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# JAPANESE ENCEPHALITIS VACCINATION IN THAILAND

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SUMMARY In 1971 and 1972, vaccination against Japanese encephalitis (JE) was carried out in the Chiang Mai Province, in the northern region of Thailand, and about 3,000 school children were vaccinated in each year. The vaccine used in 1971 was supplied in a liquid state and that used in 1972 in a lyophilized form.

The antibody-negative ratios of pre-vaccination sera in Chiang Mai in 1971 and 1972 were 40% and 56% respectively measured with neutralizing (NT) antibody, and 54% and 68% measured with hemagglutination inhibition (HI) antibody. The seroconversion rate measured by the NT-antibody response after vaccination with liquid vaccine in 1971 was 86% and that with lyophilized vaccine in 1972 was 96%. Measured by the HI-antibody response, the rates were 60% and 79%, respectively. In initially antibody-negative children the average antibody titers after vaccination were 1.5 and 2.2 as log NT-titers and 19 and 32 as HI units, in 1971 and 1972, respectively.

The cross responses to JE vaccination against Dengue type 2 and type 4 viruses, examined by the HI-test, were not much compared with those against the homologous Nakayama and JaGAr#01 strains of the JE virus.

These field trials show that it is better to use lyophilized vaccine in tropical zones because it is more stable than liquid vaccine.

## INTRODUCTION

In Thailand, some predominant virus diseases, Thai hemorrhagic fever and JE for example, are caused by arbovirus infection. No vaccine has yet been developed for Dengue virus and/or Chikungunya virus, which are considered to be

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the causative agents of the hemorrhagic fever (Hammon, Rudnick and Sather, 1960; Halstead, 1966). However, JE vaccine has been produced and widely employed with satisfactory results in several countries such as Japan. Therefore we tested it in the northern region of Thailand where JE is prevalent and where big epidemics of JE occurred in 1969 and 1970 (Yamada et al., 1971).

This was the first large scale trial of JE vaccination in Thailand. It was aimed at prevention of dreadful disease for the people of this country in accordance with the object of the Virus Disease Control Project, as a part of the scheme for medical cooperation between Thailand and Japan under the Colombo Plan.

This paper describes the antibody response to vaccination against homologous JE virus. The response to Dengue viruses was also examined as it is known that JE and Dengue viruses often show serological cross reaction with each other in cases of natural infection (Halstead, 1966; Igarashi et al., 1969).

## MATERIALS AND METHODS

### 1. *Vaccine*

Killed JE vaccine (Nakayama strain) in liquid (lot #184) and lyophilized (lot #15) forms was supplied from the Research Foundation for Microbial Diseases of Osaka University. The vaccine was obtained from mouse brain infected with the Nakayama strain by protamine treatment, inactivation with formalin and several chemical treatments and was finally purified by ultracentrifugation (Takaku et al., 1968). The vaccine passed the national assay test in the National Institute of Health, Japan, before shipment to Thailand.

### 2. *Vaccine administration*

In 1971, the vaccine was used in Sarapee district, Chiang Mai Province, and Bangkok-Thonburi Metropolitan Area. About 3,000 children of 1 to 16 years old, mainly in Sarapee, were given two subcutaneous injections of 1 ml (0.5 ml for children under 5 years old) of the liquid vaccine with an interval of one week between injections.

In 1972, the vaccination was carried out on about 3,000 more children in Sarapee district only. Vaccination was done in the same manner as in the preceded year except that lyophilized vaccine was employed. In both years vaccination was done in February–March, as the epidemic season of JE begins in May and the school summer vacation in Thailand starts in April.

### 3. *Collection of blood specimens*

Paired blood specimens were collected just before the first injection (pre-vaccination sera) and four weeks after the second injection (post-vaccination sera) from randomly selected vaccinees. In all, 168 paired sera were obtained in 1971 and 97 in 1972.

### 4. *Neutralization (NT) test*

For measurement of the serum NT-antibody titer, the 50% plaque reduction test was performed using primary chick embryo cell cultures. Primary cultures of chick embryo cells were prepared by the method of Porterfield (1959). All sera were heated at 56 C for 30 min before the test.

Briefly the procedure was as follows: 0.4 ml of appropriately diluted test serum was mixed with the same volume of JE virus suspension containing about 400 PFU and the mixture was kept in a water bath at 37 C for 90 min. As virus and serum controls, the test serum was replaced by 0.4 ml of the diluent (TC medium 199 containing 2% fetal calf serum) or 0.4 ml of diluted hyperimmune serum, respectively.

After incubation for 90 min, the mixtures were transferred to an ice bath to stop the reaction and 0.2 ml of each mixture was inoculated onto a monolayer of chick embryo cells in a plaque bottle. Two plaque bottles were used for each serum specimen. After incubation at 37 C for 90 min to allow adsorption, monolayers were washed with Hanks' solution and the first overlay was applied.

On the third day after inoculation, the second overlay with neutral red was added. As a double cheque plaques were counted on the 4th and 5th days.

The NT-antibody titer was calculated by the standard method of the National Institute of Health, Japan, as follows (Oya et al., 1967):

$$Z=\frac{y-50}{47.7622}+\log_{10}X$$

where: $Z$: log NT-antibody titer giving 50% plaque reduction
$y$: Per cent of plaque reduction obtained in the experiment
$X$: Reciprocal of dilution of test serum
(This formula is valid for a range where $10 \leqq y \leqq 90$ is satisfied.)

