AN ACCELERATED SCHEDULE FOR TICK-BORNE ENCEPHALITIS VACCINE: THE AMERICAN MILITARY EXPERIENCE IN BOSNIA

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Abstract. Tick-borne encephalitis (TBE) is a viral illness endemic to the Balkan region. United States military forces were deployed to Bosnia in early 1996 as part of Operation Joint Endeavor, a U.S.-led multinational peacekeeping operation. To counteract the TBE threat, an inactivated, parenteral vaccine (FSME-Immun Inject®; Immuno AG, Vienna, Austria) was offered to soldiers at high risk on a volunteer basis in an accelerated, 3-dose schedule (0, 7, and 28 days). Passive adverse reaction surveillance was conducted on 3,981 vaccinated personnel. Paired sera from a randomly selected group of 1,913 deployed personnel (954 who received vaccine and 959 who were unvaccinated) were tested for antibodies to TBE by an ELISA. Three-dose recipients demonstrated an 80% seroconversion rate (4-fold or greater increase in anti-TBE titers). By comparison, the TBE infection rate in the unvaccinated cohort was found to be only 0.42% (4 of 959). Only 0.18% of vaccinees reported self-limited symptoms. An accelerated immunization schedule appears to be an acceptable option for military personnel or travelers on short-term notice to TBE-endemic areas.

The central European encephalitis virus (CEE) is one of a complex of flaviviruses that causes tick-borne encephalitis (TBE).1 Transmitted by Ixodes ricinus ticks, these viruses are endemic to central, eastern, and southeastern Europe and have become a significant public health problem since the early 1950s.1-3 In previous studies conducted in Europe, risk factors for infection have included being male, 20-40 years of age, and having an occupational exposure in forested, tick-endemic areas.1,3 The United States military interest in TBE began in the mid-1980s.3 This interest was heightened when U.S. soldiers were alerted for deployment to the Balkans as part of Operation Joint Endeavor in December 1995. The risk of TBE to American forces was considered to be significant and immediate. To counteract this threat, an inactivated, parenteral TBE vaccine (FSME-Immun Inject®; Immuno AG, Vienna, Austria), previously found to be safe and efficacious through 25 years of routine use in Central Europe,4,5 was made available on a voluntary basis to all Department of Defense (DoD) personnel at high risk of tick exposure using an accelerated, 3-dose schedule (0, 7, and 28 days). This communication presents a summary of the U.S. military’s experience with this vaccine to include an estimation of its safety and immunogenicity profile when administered to a population of military personnel deployed on a peacekeeping mission to the Former Republic of Yugoslavia.

MATERIALS AND METHODS

Vaccine. The vaccine used (FSME-Immun Inject®), is a highly purified, formaldehyde-inactivated vaccine, with each dose containing 2 μg of TBE virus antigen and 1 mg of Al(OH)₃. Currently unlicensed in the United States, this vaccine has been proven safe and efficacious for over a 20-year period in central Europe.5 Vaccine was stored between 2°C and 8°C in pre-packaged injectors and administered as a 0.5-ml dose by the intramuscular route to the deltoid. The vaccination schedule recommended by the manufacturer is for 3 doses to be given at 0, 1–3, and 9–12 months.5 However, under the terms of an investigational new drug application, the vaccine was offered to soldiers on alert for deployment or already deployed to high risk areas of Bosnia under an accelerated schedule (0, 7, and 28 days). The timing of this schedule was based on previous accelerated schedule studies6-7 and military operational requirements. For purposes of this report, and due to practical constraints and the wide range of intervals between doses, we considered an interval of 4–30 days between doses to be acceptable.

Subjects. The vaccine administration protocol was reviewed and approved by the Institutional Review Board at the U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID) (Fort Detrick, Frederick, MD) and by the Office of the Army Surgeon General’s Human Subjects Research Review Board. Written informed consent was provided by all vaccine recipients in accordance with research guidelines for studies in humans. Soldiers stationed in high-risk areas of Bosnia were offered the vaccine on a voluntary basis; benefits and risks of vaccination were explained in oral and written form. A total of 3,981 of an estimated 20,000 soldiers initially deployed were immunized. Pre-deployment serum samples (obtained within 12 months prior to the initiation of the TBE protocol) were retrieved from the inventory of the Department of Defense Serum Repository (DoDSR), which is maintained by the Army Medical Surveillance Activity (AMSA) at the U.S. Army Center for Health Promotion and Preventive Medicine. Post-deployment serum samples were collected as part of the service member medical examination immediately prior to departure from Bosnia. All military personnel who deployed for at least 30 days in Bosnia and who were also found to have both pre- and post-deployment serum samples in the DoDSR were identified. From this subset of 2,096 individuals, 1,913 serum pairs (91%) were finally obtained, with each pair representing 1 individual. Of these individuals, 954 received from 1 to 3 doses of TBE vaccine during the 6–9 months
they spent in Bosnia. The remaining 959 individuals constituted a comparison, unvaccinated cohort.

