

Effectiveness of live-attenuated Japanese encephalitis vaccine (SA14-14-2): a case-control study

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Summary

Background Japanese encephalitis is a major cause of death and disability throughout Asia, including the Indian subcontinent. Although an effective vaccine for Japanese encephalitis is available, hundreds of millions of susceptible individuals remain unimmunised because of the vaccine's cost. In 1988, an inexpensive live-attenuated vaccine (SA14-14-2) was licensed in China. We have measured the effectiveness of this vaccine.

Methods In a case-control study in rural Sichuan Province, China, the 56 cases consisted of children admitted to hospital with acute Japanese encephalitis, and were confirmed serologically. 1299 village-matched and age-matched controls were identified, and vaccination histories obtained from pre-existing written records.

Findings The effectiveness of one dose was 80% (95% CI 44 to 93%); that of two doses was 97.5% (86 to 99.6%). Controlling for multiple potential confounders did not alter these results.

Interpretation We conclude that a regimen of two doses of live-attenuated Japanese encephalitis vaccine, administered 1 year apart, is effective in the prevention of clinically important disease. Subsequent study is needed to assure the safety of this vaccine.

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Introduction

Japanese encephalitis is an acute central-nervous-system infection that occurs over a vast geographic area (including India, China, Japan, and virtually all of Southeast Asia). The disease causes 35 000 cases of encephalitis and 10 000 deaths each year, and about 30% of survivors develop serious permanent sequelae.¹ Japanese encephalitis is an RNA flavivirus transmitted by *Culex tritaeniorhynchus* mosquitoes. Swine, birds, and other vertebrates are amplifying hosts. In most regions, the disease is seasonal, with most cases appearing between May and September. Japanese encephalitis is more common in rural than in urban areas, because the vector mosquito breeds in rice paddies and other standing water.¹ Because immunity to the disease is acquired naturally over time, Japanese encephalitis is primarily a childhood disease, with most cases occurring before age 15. The incidence is also elevated in the elderly, possibly because of waning immunity.

The incidence of Japanese encephalitis has declined greatly over the past three decades, presumably because of the widespread use of vaccines and because of mosquito-control efforts.² Inactivated weanling-mouse brain-derived vaccine is manufactured by Biken (Japan) and distributed internationally by Pasteur Merieux and Connaught Laboratories. In addition, a limited amount of a killed Japanese encephalitis vaccine is available from Green Cross (Korea). The efficacy of a two-dose regimen of the Biken vaccine was 91% (95% CI 70 to 97%) in a randomised controlled trial.³ Currently, a three-dose regimen is recommended in the US,⁴ where the average wholesale price for three doses is \$147.⁵ Because of the cost of the currently available vaccine, many countries in endemic areas cannot implement large-scale immunisation.

In 1988, the Chinese National Institute for the Control of Pharmaceutical and Biologic Products approved a live-attenuated, primary baby-hamster-kidney-cell-derived vaccine for Japanese encephalitis that is produced from the SA14-14-2 viral strain.⁶ This vaccine is manufactured by the Chengdu Biological Products Institute, and in some provinces has replaced the older, killed cell-culture vaccine because of the newer vaccine's higher putative efficacy (98%¹ versus 78%⁷) and slightly lower production costs (US\$0.02-0.03 versus 0.03-0.04 per dose¹). The Chengdu Biological Products Institute says that the vaccine has been administered to over 100 million Chinese children. Although the safety of this vaccine with respect to common adverse events has been shown in a study of 1026 vaccinated children,⁶ its safety with respect to rare events has not been studied. Non-randomised field trials in China suggest the efficacy of a single dose of the live-attenuated vaccine may be approximately 95%, and that of two doses one year apart may exceed 98%.¹

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However, the role of confounding in these non-randomised studies is unknown. We aimed to replicate these findings while controlling for potential confounders.

