Immunisation against Japanese Encephalitis in Nepal: Experience of 1152 subjects

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SUMMARY: During the summer of 1983, 1152 subjects were immunized against Japanese Encephalitis at BMH Dharan. This was the first use of the BIKEN killed lyophilised vaccine in the British Army. Three doses of 1 ml (0.5 ml for children under 3 years) given 10 days apart produced a protective neutralizing antibody response (titre more than 1:10) in almost 90% of subjects. Two doses of vaccine however seemed inadequate in that less than 40% of subjects seroconverted. Side-effects were minimal and trivial. Studies of neutralizing antibodies before vaccination showed up to 30% of Nepalese subjects tested were already immune due to previous inapparent infection while all but one of the British Nationals were fully susceptible.

Introduction

Japanese Encephalitis (JE) is a major health hazard in the Terai region of Southern Nepal. The area around the British Military Cantonment at Dharan, in the Koshi Zone of South Eastern Nepal, is ideal for JE virus transmission. Low level virus activity continues throughout the year with major epidemics during the summer monsoon. Previous serological studies involving British military personnel and families stationed in Dharan indicated all were fully susceptible to JE virus while many local Nepalese were already immune due to previous inapparent infection. In response to the JE threat a large scale immunization programme using the BIKEN killed lyophilised vaccine was introduced at BMH Dharan during the summer of 1983. This study reports experience of the first use of BIKEN vaccine in the British Army.

Methods

1. Immunization Programme

The BIKEN killed, lyophilised vaccine manufactured by the Research Foundation for Microbiological Diseases of Osaka University, Japan, was used. The vaccine was transported at ambient temperature to Nepal but stored in a deep freezer at BMH Dharan. It was reconstituted according to the manufacturer’s instructions and used immediately. Any unused vaccine was discarded. The manufacturer’s contraindication guidelines were observed. The vaccine was injected subcutaneously into the deltoid region of the left arm. A dose of 1 ml was given to subjects over 3 years of age, 0.5 ml for those aged 1-3 years, while children under 1 year of age were not vaccinated. The following groups were immunised:

(a) Serving soldiers and their families. Each received 3 doses at intervals of 10 days.
(b) Serving Gurkha soldiers. Each received 3 doses at intervals of 10 days.
(c) Locally employed Nepalese civilians (LEC). Each received 2 doses 10 days apart.
(d) Gurkhas in transit (serving Gurkha soldiers and families on leave in Nepal). First dose was given at their Base Station with a second dose given 10 days later at BMH Dharan.
(e) Other UK Nationals. Each received 2 doses 10 days apart.

2. Assessment of Safety

A register of all subjects vaccinated was kept. Subjects were questioned about side-effects after each dose and asked to report any untoward effects to a designated nursing assistant at BMH Dharan.

3. Assessment of Susceptibility To Infection

Serum was taken from a sample of subjects from groups A, B and C before vaccination. The serum was dispatched to the U.S. Army Medical Component, Armed Forces Research Institute of Medical Sciences (AFRIMS) in Bangkok for assay of JE virus neutralizing antibody titre by plaque reduction neutralization using YOKEN-BIKEN and KE-094 strains of JE virus.

4. Assessment of Immune Response to Vaccination

Serum was taken from a sample of subjects in groups A, B and C before and 10 days after the final dose of vaccine for assay of the neutralizing antibody response. The assay by plaque reduction neutralization was carried out at AFRIMS.
Results

1152 subjects were vaccinated including 59 children with a mean age of 8.7 years (range 2-15 years). The distribution of the vaccinated subjects by group is shown in Table 1.

Table I

<table>
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<th>Group</th>
<th>Male</th>
<th>Female</th>
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<td>68</td>
<td>132</td>
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<tr>
<td>B</td>
<td>223</td>
<td>0</td>
<td>223</td>
</tr>
<tr>
<td>C</td>
<td>693</td>
<td>19</td>
<td>712</td>
</tr>
<tr>
<td>D</td>
<td>62</td>
<td>13</td>
<td>75</td>
</tr>
<tr>
<td>E</td>
<td>10</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>Total</td>
<td>1052</td>
<td>100</td>
<td>1152</td>
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Side-Effects

15 subjects left the cantonment immediately after the last dose of vaccine. Although none reported any side-effects their subsequent fate was unknown. 1137 subjects were fully documented. Mild discomfort at the injection site similar to that experienced after tetanus toxoid or HDC rabies vaccine was very common. It rarely lasted more than a few hours and was not associated with swelling, erythema or fever. One subject felt dizzy after each injection while another complained of a rash after the first but not the second injection. It is doubtful that either reaction was related to the vaccine itself. No other side-effects were reported.

Susceptibility to Infection

**Group A** 35 subjects were tested of whom 33 had no evidence of neutralizing antibody (NA) in their serum and were therefore fully susceptible to infection. Two had positive titres (1:78 and 1:84). One, however, was a Nepalese National while the other had previously served in JE endemic areas elsewhere in Asia.

**Group B** 20 subjects were tested. Six (30%) had neutralizing titres ranging from 1:23 to 1:640.

**Group C** 20 subjects were tested. Six (30%) had NA with titres 1:26 to 1:145.

These results indicate up to 30% of the Nepalese tested were already immune to JE virus due to previous inapparent infection.

Immune Response to Vaccination

The neutralizing antibody response to BIKEN vaccine was evaluated in 75 subjects. The results are shown in Table II.