5. *Hemagglutination inhibition (HI) test*

The HI-antibody titers against the Nakayama and JaGAr#01 strains of JE virus and also against Dengue type 2 and type 4 viruses were determined by the method of Clarke and Casals (1958). Serum specimens were treated with acetone twice before the test. The antigens used were extracted from infected suckling mouse brain with acetone-ether or sucrose-acetone method.

## RESULTS

1. *NT-antibody response to vaccination*

In 1971, a total of 161 paired sera (68 pairs from Chiang Mai and 93 pairs from Bangkok) were tested for their NT-antibody titers against the Nakayama strain of JE virus. In 1972, 97 paired sera from Chiang Mai were examined in the same way.

Fig. 1 shows the distribution of the log NT-antibody titers in pre- and post-vaccination sera in 1971 and 1972.

In 1971, 40% (65/161) of all the pre-vaccination sera tested were negative (log NT-titer, $<1.0$). When the rates of negative cases in Bangkok and Chiang Mai areas were compared, the former was 33% (31/93) which was a little lower than that in the latter (50%; 34/68). The distribution of the log NT-titers of pre-vaccination sera in the Bangkok area was slightly different from that in the Chiang Mai area, showing a peak at $1.0 \leqq z < 2.0$ while the highest frequency in the Chiang Mai area was observed at $Z < 1.0$.

In 1972, the experiment was performed only in the Chiang Mai area and the NT-negative rate of the pre-vaccination sera was 56% (54/97). This figure and the distribution of the log NT-titer of pre-vaccination sera were very similar to those in the same area in 1971.

In post-vaccination sera, 41% (65/161) and 17% (27/161) of the vaccinees showed log NT-titers of $2.0 \leqq Z < 3.0$ and $3.0 \leqq Z < 4.0$, respectively in 1971, while in 1972, these percentage were 50% (48/97) and 32% (31/97). No significant difference was observed in the distributions of post-vaccination NT-titers in

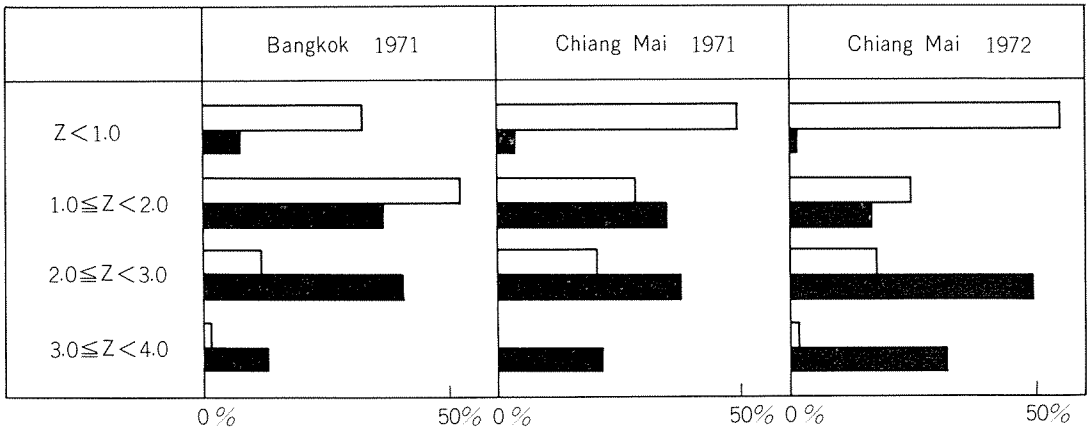


FIGURE 1. *Distribution of NT-antibody titers against Nakayama strain before and after vaccination in 1971 and 1972.* Z, $log_{10}$ *antibody titer which gave 50% plaque reduction*; □, *pre-vaccination sera*; ▬, *post-vaccination sera.*

the Bangkok and Chiang Mai area in 1971, although the distributions of pre-existing NT-antibody in these areas were different, as described above.

The NT-antibody response to vaccination can be most clearly seen from the cumulative curves shown in Fig. 2. In the figure, the percentages were accumulated from the right to the left, namely from the frequency of higher log NT-titers towards those of lower titers. In both 1971 and 1972, the cumulative percentage curves for post-vaccination sera shifted moderately to the right (i.e., to higher log NT-titer side) when compared to the curves for pre-vaccination sera. Thus, both liquid and lyophilized JE vaccines could elicit a responses to vaccination in human subjects.

In 1971 (using liquid vaccine) the cumulative percentage of vacciness having $Z \geqq 2.0$ was increased from 17% in pre-vaccination period to 58% in post-vaccination period, while in 1972 (using lyophilized vaccine) such percentage was increased from 20% to 82%. Observation confined to vaccinees showing $Z \geqq 3.0$, the cumulative percentage of 1% in pre-vaccination sera was increased to 17% in post-vaccination sera when using liquid vaccine in 1971. In the similar observation in 1972 when lyophilized vaccine was used, this rise was greater, i.e. from 2% to 32%.