**Serologic testing.** In an initial screening for IgM antibodies in serum samples performed by one of the authors (CAR), no CEE-specific antibodies were detected in a sample of TBE vaccine recipients. Therefore, all subsequent seroconversion data analyses on serum pairs were performed based only on detection of IgG antibodies. Sera were tested for IgG antibodies in an ELISA with TBE antigen bound directly to a microtiter plate. Sera were screened at 1:100 dilution against the CEE strain (Hypr) of TBE. The secondary antibody used was a horseradish peroxidase–labeled antibody directed against theFc portion of the human IgG molecule. Sera were considered positive if the optical density (OD) exceeded the mean OD of 4 negative sera obtained from military personnel free of flavivirus antibodies, plus 3 SD, rounded to the nearest tenth. Sera exhibiting reactivity directly to a microtiter plate.

**Side effect monitoring.** Adverse reactions were passively monitored. Vaccinees were instructed to report any reaction that they believed to be secondary to the vaccine to the local military medical officers at each site. Adverse reaction reports were collected for a period of up to 30 days after each vaccine dose. No comparison adverse event data was collected in non-vaccinees.

**Data management.** Demographic and deployment data were provided to the investigators in a coded fashion (i.e., free of personal identifiers) by the AMSA. All serologic testing was done in a blinded fashion by USAMRIID collaborators. Differences in proportions for the demographic variables analyzed were tested for significance by chi square and Fisher’s exact tests. Geometric mean titer (GMT) responses were compared by grouped t tests and analysis of variance using SPSS version 7.5 statistical software (SPSS, Inc., Chicago, IL).

**RESULTS**

The demographic variables of the 1-, 2-, and 3-dose groups and non-vaccinees are shown in Table 1. There were no statistically significant differences in age, gender, race, or military rank distribution between groups. The response to vaccine did not differ significantly among Caucasians and non-Caucasians. It is important to note that there does not appear to be a genetic-based (i.e., race) difference in vaccine response rates. There were significantly more males represented in the vaccine group (914 of 954, 95.8%) than in the unvaccinated group (853 of 959, 88.9%), reflecting a higher refusal rate by females (P < 0.001).

Of the 954 individuals in the vaccinated cohort, 794 (83%) received their first, second, and/or third doses in the appropriate interval of 4–30 days and were included in the final vaccine immunogenicity analysis. The remaining 160 individuals were excluded from the final immunogenicity analysis because 1) 38 demonstrated pre-existing anti-TBE titers ≥ 1:100, indicative of past flavivirus exposure; and 2) 122 received vaccine doses outside of the 4–30-day acceptable range. The breakdown of doses and intervals is shown in Table 2. Significantly more of the 3-dose recipients were immunized during the first 60 days in-country compared with 1- and 2-dose recipients (98.4% versus 89.1%; P < 0.05).

Only 4 (0.42%) unvaccinated individuals, all males, demonstrated a 4-fold seroconversion. All 4 were infected with TBE virus (or a closely-related variant) during their 6–9-month deployment period in Bosnia, but did not report with symptoms to any health care provider. No clustering of infections was noted to occur in any particular military camp(s).

Table 3 shows the GMTs, mean-fold GMT conversions, and seroconversion rates (in %) by gender, race, and military rank for individuals who received 1, 2, and 3 doses at the acceptable interval(s). The comprehensive seroconversion rates for all individuals receiving 1, 2, and 3 doses were 20%, 60%, and 80%, respectively. The GMTs and seroconversion rates for males and females receiving 2 or 3 doses were found to be similar. However, a higher seroconversion rate was noted for females (60%) compared with males (17%) receiving only 1 vaccine dose (P = 0.05). Caucasian individuals who received only 1 vaccine dose sustained a higher GMT response than non-Caucasians (GMT = 98 for Caucasians and 68 for non-Caucasians; P = 0.003). For 2-dose recipients only, it was noted that the seroconversion rates among enlisted personnel were higher than for officers.