All the subjects who had positive pre-vaccination NA titres showed a brisk secondary response to both 2 and 3 doses of vaccine. Of those without evidence of NA pre-vaccination 89% showed a satisfactory primary response (to a titre at least 1:10) when given 3 doses of vaccine. However, when only 2 doses were employed only 36% achieved seroconversion.

Discussion

BIKEN vaccine has been extensively used in Japan and other parts of Asia. Since 1966 over 82 million doses have been issued without any reports of serious side-effects. Several reports attest to its efficacy and safety. Despite the reassuring evidence regarding safety some concern was felt about using a vaccine prepared from mouse brain because of the possibility of hypersensitivity and encephalopathy. In this study 1137 vaccinated subjects were carefully followed up. Apart from minor local discomfort at the injection site no significant side-effects were reported, confirming the excellent safety record of this vaccine which is highly purified with regard to potentially immunogenic basic proteins.

In animals NA titres 1:10 are highly protective against challenge with virulent JE virus. In this study a satisfactory primary seroconversion was defined as a NA titre of at least 1:10. With three doses of vaccine almost 90% of subjects achieved seroconversion. Why 10% failed to seroconvert is not clear. Careful examination of the records however revealed vaccine batch, injection technique and other technical factors were not responsible. Two doses of

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<td>3</td>
<td>K</td>
<td>2</td>
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<td>162</td>
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<tr>
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<td>6</td>
<td>14</td>
<td>5</td>
<td>9</td>
<td>31</td>
<td>25-70</td>
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Prevac. — prevaccination neutralizing antibody
Primary response — NA response in those without NA in serum before vaccination
Positive response — Titre of at least 1:10
YB — YOKEN-BIKEN
KE — KE-094
vaccine proved very inferior with under 40\% seroconversion. Secondary responses in those with prevaccination NA were brisk, suggesting booster doses will maintain adequate protection. It is likely that annual booster doses will be required.

With regard to susceptibility of the unvaccinated subjects this study confirms the earlier impression that British military personnel are nearly always fully susceptible and at considerable risk of devastating encephalitis\textsuperscript{1,4,12}. The situation regarding Nepalese Nationals is very different, up to 30\% of whom are immune due to previous inapparent infection. This high level of community immunity is typical in JE endemic areas\textsuperscript{3,4,13-15}. High grade community immunity combined with the low risk of clinical encephalitis in patients from endemic areas (1:300-1:1000) suggests that immunization is unnecessary\textsuperscript{3,16,17}.

It must not be assumed that satisfactory seroconversion in response to BIKEN vaccine implies immunity to Nepalese wild strain JE virus. While there is no doubt that NA raised against BIKEN vaccine virus protects against Japanese JE virus the protection afforded against other wild strains will depend upon the antigenic variation of the wild strains. That such antigenic variation exists is undoubted\textsuperscript{18,19}. How antigenically close Nepalese JE virus is to Japanese JE virus is unknown. This underlines the urgent need for further research in Nepal to isolate the local strain and test it against NA raised by BIKEN vaccine. Some reassurance is possible however as a study in India\textsuperscript{6} revealed that although Indian wild strain was different antigenically from Japanese virus the BIKEN vaccine still affords useful if somewhat degraded protection. As it is probable that the JE virus invaded Nepal from India\textsuperscript{a}, Nepalese and Indian strains are likely to be antigenically close.

This study concerned only vaccine prophylaxis. As important are personal and community measures to reduce the mosquito vector population and the risk of bites (which is highest out of doors after sunset) and probably measures to reduce the local virus pool, particularly active immunization of young pigs kept on the Dharan cantonment farm.

Conclusions
1. BIKEN vaccine appears to be very safe.
2. Three doses produce high grade immunity in about 90\% of subjects but 2 doses are ineffective in more than 60\%.
3. Booster doses should be given annually.
4. Locally employed Nepalese civilians need not be vaccinated.
5. All British military personnel and families posted to or visiting endemic areas should be vaccinated with three doses before travelling: vaccination should be offered regardless of season. Because of a WHO recommendation\textsuperscript{20} children under 1 year were not vaccinated. This report however refers to children living in endemic areas in whom transplacental antibody transfer confers immunity in the first months of life.

This is not the case in babies travelling to Nepal from non-endemic areas and it seems wise to offer these infants vaccination with three 0.5 ml doses.
6. Despite difficulties with the cold chain during transportation the vaccine remained potent.
7. Intense anti-mosquito measures should be undertaken and vaccination of pigs considered.
8. Urgent attempts to isolate the local virus are required.

Acknowledgements
I am grateful to Major D S Burke US Army MC and Dr C J Leake for their assistance with antibody assays, and to Mr T B Gurung and the nurses of BMH Dharan who helped manage the study.

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Major A Henderson MRCP RAMC

Special Commendation at "Doctor of the Year" Awards

Major Alan Henderson MRCP RAMC was given a "Special Commendation" at the BUPA Medical Foundation "Doctor of the Year" Awards held at the Savoy Hotel, London on the 29th March 1984.

The award was for his excellent work at BMH Dharan, Nepal, on the cause, epidemiology and prevention of Japanese Encephalitis in that part of the world. His work, the subject of several recent publications, has already had a significant practical spin-off: all military and sponsored personnel proceeding to and through the area must now receive the appropriate vaccine.

Major Henderson, married, with two children, is presently undertaking a one-year secondment in cardiology at St Thomas' Hospital, London, prior to posting to BMH Hong Kong in October 1984.

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