### 2. *Seroconversion as shown by NT- and HI-titration*

Fig. 3 shows the distribution of post-NT-titers in pre-NT-negative vaccinees. In pre-vaccination sera, 40% (65/161) of the vaccinees showed negative titers on NT-titration in 1971 while 56% (54/97) showed negative titers in 1972. As shown in the figure, after vaccination in 1971 trial 14% (9/65) of those who were initially NT-negative remained NT-negative, while in 1972 only 4% (2/54) remained NT-negative after vaccination. Thus, the seroconversion rates in 1971 and 1972 were 86% and 96%, respectively.

The geometric mean post-NT-titers of pre-

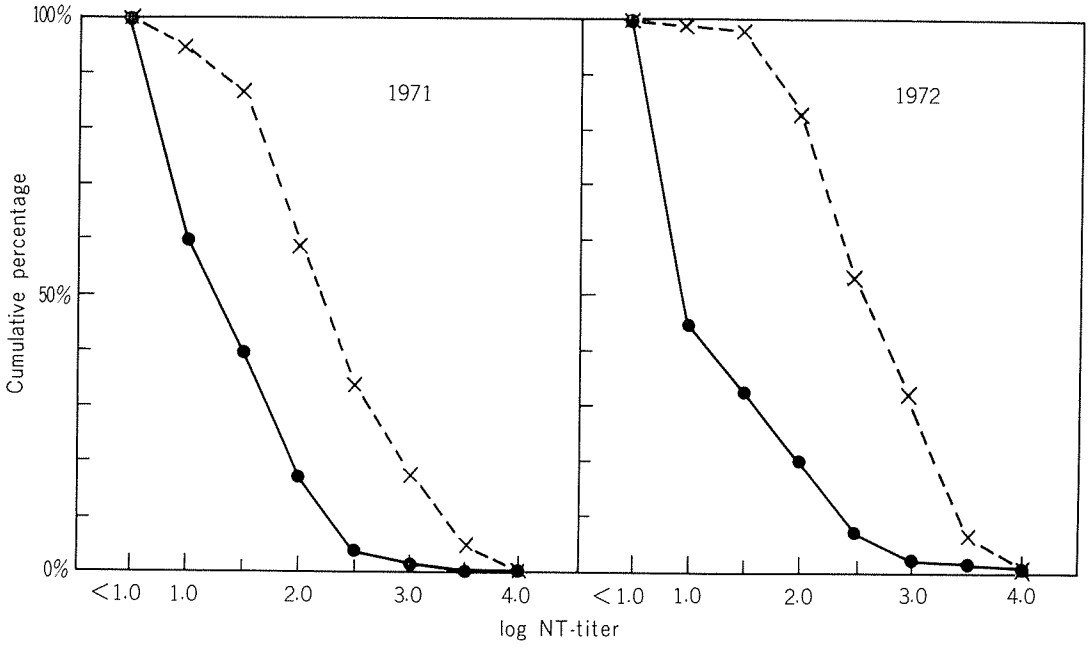


FIGURE 2. *NT-antibody responses of vaccinees in 1971 and 1972.* ●, *pre-vaccination;* ×, *post-vaccination.*

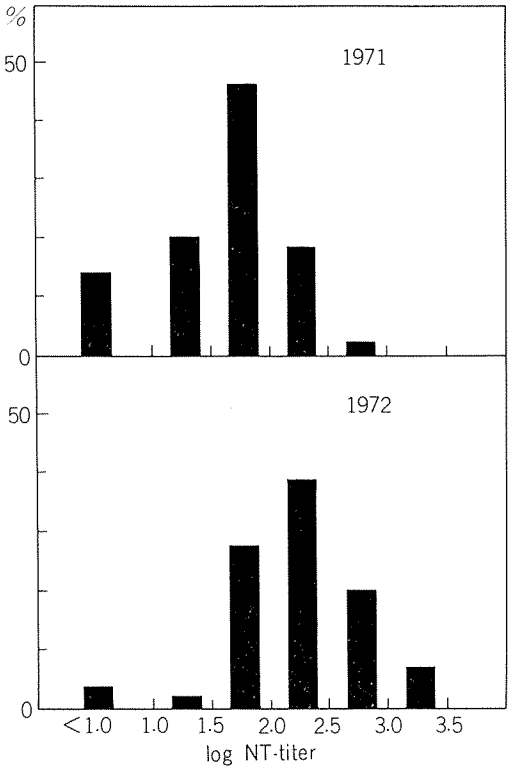


FIGURE 3. *Distribution of log NT-titers after vaccination of pre-NT-negative ($Z<1.0$) vaccinees.*

