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Single dose of SA 14-14-2 vaccine provides long-term protection against Japanese encephalitis: A case–control study in Nepalese children 5 years after immunization ☆

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Abstract

In July 1999, a single dose of live-attenuated SA 14-14-2 Japanese encephalitis (JE) vaccine was administered to children living in the Bardiya, Banke and Kailali districts of Nepal. In 2004, the original vaccinated population experienced a fifth seasonal exposure to JE. We performed a case–control study comparing the prevalence of the administration of vaccine in patients with JE hospitalized in the Bardiya and Bheri Zonal hospitals and in age–sex matched controls resident in the Bardiya district. Among the 219 village controls, 114 had been vaccinated (52.1%) while only one of 20 JE cases had received live-attenuated JE vaccine. Five years after administration of a single dose, SA 14-14-2 provided a protective efficacy of 96.2% (CI 73.1–99.9%).

Keywords: Japanese encephalitis; Flavivirus; Vaccine

1. Introduction

Between 1978 and 2003, 26,667 cases and 5381 deaths due to acute encephalitis have been reported in Nepal [1]. Mortality and long-term sequellae rates are high in the age group below 15 years [2]. Although Nepal health authorities favored protecting the population by administering JE vaccines, budgetary constraints made it impossible to implement an immunization program. The donation in 1999 of a large quantity of SA14-14-2 live-attenuated JE vaccine (live JE vaccine) offered an opportunity to provide for some of those needs and to evaluate efficacy [3].

During 11–24 July 1999, nearly 160,000 doses of live JE vaccine were administered to children, ages 1–15 years, resident in 3 districts of Nepal [2,3]. In Bardiya district 79.9% of children in this age group were vaccinated, 34% in Banke and 18% in Kailai districts. When measured during the year of administration, the efficacy of a single dose of JE vaccine was 99.26% (CI 94.9–100%) while 1 year later it was 98.5% (CI 90.1–99.2%) [3,4]. Here we report vaccine efficacy observed after 5 years of seasonal exposure.

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2. Materials and methods

2.1. Study locations and subjects

Using methods described previously [3,4], a case–control study was performed in Nepalese children, adolescents and young adults, ages 6–20 years, residents in Bardiya district. Controls were selected from individuals of the same sex and ages living in the same village as JE cases. Study protocols were submitted to Nepal Health Research Council in November 2004 for approval of this case–control study. Because of the Maoist insurgency in Nepal, interview of controls was initiated in October/November 2005 almost 1 year after the end of 2004 epidemic. Written informed consent forms were completed by parents or guardians of cases enrolled in hospitals and age–sex matched village control cases.

2.2. Selection of cases

The case–control study was restricted to residents of the Bardiya district who were admitted to the Bheri Zonal or Bardiya district hospitals in July/October 2004 as the only JE vaccine administered in this district was the live vaccine given in July 1999.

In May 2004, JBT visited the Bheri Zonal hospital and Bardiya district hospital to select study physicians, lab technologists and nurses and conduct a 2-day training program in specimen collection/storage, interview methods and clinical record-keeping. The inclusion criteria and the standard procedures for selecting and reporting cases were defined and discussed with study staff.

Cases of JE complied with three criteria—(1) acute onset of fever of up to 7 days duration; (2) one or more of the following: change in consciousness, stiff neck, limb weakness, or long track neurological signs; and (3) IgM positive JE ELISA in a serum obtained at least 5 days after onset of fever. History of JE vaccination was elicited at the time of hospitalization and noted on a specially designed protocol in the hospital chart. After serological results were available on hospitalized cases, JBT HCO, YMS and SY met in Bangkok on September 2005 to review the clinical records for conformance with the JE case definition and to obtain records of name, age, sex, home address, admission history, physical examination and data on hospital course.

Inclusion criteria for JE cases in the case–control study were as follows: year of birth between June 1984 and 1998; continuously resident in Bardiya district since June 1999; admitted to Bheri Zonal or Bardiya district hospitals with a primary diagnosis of JE from July to October 2004; clinical JE (see case definition); laboratory confirmation (paired or single sera positive in a IgM capture ELISA and/or plaque reduction neutralization test).

Exclusion criteria: Bacterial encephalitis or meningitis based upon CSF culture or smear; immigrants to Bardiya district after 1999 vaccine campaign.

2.3. Serological determinations

All serological tests were performed on coded samples at the Center for Vaccine Development, Mahidol University, Bangkok, Thailand.

2.3.1. Plaque reduction neutralization tests (PRNT)

Separated sera were kept under −20 °C before testing. Neutralizing antibody was determined in LLC-MK_2 cells by PRNT using the 50% plaque reduction endpoint method described by Russell et al. [5]. Serial four-fold dilutions of serum were made (1:10, 1:40 and 1:160). An equal volume of diluted Beijing strain JE virus (genotype III) to contain about 40–50 pfu/well were added to each serum dilution tube. Following incubation at 37 °C for 60 min, 0.2 ml was removed from each tube and inoculated onto triplicate six-well plates of confluent LLC-MK_2. Each plate was incubated at 37 °C for 90 min and the monolayers were then overlaid with 4 ml of 3.5% carboxy methyl cellulose/minimum essential medium, Earles. Plates were incubated for 7 days at 37 °C with 5% CO_2. Plaques were counted and PRNT 50s were determined using SPSS. An antibody titer of 1:10 or above was considered positive. JE viruses isolated from Nepal and India also belong to genotype III [6].

2.3.2. IgM/IgG ELISA

IgG or IgM assays as described by Innis et al. [7] were modified by performing tests in microtiter plates coated with goat antihuman IgG or IgM. Diluted serum samples (1:100) were added to 96 well plates coated with antihuman-μ or -δ chain antibody. JE viral antigen prepared in Vero cells (Nakayama strain) or control Vero cell culture supernatant was added, followed by enzyme-labeled antiflaviviral monoclonal antibody and substrate. Absorbance values of viral and control wells were measured. Results were expressed as a ratio of adjusted optical density divided by 0.5. A positive value was equal to or greater than 1.00.

2.4. Selection of controls

Age–sex matched village controls were selected as described previously [3]. We recruited all available matches, or between 6 and 20 controls/case to avoid potential sampling bias. Inclusion criteria for controls: individuals resident in the same village as a serologically confirmed JE case; the same sex and age as index case (born between June 1984 and 1998).

In October 2005, we organized a 2-day training course to prepare field staff in the procedure of selecting controls. From October/November 2005, JBT and field staff visited villages of confirmed JE cases and identified/confirmed the JE case by his/her name, age, sex and father's name as recorded in hospital during the time of hospitalization. Vaccination status of JE cases was again checked by re-interviewing parents. Controls were identified by starting at the home of the index case and visiting sequential houses in a clockwise direction.