**TABLE 1**

Demographic characteristics of tick-borne encephalitis vaccine recipients by dose*

<table>
<thead>
<tr>
<th>Demographic Characteristics</th>
<th>1 Dose (n = 66)</th>
<th>2 Doses (n = 163)</th>
<th>3 Doses (n = 728)</th>
<th>Unvaccinated (n = 959)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (SD) age in years</td>
<td>24.8 (5.4)</td>
<td>26.3 (6.1)</td>
<td>26.7 (6.0)</td>
<td>26.9 (6.1)</td>
</tr>
<tr>
<td>Male gender (%)</td>
<td>60 (90.9)</td>
<td>155 (95.1)</td>
<td>699 (96.4)</td>
<td>853 (88.9)</td>
</tr>
<tr>
<td>Caucasian race (%)</td>
<td>42 (63.6)</td>
<td>110 (67.5)</td>
<td>516 (71.2)</td>
<td>5568 (59.2)</td>
</tr>
<tr>
<td>Military rank (%)</td>
<td>Lower enlisted</td>
<td>40 (60.6)</td>
<td>98 (60.1)</td>
<td>359 (49.5)</td>
</tr>
<tr>
<td></td>
<td>Higher enlisted</td>
<td>18 (27.3)</td>
<td>48 (29.4)</td>
<td>251 (34.6)</td>
</tr>
<tr>
<td></td>
<td>Officers</td>
<td>8 (12.1)</td>
<td>27 (16.6)</td>
<td>115 (15.9)</td>
</tr>
</tbody>
</table>

* Values in parentheses are standard deviations.
Dosing characteristics of tick-borne encephalitis vaccine recipients by dose*

<table>
<thead>
<tr>
<th>Month of 1st dose (%)</th>
<th>1 Dose (n = 66)</th>
<th>2 doses (n = 163)</th>
<th>3 Doses (n = 725)</th>
</tr>
</thead>
<tbody>
<tr>
<td>April 1996</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>May 1996</td>
<td>25 (37.9)</td>
<td>79 (48.5)</td>
<td>564 (77.8)†</td>
</tr>
<tr>
<td>June-August 1996</td>
<td>7 (10.6)</td>
<td>18 (11.0)</td>
<td>13 (1.6)</td>
</tr>
</tbody>
</table>

Median (range) of days between first and second doses

<table>
<thead>
<tr>
<th>4–10 (%)</th>
<th>11–20 (%)</th>
<th>21–30 (%)</th>
<th>31–60 (%)</th>
<th>61–90 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>7 (4–70)‡</td>
<td>135 (83.3)</td>
<td>8 (4.9)</td>
<td>4 (2.5)</td>
<td>3 (1.9)</td>
</tr>
</tbody>
</table>

Median (range) of days between second and third doses

<table>
<thead>
<tr>
<th>4–10 (%)</th>
<th>11–20 (%)</th>
<th>21–30 (%)</th>
<th>31–60 (%)</th>
<th>61–90 (%)</th>
<th>&gt;90 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>21 (8–147)</td>
<td>203 (28.0)</td>
<td>407 (56.1)</td>
<td>87 (12.0)</td>
<td>20 (2.8)</td>
<td>3 (0.4)</td>
</tr>
</tbody>
</table>

*NA = not applicable.
† P < 0.05 for comparison of 3-dose versus 1- and 2-dose recipients.
‡ One observation with an unknown interval.

We noted slight differences in vaccine response among military personnel with higher second dose seroconversion rates for enlisted personnel compared with officers. This probably represents a random (chance) variation given the number of statistical tests performed. The fact that fewer women volunteered to receive the vaccine is most likely due to concerns of potential vaccine teratogenicity. These concerns, although understandable, are unwarranted given the safety record of this vaccine in Europe, to include its use in vaccinating pregnant women (Pittman PR, unpublished data).