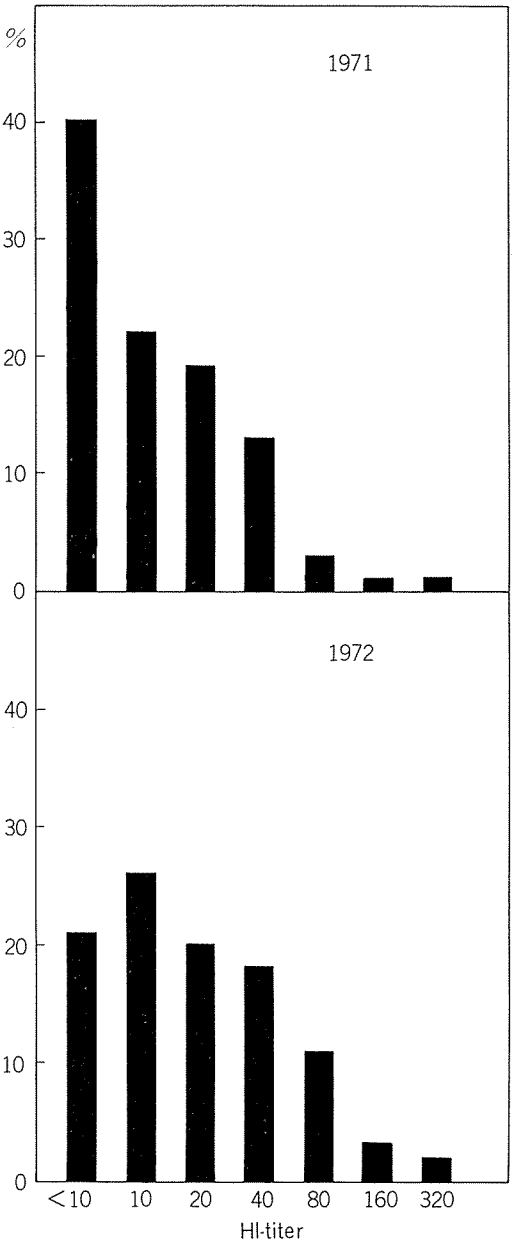


FIGURE 4. *Distribution of HI-titers after vaccination of pre-HI-negative ($<10$) vaccinees.*

NT-negative vaccinees as calculated in log NT-titers were 1.5 in 1971 and 2.2 in 1972. (In the calculation $Z<1.0$ was treated as $10^0$.)

In 1971, 54% (90/168) of the pre-vaccination sera tested were found to be negative ($<10$) on HI-titration while in 1972, 68% were HI-negative (66/97).

Fig. 4 shows the distribution of HI-titers of post-vaccination sera (post-HI-titer) of pre-HI-negative vaccinees against Nakayama strain antigen. The HI-seroconversion rates judged by the HI-titer was 60% (90–36/90), in 1971 and 79% (66–14/66), in 1972. The average post-HI-titers of pre-HI-negative vaccinees were 19 and 32 ($<10$ was regarded as 0 in the calculation) in 1971 and 1972, respectively.

### 3. *Cross responses due to vaccination*

The data shown in this section were all obtained in 1972 with lyophilized vaccine, as results in 1971 were less satisfactory for analysis of cross responses, as shown above.

TABLE 1. *Distribution of post-HI-titers of pre-HI-negative vaccinees against Nakayama, JaGAr #01, Dengue type 2 and Dengue type 4 viruses*

| Post-HI-titer | Anti-Nakayama | Anti-JaGAr#01 | Anti-Dengue-2 | Anti-Dengue-4 |
|---|---|---|---|---|
| <10 | 14 (21%) | 12 (20%) | 42 (68%) | 40 (71%) |
| 10 | 17 (26%) | 9 (15%) | 8 (13%) | 8 (14%) |
| 20 | 13 (20%) | 11 (18%) | 8 (13%) | 5 ( 9%) |
| 40 | 12 (18%) | 12 (20%) | 3 ( 5%) | 3 ( 5%) |
| 80 | 7 (11%) | 10 (17%) | 1 ( 2%) | 0 ( — ) |
| 160 | 2 ( 3%) | 3 ( 5%) | 0 ( — ) | 0 ( — ) |
| 320 | 1 ( 2%) | 3 ( 5%) | 0 ( — ) | 0 ( — ) |
| Total | 66 | 60 | 62 | 56 |

1) Cross HI-antibody response between Nakayama and JaGAr#01 strains of JE virus, Dengne type 2 and type 4 viruses

Table 1 shows the distribution of post-HI-titers of pre-HI-negative vaccinees. Among 97 vaccinees there were 66, 60, 62 and 56 pre-HI-negative cases against the Nakayama, JaGAr#01, Dengue type 2 and type 4 virus, respectively.

The seroconversion rates were calculated as 79% and 80% with the homologous Nakayama strain and JaGAr#01 strain of JE virus and 32% and 29% with Dengue type 2 and type 4 viruses. The highest HI-titer in post-vaccination sera of pre-HI-negative vaccinees were 320 against both strains of JE virus, and only 80 and 40 against Dengue type 2 and type 4 viruses, respectively.

Fig. 5 shows the HI-antibody responses against the Nakayama and JaGAr#01 strains of JE virus as well as against the Dengue type 2 and type 4 viruses. In the vaccinees who had been pre-HI-positive, the HI-antibody responses against the Dengue type 2 and type 4 viruses seem rather poor compared with those against the Nakayama and JaGAr#01 strains of JE virus.

The correlations between the HI-titers against JaGAr#01 and the HI-titers against Nakayama, Dengue type 2 and type 4 are summarized in Figs. 6A-F. Before vaccination, the HI-titers against JaGAr#01 were slightly higher or the same as those against the Nakayama strain. The HI-titers against Dengue type 4 corresponded more closely to those against JaGAr#01 than the HI-titer against Dengue type 2.