Patients and methods

Study design

We conducted a case-control study of incident cases of Japanese encephalitis in rural areas surrounding the city of Chengdu, Sichuan Province, China. Our overall strategy was to identify incident cases and to compare their vaccination histories with those of non-diseased controls. The study was approved by the University of Pennsylvania's Committee on Studies Involving Human Beings.

Sichuan Province

Sichuan, in southwestern China, is the largest and most populous Chinese province, with a 1990 population of 106 370 000. The Dujiangyan irrigation on the Min River irrigates 3 million hectares of rice and wheat crops, a vast expanse of flooded fields, which are the favoured habitat of *C. tritaeniorhynchus*. As a result, the annual incidence of Japanese encephalitis is estimated by Sichuan Province public-health officials to be 1 per 100 000, despite an existing vaccination programme.

Nearly all inhabitants of rural Sichuan are subsistence farmers, organised into geographically and economically homogeneous agricultural villages. Each village is associated with a government-supported county hospital that meets the inpatient needs of villagers. In addition, each village is served by a government-supported county anti-epidemic station that provides centralised support for village-run immunisation campaigns.

Children in rural Sichuan are immunised with Japanese encephalitis vaccine at 1 and 2 years of age, and receive a booster at 6. Immunisation occurs during annual campaigns each spring, with vaccine that was produced earlier that year. No other vaccine is given concurrently. Vaccines are administered by the village doctor, who provides primary health-care for that village and maintains a village registry that includes documentation of all immunisations. The structure of these registries is specified by the Province, with children being entered shortly after birth. Although parents are charged a small fee of 2 yuan (US\$0.24, typically 0.02–0.05% of an annual rural household income) to have a child immunised against Japanese encephalitis, it is believed that absence from the village during the brief vaccination campaign, rather than inability to pay this small sum, is the major reason that vaccination campaigns miss some children.

Identification of cases

One of us (LZ) recruited and trained physicians and staff from twenty-four country hospitals and two urban hospitals that serve rural areas surrounding urban Chengdu (the location of the coordinating centre, West China University of Medical Sciences [WCUMS]) to identify suspected Japanese encephalitis cases. Suspected cases were identified during the period of peak transmission (May 1 to Sept 30) of 1993, and consisted of all children under 15 who presented to one of the participating hospitals with acute central-nervous-system findings (headache, lethargy, ataxia, delirium, seizure, or coma) accompanied by a temperature of 37.5° or higher. Potential cases were enrolled without regard to immunisation status. For serological confirmation, we enrolled only suspected cases who had at least one serum or cerebrospinal-fluid (CSF) sample drawn as part of clinical care. Clinicians were asked to obtain a convalescent serum sample when possible.

Serological confirmation

Serum and CSF specimens were kept cold and transported to WCUMS, where they were stored at –20°C until samples were shipped to the Centers for Disease Control and Prevention

laboratory in Fort Collins, Colorado, USA. Neutralising antibodies against Japanese encephalitis were measured with a plaque-reduction test.⁸ Cases satisfying the clinical criteria were classified as presumptive Japanese encephalitis if the serum neutralising-antibody-titre was 80 or higher and as confirmed Japanese encephalitis if paired serum samples demonstrated a fourfold change in neutralising antibody titre or the CSF neutralising antibody titre was 10 or more.⁹

Controls

To select controls, investigators travelled unannounced to the village of residence of each confirmed case, where they located the village doctor, and examined the village registry. Controls were identified from this registry, and consisted of all children listed on the village registry who were born the same year as the case, and who did not develop clinical encephalitis during the study period. Appearance on the village registry was also an inclusion criterion for cases. Because each village experienced only one case during the study period, the control group consisted of all children of a given age who were at risk of developing disease at the time that the case occurred. This scheme is termed density sampling.¹⁰

We matched on village (rather than region-wide population-based sampling) for two reasons. First, because there is no overall roster of children living in the region, identifying unmatched population-based controls was not feasible. Second, we wished to avoid potential confounding that would occur if vaccination programmes tended to focus on villages with a high concentration of infected vectors, which could create an artificial positive association between vaccination and disease. We enrolled all potential controls, rather than sampling among them, because sampling would have required more effort than including all controls yet would have resulted in somewhat less statistical power.