Previous smaller-scale studies with inactivated TBE vaccines conducted among European and American civilians have indicated that conventional and accelerated schedules induce comparable seroconversion rates. In addition, animal studies with these inactivated vaccines also support the...
idea that accelerated schedules produce adequate antibody responses.\textsuperscript{11} In 2 studies conducted by Kunz and others\textsuperscript{12,13} using the same vaccine we used, high seroconversion rates were detected 14 days after 2 (85–95\%) and 3 doses (95–100\%). This same vaccine, tested using an accelerated schedule of 0, 14, and 28 days among U.S. military personnel at USAMRRIID, also demonstrated seroconversion rates of 25\%, 95\%, and 97\% one week after each of 3 doses (Pittman PR, unpublished data). Harabac\textsuperscript{z} and others\textsuperscript{6} conducted trials of conventional and accelerated schedules using a competing, but antigenically similar vaccine (FSME-Vaccine Behring; Behringwerke AG, Marburg, Germany). When administered in the conventional schedule (0, 28, and 300 days) high seroconversion rates of 80\%, 100\%, and >90\% after each of 3 doses were seen. Seroconversion rates for the accelerated schedule (0, 7, and 21 days) were 90\% fourteen days after the second dose, 100\% fourteen days after the third dose, and >90\% ten months after the third dose.\textsuperscript{5} Both Kunz and others\textsuperscript{12} and Harabac\textsuperscript{z} and others\textsuperscript{6} noted a significant decrease in antibody titers from 9 to 10 months after the second dose on the conventional schedule.

It should be noted that the lower seroconversion rates of 60\% (for 2 doses) and 80\% (for 3 doses) in our study probably reflect the natural decrease in antibody titer after the second and third doses noted in these earlier studies. In addition, it is possible that variations in the actual vaccine schedule, such as extended (3–4 weeks) or shortened (4–7 days) periods between doses, may have accounted for a lower response (see Table 2).

For the military and travel community from non-endemic areas of the world that are required to deploy large numbers of individuals to areas where TBE is endemic, a safe, effective, and rapidly immunizing vaccine(s) is (are) highly desirable. Although TBE vaccine has not been formally tested, the current literature supports immunization in these situations.\textsuperscript{14} Results from our field study indicate that FSME-Immun Inject\textsuperscript{8} vaccine can be administered safely on an accelerated schedule and still confer significant antibody levels to at least 80\% of 3-dose recipients. Our data also suggest that the antibody response to this vaccine may be blunted in those individuals with pre-existing anti-TBE immunity, whether due to previous exposure to TBE virus or vaccine, or to other flaviviruses.

U.S. soldiers were initially deployed to Bosnia during February and March of 1996, approximately 1–2 months prior to implementation of the TBE vaccine protocol on-site. Tick activity in Bosnia begins in late April and the Western subtype of TBE endemic to the area is seen predominantly in early autumn.\textsuperscript{15} These facts and the low seroconversion rate (0.42\%) noted in the unvaccinated cohort in our study suggests that the risk of TBE infection in areas occupied by U.S. troops was not as great as previously suspected. The low seroconversion rate among unvaccinated personnel clearly demonstrates a very low risk of infection with the TBE complex (or other cross-reacting flaviviruses) among deployed personnel. Our data replicate similar findings by McNeil and others\textsuperscript{3} in American service members training in southern Germany where a low rate of infection (0.9 per 1,000 person-months) was documented. During Operation Joint Endeavor in 1996, a comparable low infection rate (0.7 per 1,000 person-months) was noted. Moreover, all 4 infections in the unimmunized group were subclinical in nature, a finding that correlates well with documented TBE subclinical infection rates of 70–98\%.\textsuperscript{15,16} Our previous finding of no IgM antibodies in tested sera further documents the infrequent occurrence of acute TBE infections among military personnel in Bosnia.\textsuperscript{17} While these data support the notion that continued, widespread use of TBE vaccine for American troops deployed to Bosnia is not warranted, it must be remembered that the soldier’s exposure to potentially infected ticks could increase very rapidly if hostilities were to ensue.

The low rates of natural infection are most likely due to a combination of factors that include 1) a low virus endemicity in areas occupied by U.S. forces, 2) a low tick exposure rate in the military compounds occupied by service members, and/or 3) an absence of the principal tick vector, *Ixodes ricinus*, in areas contiguous to military compounds. The absence of trees and underbrush on many of the military compounds supports the absence of tick vectors. It seems unlikely, however, that compliance with personal protection measures (PPM) such as repellents, bed nets, and permethrin-impregnated clothing played a significant role in decreasing soldier risk.\textsuperscript{18}

Finally, it should be noted that the ELISA used in this study is not able to specifically distinguish between anti-TBE and cross-reacting antibodies to other flaviviruses (dengue virus and yellow fever virus). Nonetheless, given the low seroconversion rate noted in the unvaccinated cohort and the documented lack of transmission of dengue or yellow fever in Bosnia, we feel certain that the antibodies to TBE measured represented a true reflection of the background infection rate in Bosnia with TBE-complex flaviviruses.

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