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Follow-Up Survey of Japanese Encephalitis Virus Infection in Kumamoto Prefecture, South-West Japan: Status during 2009–2011

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Japanese encephalitis (JE) is a major public health concern in Asia with 30,000–50,000 cases, occurring annually, including 10,000 deaths (1). The causative agent is the Japanese encephalitis virus (JEV), a mosquito-borne flavivirus. Despite a high rate of occurrence in many parts of Asia, the incidence of JEV has decreased in countries that implemented a national JE vaccination program (2).

In Japan, over 1,000 human JE cases were recorded annually before 1967. Following the initiation of a nationwide distribution of mouse brain-derived, high-purity, inactivated JE vaccine in 1967, the annual number of JE cases decreased and remained at less than 10 per year after 1992 (3). However, a decrease in the number of vector mosquitos and the relocation of many pig farms to areas far from residential zones may also have had an impact on the reduced number of JE cases (4). Because of these factors, and the occurrence of a case with severe acute disseminated encephalomyelitis following JE vaccination, the Japanese Government withdrew its strong recommendation for the JE vaccination in 2005 (5,6). Although the Japanese Government

reinstated its strong recommendation for the routine immunization of children aged 3 years using the new Vero cell-derived vaccine from April 2010 and immunization of children aged 4, 9, and 10 years from April 2011, the JE vaccination rate of children aged 3–4 years decreased to approximately 10% in 2007, following this suspension (7).

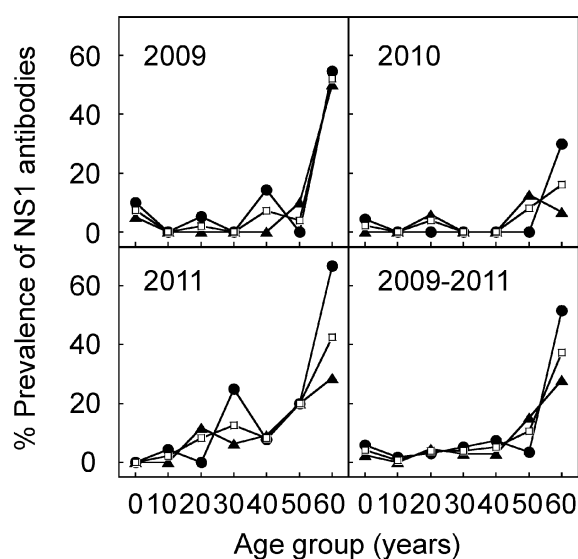
A critical factor arguing for the necessity for a vaccination program is the JEV activity in nature. With the exception of the non-endemic northern areas, annual infection rates in humans ranged from 3 to 17% before 1960, 5 to 10% in the early 1980s and mid-1990s, and 0.2 to 3.4% between 2001 and 2004 (8). We previously reported mean annual infection rates of 1.8–2.6% between 2004 and 2008 among the inhabitants of Kumamoto Prefecture, which is located in south-west Japan and has a relatively high incidence of JE cases (9). This study tracks the status of natural JEV infections in Kumamoto Prefecture between 2009 and 2011.

Signed informed consent was obtained, and sera were collected from 716 inhabitants of Kumamoto Prefecture from 2009 to 2011 as part of a national JE surveillance program. The subjects were aged 0–99 years and were grouped in 10-year increments, except for those aged over 60 years, who were analyzed as one age group. Serum samples from babies aged <6 months, who can contain maternally transferred antibodies, were not used. The use of all human samples in the present study was approved by the Ethical Committee of the Kobe University School of Medicine. Natural infection was detected by measuring the antibodies against nonstructural protein 1 (NS1) of JEV, and this was used for differentiating infected from vaccinated individuals.