Vaccination history

Vaccination histories were obtained from village registries for cases and controls. Because pilot experience revealed that the quality of vaccination records was variable among villages, a subjective score, ranging from one (poor) to five (good), was assigned to the records of each village, based on the impression of researchers from WCUMS.

Statistical analysis

In randomised trials, vaccine efficacy is calculated as $1 - RR$, where RR is the rate ratio, or the incidence of disease in the vaccinated group divided by the incidence in the unvaccinated group. When density sampling is used in a case-control study, the exposure odds ratio (OR) is an unbiased estimate of RR .¹¹ Therefore, vaccine effectiveness was calculated as $1 - OR$.¹¹

We used conditional logistic regression¹² to calculate OR s and 95% CI s, conditioned on matched set, for one, two, and three doses of live-attenuated vaccine, each compared with zero doses. We used multivariate conditional logistic regression to evaluate and control for the effects of potential confounding variables. In the primary analysis, we evaluated the effectiveness of two versus zero doses. We used EGRET.

Results

158 cases of potential Japanese encephalitis were identified clinically and after having had at least one biological sample drawn. 59 potential cases (37%) were excluded because the serological evidence for recent infection with Japanese encephalitis virus did not support classification as presumptive or confirmed. 39 (25%) potential cases were serologically presumptive and therefore excluded from the analysis of vaccine effectiveness. 60 potential cases (38%) were serologically confirmed.

Doses received	Cases (n=56)	Controls (n=1299)	Effectiveness (95% CI)
0	38 (68%)	615 (47%)	..
1	11 (20%)	332 (26%)	80% (44 to 93%)
2	6 (11%)	308 (24%)	97.5% (86 to 99.6%)
3	1 (2%)	44 (3%)	NE

NE=not evaluable because of insufficient data.

Table: Effectiveness of live-attenuated Japanese encephalitis vaccine (SA14-14-2)

52% of confirmed cases were female, and the mean age of all cases was 4.7 years. Eligible controls were unavailable for four confirmed cases. For the remaining 56 confirmed cases, 1299 matched controls were identified. Controls' average age was also 4.7 years; 57% of controls were female.

Previous vaccine exposure among cases and controls is shown in the table. 68% of cases received no vaccine, compared with 47% of controls. The effectiveness of one dose of the vaccine was 80% (95% CI 44 to 93%), and that of two doses was 97.5% (86 to 99.6%). Simultaneously adjusting for gender and past receipt of killed vaccine, effectiveness of one dose of live-attenuated vaccine was 71% (21 to 90%), and that of two doses was 97.6% (86 to 99.6%). Effectiveness was similar in boys and girls (p for interaction=0.35).

After exclusion of subjects who had received any killed vaccine, effectiveness for one dose was 61% (-14 to 86%), and for two doses was 97.5% (86 to 99.6%). For only subjects whose village had a vaccination-record quality-score of five (ie, those with the best vaccination records), effectiveness of one dose was 82% (12 to 96%), and that of two doses was 94% (16 to 99%). Because few subjects received three doses, the effectiveness of three doses could not be estimated.

Discussion

Given the evidence for the immunogenicity and efficacy of live-attenuated Japanese encephalitis vaccine,¹ and the known safety and efficacy of the Biken vaccine,² we decided that a placebo-controlled trial of SA14-14-2 vaccine would have been unethical. Therefore we did a case-control study to provide the best estimate of vaccine effectiveness.