In plots of titers after vaccination against JaGAr#01 versus those against Dengue type type 2 and Dengue type 4 many values were distributed on the JaGAr#01 side (compare Figs. 6D and F with Figs. 6C and E), but in the plot of titers against Nakayama versus those against JaGAr#01 (Figs. 6A and B), this tendency was not so marked.

2) Cross NT-antibody response against the JaGAr#01 strain of JE virus

Fig. 7 shows NT-antibody responses agsinst the Nakayama and JaGAr#01 strains due to vaccination. In the case of the Nakayama strain, 74 points were above the dotted line, which means an increase of more than one $\log_{10}$ unit in the log NT-titer after vaccination.

On the other hand, in the case of NT-antibody against the JaGAr#01 strain, only 43 points were above this line, showing that the NT-antibody response was lower than that against the Nakayama strain.

Fig. 8 shows the correlation between NT-antibody titers against the Nakayama and JaGAr#01 strains before and after vaccination. The NT-titers of most pre-vaccination sera (86%) were slightly higher against JaGAr#01 than against the Nakayama strain. However, in post-vaccination sera, the distribution of values changed, 46 points (47%) being on the

Nakayama side, 15 points (16%) in the middle, and 36 points (37%) on the JaGAr#01 side.

Therefore, although the HI-antibody response to Nakayama strain vaccine appeared to be higher against JaGAr#01 than against the homologous Nakayama strain, as shown above, the NT-antibody response appeared to be higher against the homologous Nakayama strain than against JaGAr#01, one of the newer strains of JE virus.

## DISCUSSION

To control Japanese encephalitis much knowledge is required on the ecology of the virus and on the environment where the disease oc-

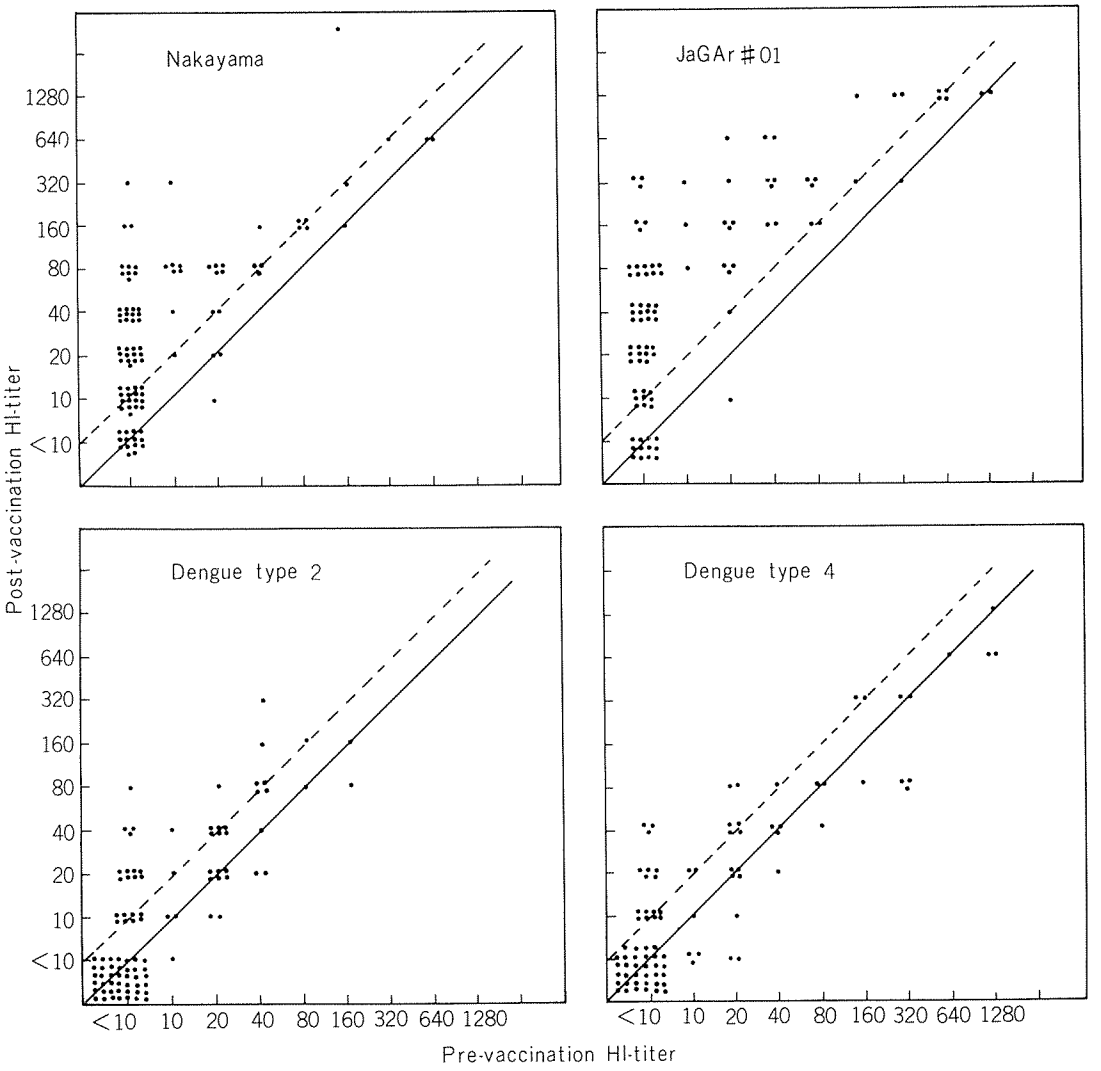


FIGURE 5. *HI-antibody response against Nakayama and JaGAr#01 strains of JE virus, Dengue type 2 virus, and Dengue type 4 virus.*