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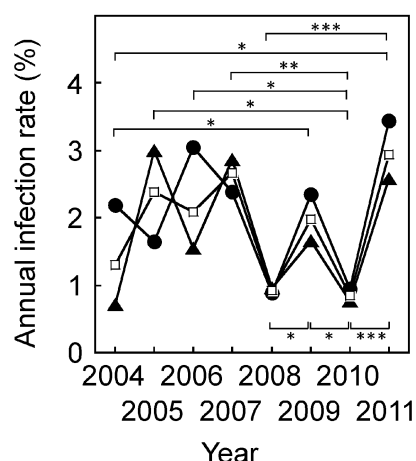


Fig. 1. NS1 antibody prevalence in Kumamoto populations during 2009–2011. (A) Age-dependent antibody prevalence curve in male (closed circles), female (closed triangles), and total (open squares) populations. (B) Annual infection rates estimated from NS1 antibody prevalence in male (closed circles), female (closed triangles), and total (open squares) populations. As a reference, this graph also shows previously reported data for 2004–2008 (9). Asterisks indicate significant differences from each year of 2009–2011 in the total population. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ by the chi-square test with the Yates' correction factor.

Enzyme-linked immunosorbent assay was performed to quantify NS1 antibodies as described previously (10). The annual infection rate was calculated by dividing the NS1 antibody prevalence by the duration of NS1 antibodies (4.2 years). Neutralizing antibodies contained in human sera were titrated using a standard 50% plaque/focus reduction method, essentially as described (11).

Figure 1A shows the results of the NS1 antibody assay. The overall prevalence of NS1 antibodies between 2009 and 2011 was 8.1% (58/716), which was similar to 7.6% (90/1,190) previously reported for 2004–2008 in the same prefecture (9). Significant differences were not

Table 1. Annual JEV infection rates in Kumamoto, 2009–2011, calculated from the number of unvaccinated children aged 0–9 years positive for JE neutralizing antibodies

Gender	Total no.	No. positive	% positive	Average survival period (y) ¹⁾	Annual infection rate (%) ²⁾
Male	48	4	8.3	3.9	2.1
Female	55	4	7.3	3.1	2.4
Total	103	8	7.8	3.4	2.3

¹⁾: Calculated from the age of the subjects. The survival period of each subject was assumed to be 0.5 years greater than the subject's age. For instance, the survival period of a subject aged 1 year was regarded as 1.5 years.

²⁾: Calculated by dividing the “% positive” by the “Average survival period.”

observed among the male and female subjects in each age group as well as in the overall prevalence in each year. Antibody prevalence increased with age; this was consistent with previous findings (9). Among the total population (2009–2011), the > 60 age group showed significantly higher prevalence than other age groups ($P < 0.001$), and the 50s age group showed significantly higher prevalence than the 10s age group ($P < 0.01$). The mean annual infection rate estimated from NS1 antibody prevalence in 2009–2011 was 2.2% in male subjects, 1.7% in female subjects, and 1.9% in the total population (Fig. 1B), which was similar to the 2004–2008 results of 1.9% in male subjects, 1.7% in female subjects, and 1.8% in the total population (9). Although the time-related mean annual infection rate during the 2004–2011 period fluctuated, and significant differences were found for some of these years, the rates in 2004–2011 were considered to be approximately 2%, based on the duration of NS1 antibodies (4.2 years).

Additionally, annual infection rates were obtained from a percentage of the population with no history of JE vaccination but who possessed neutralizing antibodies against JEV (Table 1). In the present population, 103 children aged 9 years or less had not received JE vaccination. Of these, 8 (7.8%) were positive for neutralizing antibodies, indicating natural infection with JEV. Since the average survival period of these subjects was 3.4 years, the annual infection rate was calculated to be 2.3% (2.1% for male subjects and 2.4% for female subjects), which was similar to the 2.6% results obtained in 2004–2008 (2.4% for male subjects and 2.9% for female subjects) (9).

The present follow-up survey in Kumamoto Prefecture during the 2009–2011 period found that annual infection rates estimated by NS1 antibody prevalence and the percentage of population that was neutralizing antibody-positive but had no history of JE vaccination were similar to those obtained in 2004–2008 in the same prefecture. Although correlations were not always observed among the yearly JE incidence, JEV antibody prevalence in swine (7), and annual infection rates estimated in this study, these results provide strong evidence of continuous JEV circulation in Kumamoto and suggest a recent exposure of the human population in south-west Japan to JEV infection.

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Conflict of interest None to declare.

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