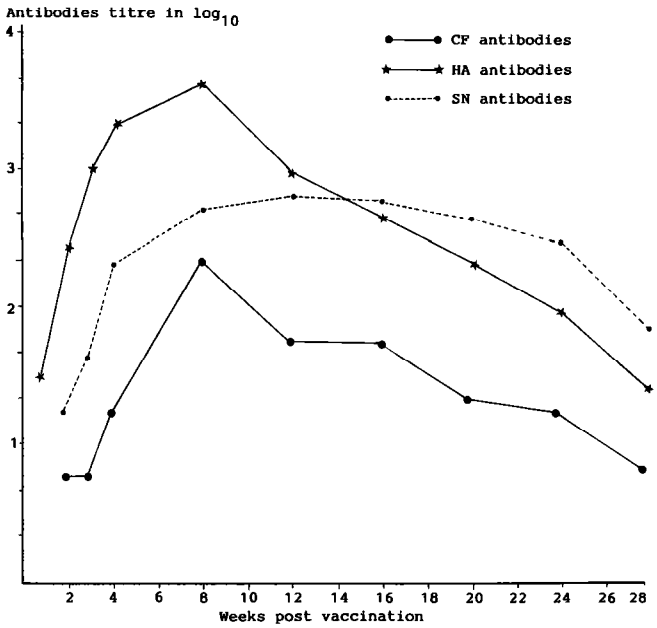

Fig. 2 : Mean antibody titres in horses vaccinated with oil adjuvant inactivated vaccine using BEI.

red for complete virus inactivation (48 h) preserving a minimum virus loss as compared with 0.002M (84 h) and preventing a high BEI concentration (0.004M) with short-time inactivation (18 h). Similar results were recommended by GRAVES (6) using formaline inactivant.

As for the antigenic changes of the virus during the inactivation process using different molarities before and after vaccination, the results obtained were almost identical.

Emulsification of the 0.003M BEI inactivated vaccine for 25 s revealed acceptable physical properties for an oil-inactivated vaccine. These properties include complete mixing, flow time of 2.2 s per 0.1 ml and stability for 6 months and 15 days at + 4°C and 37 °C, respectively.

Experimental application of oil vaccine on the two horses produced a mild local reaction. The slight rise in temperature could have been due to a reaction against the other foreign proteins included in the vaccine such as trace of cells, debris and bovine serum. Results of the immune response of the vaccinated horses indicate that immunized horses developed detectable haemagglutination antibodies by the first week post-vaccination while complement fixing and neutralizing antibodies were detected during the second week. A remarkable increase in all types of detected antibodies was observed four weeks after boosting. Although all the tested antibodies declined during the experiment, they still maintained their presence at the end of the observation period (6 months) especially the neutralizing antibodies which showed a minimum antibody titer drop (0.2 $\log_{10}$) at the 6-month check.

In conclusion, a new oil adjuvant BEI-inactivated AHS virus vaccine, that induced a transient local reaction and a minimal fever, proved to be an efficient immunogen. This type of vaccine would be of great benefit if used in countries which are affected for the first time with the disease or in those which have the intention of eradicating the disease without using live vaccines.

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M. M. Hassanain

HASSANAIN (M.M.). Preliminary findings for an inactivated African horsesickness vaccine using binary ethyleneimine. *Revue Élev. Méd. vét. Pays trop.*, 1992, **45** (3-4) : 231-234

Investigation studies on inactivated African horsesickness vaccine using binary ethyleneimine were conducted. The inactivation process of virulent type-9 strain using the above inactivant revealed complete virus inactivation at 18, 48 and 84 h post-treatment with inactivant concentrations of 0.004, 0.003 and 0.002M, respectively, without detection of residual virus. An inactivant concentration of 0.003M is recommended and no changes in viral antigenic properties were noticed in complement fixation test. The physical parameters in oil-emulsion vaccine using the incomplete Freund's adjuvant, were studied. Emulsification time of 25 s was recommended which resulted in a 100 % emulsion phase, creamy consistency, flow time of 2.2 s/0.1 ml and a stability at 4 °C and 37 °C for six months and 15 days, respectively. An experimental application of the oil vaccine in two horses (which was followed by a booster oil vaccine inoculation at 2 months post-vaccination) gave an acceptable immunity during the 6-month observation period with a maximum decline of the neutralizing antibody titer of 0.2 log_{10} at end of this period. Challenge of the vaccinated horses with the virulent virus strain at 2 months post-vaccination did not bring about any clinical symptoms. *Key words* : African horsesickness - Inactivated vaccine - Binary ethyleneimine - Protection - Egypt.

HASSANAIN (M.M.). Hallazgos preliminares para la producción de una vacuna inactivada contra la peste equina mediante la utilización de etileneimina binaria. *Revue Élev. Méd. vét. Pays trop.*, 1992, **45** (3-4) : 231-234

Se llevaron a cabo estudios con el fin de fabricar una vacuna inactivada contra la peste equina, gracias al uso de la etileneimina binaria. Dicho agente inactivante produjo la inactivación completa de la cepa tipo 9 a las 18, 48 y 84 horas post tratamiento, con concentraciones de 0,004, 0,003 y 0,002M respectivamente, sin presencia de virus residual detectable. Se recomienda una concentración de 0,003M, la cual no produce cambios en las propiedades antigénicas del virus, verificadas mediante el test de fijación de complemento. Se estudiaron los parámetros físicos en una emulsión oleosa de la vacuna, gracias al uso de adjuvante de Freund incompleto. El tiempo de emulsificación recomendado es de 25 s, el cual produce una fase de emulsificación de 100 p. 100, una consistencia cremosa, una velocidad de fluído de 2,2 s/0,1 ml y la estabilidad a 4 °C y 37 °C durante seis meses y quince días respectivamente. La administración experimental de la vacuna oleosa en dos equinos (seguida de una inoculación de vacuna oleosa en "booster" dos meses después de la primera vacunación), ofreció un grado de inmunidad aceptable durante los seis meses de observación, con una caída de los títulos de anticuerpos neutralizantes de 0,2 log_{10} hacia el final del periodo. La inoculación de la cepa virulenta en los equinos, dos meses post-vacunación, no produjo ninguna sintomatología clínica. *Palabras claves* : Peste equina - Vacuna inactivada - Etileneimina binaria - Inmunidad - Egipto.

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