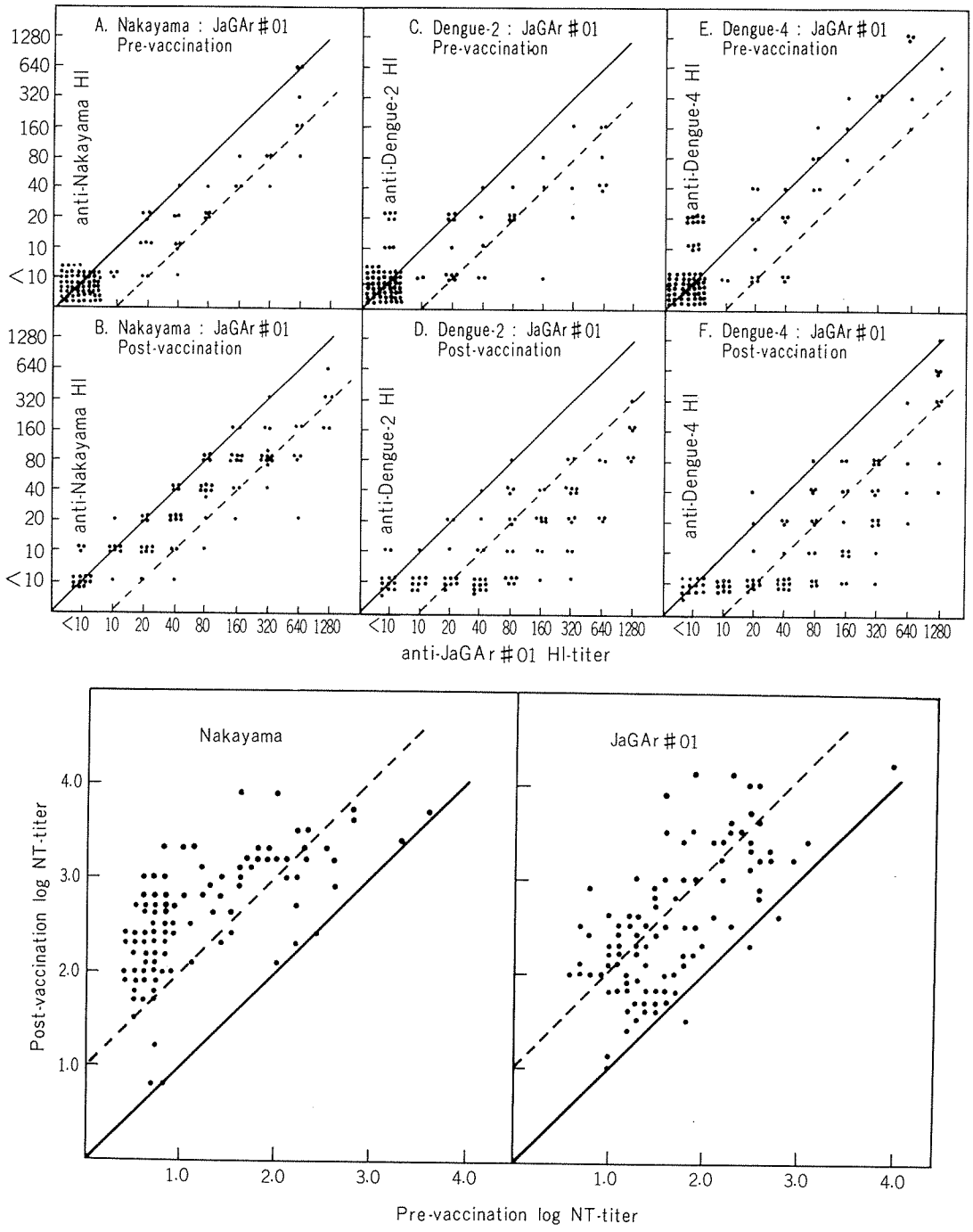


FIGURE 7. *NT-antibody response to the vaccine (Nakayama strain) against homologous Nakayama and JaGAr#01 strains of JE virus.*

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FIGURE 6. *Correlation of the HI-titers against JaGAr#01 and against Nakayama, Dengue type 2, and Dengue type 4 viruses before and after vaccination.*