Our data provide strong evidence for the vaccine's effectiveness. The effectiveness of a single dose was 80% (95% CI 44 to 93%), while that of two doses was 97.5% (86 to 99.6%). The observed 80% effectiveness of a single dose is lower than the 95% efficacy rate in prospective non-randomised trials.¹ This difference may have arisen because previous studies were conducted in more highly endemic areas than our study area; therefore, the immunity provided by a single vaccine dose in such a setting may have been reinforced by either previous or subsequent natural exposure to Japanese encephalitis virus. This explanation is supported by previous immunogenicity studies of inactivated Japanese encephalitis vaccine in which two doses produced adequate immunity among persons from areas with transmission of this disease, but three doses were necessary for persons from non-endemic areas.^{1,3} Alternative explanations for the apparent difference in the effectiveness of a single dose include inadequate refrigeration or improper administration technique during routine immunisation campaigns, which would have been reflected in our results, and unmeasured differences between vaccinated and unvaccinated groups in earlier

studies. The apparent superiority of two doses compared with one dose is consistent with previous immunogenicity studies.^{1,6}

The 97.5% effectiveness estimate is not directly evident from the proportion of vaccine exposure in the cases (table), because 13% of cases received two or more doses. However, that frequency (13%) is substantially lower than the proportion of people in the general population who have received two or more doses which, based on the control series, can be estimated to be 27%.

To interpret our study, we must look for possible bias or confounding. Selection bias would have occurred if cases were chosen on the basis of vaccination status or if they did not truly have the disease of interest. We believe that the use of an organised surveillance system and an explicit clinical case definition, combined with a rigorous serological case definition, served to minimise this possibility. Because controls should represent the population of individuals who would have been included as cases had they developed disease,¹⁴ and because there is universal access to government-supported hospitals in rural Sichuan, the use of matched population-based controls should minimise the possibility of bias in the selection of controls. Information bias occurs in a case-control study when exposure status is misclassified differentially between cases and controls. We attempted to avoid this bias by relying on the same pre-existing written records to ascertain the exposure status of cases and controls.

A confounder is a factor that is associated with the exposure of interest and the outcome of interest to artificially inflate or deflate the true association. In Japanese encephalitis, the known determinants of disease are age, sex, vaccination history, and exposure to infected vectors. We accounted for potential effects of age by matching on year of birth and performing the corresponding matched analysis. We controlled for sex and past receipt of killed vaccine by stratified and multivariate analyses. We matched on village (and did the corresponding matched analysis) to control for exposure to infected mosquitoes, and for potential confounders (eg, socioeconomic status) that might operate through this mechanism. To do so, we used residence in a particular village (within age and sex strata) as a proxy for factors determining exposure to infected vectors. To the degree that this proxy does not capture differences in exposure to infected vectors and that these differences are associated with vaccination status, residual confounding may remain. However, we believe that the magnitude of any such residual confounding is probably small.

Provided that a case-control study is free from bias and confounding, it may offer some advantages over a randomised controlled trial. First, it provides a measure of the clinical effectiveness of the vaccine as actually used, whereas most randomised controlled trials measure the efficacy of the vaccine under carefully controlled experimental conditions, and do not reflect the effects of breaches of acceptable protocol, such as inadequate refrigeration, that may occur in usual practice. A second advantage is that an unbiased case-control study can provide a more precise estimate of vaccine effectiveness (ie, narrower 95% CIs) than could have been achieved by a similarly sized randomised trial.

Our study was not designed to measure the safety of live-attenuated Japanese encephalitis vaccine. However, since the principal safety concern about this live vaccine is

the possibility of inadequate attenuation and the resultant possibility of vaccine-induced encephalitis, our observation of a negative association between vaccination and hospital admission for Japanese encephalitis supports previous observations of the vaccine's safety.⁶ Nevertheless, further study is needed to assure the safety of this vaccine with respect to other adverse events.

In conclusion, we conducted a case-control study of incident cases of hospital admission for Japanese encephalitis to measure the effectiveness of live-attenuated hamster-kidney-cell-derived Japanese encephalitis virus (SA14-14-2). A regimen of two doses of vaccine administered 1 year apart prevented clinically important disease. If the safety of this vaccine is confirmed in a sufficiently large study, and it is made available internationally at an affordable price, the widespread administration of SA14-14-2 vaccine could prevent thousands of deaths and cases of permanent disability each year throughout Asia.

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