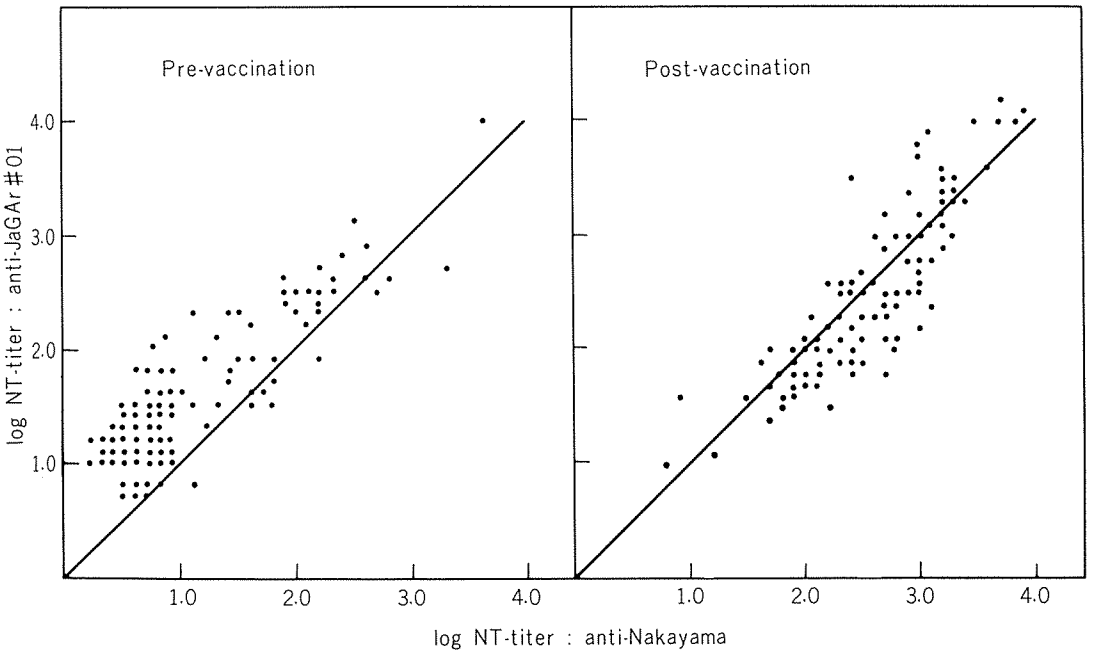


FIGURE 8. *Correlation between anti-Nakayama and anti-JaGAr#01 NT-antibody titers before and after vaccination.*

currs. JE virus is known to have vectors and amplifiers. Three species of *Culex* mosquitoes, *C. tritaeniorhinchus*, *C. gelidus* and *C. fuscocephala* are considered to be vectors of JE virus in Thailand (Grossman et al., 1972). Pigs are considered to be the most important amplifier of JE virus in Japan (Scherer et al., 1959; Nakamura et al., 1964) and this also seems to be so in Thailand, especially in the Chiang Mai area (Yamada et al., 1971).

Thus mosquito control and measures to control amplifier, that is mainly pigs, should prevent JE in Thailand. However, at present such controls seem to be impractical. Frequent and rather wide-spread spraying of insecticide, as is usually done to control *Culex* mosquitoes is not possible as it may cause critical socio-agricultural problems and disturbances in ecological systems, such as those suffered in several other countries. The possibility of establishing an immune barrier in pigs against JE through vaccination is under investigation in Japan (Ueba et al., 1972) but no suitable vaccine is yet available. Therefore, since the age incidence of JE in Chiang Mai is between 10 and 14 years and 70% of the cases of JE in the 1969 and 1970 epidemics were under 15 years old, we chose vaccination of school children as a more direct way of preventing JE.

In 1971, a liquid JE vaccine was employed in the trial and the antibody response was rather unsatisfactory compared to results reported in Japan (Kanamitsu, Hashimoto and Katsurada, 1966).

The cause of this discrepancy was investigated in the Research Foundation for Microbial Diseases of Osaka University (Fukai;

personal communication). All the liquid vaccine supplied had been used in the field trial, so no residual vaccine was available for examination. Therefore, a sample of vaccine of the same lot which had been kept in the Research Foundation was divided in half. One half was subjected to violent shaking and an elevated temperature (30C) several times to simulate conditions during transporatation. Then the potencies of the treated and control samples were assayed. Moderate loss of potency was detected in the treated sample, suggesting that potency of deteriorated during long-distance transportation from Japan to the site of the field trial.

Thus it seems that liquid JE vaccine for field work must be treated very carefully, especially in tropical zones, or when long-distance transportation is necessary. (Killed liquid JE vaccine has been found to lose potency on freezing.)

To avoid this decrease in potency lyophilized JE vaccine was employed in 1972 and results were much better.

When the distribution curves of the log NT-titers of pre-vaccination sera from the Bangkok area and Chiang Mai area in 1971 were compared, a slight difference was found between them. The latter curve was very similar to that obtained in Chiang Mai in 1972. The cause of the difference in the pre-vaccination log NT-titers is not clear. That obtained in Bangkok may reflect the status of an urban area, or of central Thailand, while those obtained in Chiang Mai may express the rural or northern Thailand epidemiological status.

The seroconversion rates after vaccination based on the NT-titers were 86% in 1971 and 96% in 1972. Based on the HI-titers, the rates were rather lower, being 60% and 79%, respectively.

This seroconversion was due entirely to vaccination, not to latent infection, because the observations were made in an inter-epidemic season.

The NT-antibody level has been proved the more essential for protection of animals against JE infection in direct challenge experiments. Therefore, the seroconversion rate based on the NT-titer is probably the more reliable estimate, especially with JE vaccine.

The geometrical mean log NT-titers of post-vaccination sera of pre-NT-negative vaccinees were $Z=1.5$ in 1971 and $Z=2.2$ in 1972. In Japan it is considered that a log NT-titer Z (measured by the 50% plaque reduction method) of $\geqq 1.0$ indicates immunity against JE infection (Oya, 1967). Thus the vaccination against Japanese encephalitis were probably successful. None of the vaccinees has developed JE since the time of vaccination, but the effectiveness of the vaccination is still not proved because there have been no epidemics in the area since 1971.

With regard to seroconversion, it must be mentioned that 14% and 4% of the pre-NT-negative vaccinees remained NT-negative after vaccination in 1971 and 1972, respectively. The percentage observed in 1972 is not unusual but that in 1971 is high, probably due to instability of the liquid vaccine used. Further analysis of the cases who showed no conversion should provide useful information with regard to JE immunity.

Cross response experiments showed that JE vaccination did not stimulate any secondary response against Dengue viruses, even in vaccinees who had high titers of antibody against dengue viruses before vaccination.

Post-vaccination sera were found to have higher HI-titers to the newer strain, JaGAr#01, than to the classical Nakayama strain used for production of the vaccine. However, the response of the NT-titer to the homologous strain (Nakayama) appeared to be higher than that to JaGAr#01. At present, there is no adequate explanation on this. But the fact that pre-vaccination sera showed more affinity to JaGAr#01, as shown in Fig. 8 may be related to this point.

The cross responses between JE viruses and Dengue viruses were measured by HI-titration only owing to limitation in the present tech-

nique for measuring the NT-titer. These should be made using the NT-titer as an indicator and for this purpose it is hoped that a reproducible plaque reduction technique for Dengue antibody titration may be established.

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REFERENCES

Clarke, D. H., and J. Casals. 1958. Techniques for hemagglutination and hemagglutination-inhibition with arthropod-borne viruses. Am. J. Trop. Med. Hyg. 7: 561–573.

Grossman, R. A., D. O. Johnsen, D. J. Gould, R. Edelman, and C. L. Bailey. 1972. The ecology of Japanese encephalitis virus infections in Chiangmai. Ann. Progress Report, The SEATO Medical Research Laboratory, Bangkok, Thailand 1972: 3–15.

Halstead, S. B. 1966. Mosquito-borne haemorrhagic fever of South and South-East Asia. Bull. WHO 35: 3–15.

Hammon, W. M., A. Rudnick, and G. E. Sather. 1960. Viruses associated with epidemic hemorrhagic fevers of the Philippines and Thailand. Science 131: 1102–1103.

Igarashi, A., K. Fukai, S. Ahandrik, and P. Tuchinda. 1968. Antibody against Japanese encephalitis in sera of Dengue hemorrhagic fever patients in Thailand. Biken J. 11: 41–49.

Kanamitsu, M., N. Hashimoto, and M. Katsurada. 1966. Field survey on the improved Japanese encephalitis vaccines in Hokkaido. *In* Proceedings of the Committee on Japanese Encephalitis Vaccine. Vol. 1: 53–59. [In Japanese]

Nakamura, J., N. Nakamura, and I. Nozaki. 1964. Studies on Japanese encephalitis among pigs and lambs. Virus 14: 252–253. [In Japanese]

Oya, A., G. Han, M. Kurokawa, and M. Ishida. 1967. Experimental formula for the direct calculation of neutralizing antibody titer from the plaque reduction rate. *In* Proceedings of the Committee on Japanese Encephalitis Vaccine. Vol. 2: 5–10. [In Japanese]

Oya, *A.* 1967. Japanese encephalitis vaccine. p. 161–175. *In* Vaccines used in Japan. Maruzen Co. [In Japanese]

Porterfield, J. S. 1959. Plaque production with yellow fever and related arthropod-borne viruses. Nature 183: 1069–1070.

Scherer, W. F., J. T. Moyer, T. Izumi, I. Gresser, and J. McCown. 1959. Ecologic studies of Japanese encephalitis virus in Japan. VI. Swine infection. Am. J. Trop. Med. Hyg. 8: 698–706.

Takaku, K., T. Yamashita, T. Osanai, I. Yoshida, M. Kato, H. Goda, M. Takagi, T. Hirota, T. Amano, K. Fukai, N. Kunita, K. Inoue, K. Shoji, A. Igarashi, and T. Ito. 1968. Japanese encephalitis purified vaccine. Biken J. 11: 25–39.

Ueba, N., A. Maeda, K. Otsu, B. Mitsuda, T. Kimoto, S. Fujito, and N. Kunita. 1972. Natural infection of swine by Japanese encephalitis virus and its modification by vaccination. Biken J. 15: 67–79.

Yamada, T., S. Rojanasuphot, M. Takagi, S. Wungkobkiat, T. Hirota, T. Yamashita, S. Ahndarik, S. Pisuthipornkul, S. Sawasdikosol,, N. Sangkawibha, P. Tuchinda, S. Washarothal, S. Jatanasen, S. Hiranniramon, V. Laosuthibongse, P. Chiowanich, C. E. Robert, Jr., P. Oesawadi, S. Bukkavesa, M. Gaew-im, A. Shimizu, and M. Kitaoka. 1971. Studies on an epidemic of Japanese encephalitis in the northern region of Thailand in 1969 and 1970. Biken J. 14: 267–296.