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# A community-based study of subclinical flavivirus infections in children in an area of Tamil Nadu, India, where Japanese encephalitis is endemic\*

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*A characteristic feature of the epidemiology of Japanese encephalitis (JE) is the occurrence of a large number of subclinical infections. The reporting of only overt cases underestimates the total level of virus transmission, a knowledge of which is essential for the evolution of control strategies. We carried out a 3-year prospective serological study between 1989 and 1991 in a primary health centre in Tamil Nadu where JE is endemic. Each year paired specimens, taken before and after the transmission season from a cohort of schoolchildren aged 5–9 years, were tested for haemagglutination inhibition (HI) antibody titres in order to study seroconversion.*

*The seroconversion rates in the successive years were 37.5, 42.1 and 25 percentage points, and in a third of such seroconversions it was possible to establish a specific diagnosis. Seroconversion was attributable predominantly to JE virus and minimally to West Nile virus. Relatively high dengue virus activity occurred only in 1991. There were statistically significant differences in seroconversion rates between villages and this was related to variations in the ratio cattle:humans:pigs. Very high seroconversion rates occurred among children who were negative for HI antibodies before the transmission season. HI antibodies declined to undetectable levels 6–8 months later in half the children who had seroconverted. The average net annual increase of 16.2 percentage points in seropositivity was nevertheless much higher than values reported from other areas of endemicity. The overall incidence of JE cases was 15 per 10 000 children aged 5–9 years, and the estimated ratio overt:inapparent infection was 1:270.*

## Introduction

Japanese encephalitis (JE) is an important public health problem in south-east Asia, and its transmission appears to be increasing in several countries (1). In India the disease was first reported in 1955 (2), and subsequently many epidemics have occurred in different parts of the country. JE virus infects a large number of susceptible individuals but only a few develop overt manifestations of the disease. Differences in the virulence of virus strains, host susceptibility, background immunity, and several other factors may cause variations in the incidence of the

disease without affecting the total infection rates. Therefore the current system of reporting only cases of encephalitis does not reflect the total level of transmission, measurement of which is an essential prerequisite for planning control strategies.

Inapparent infection, resulting in the development of measurable antibodies to JE virus, can be used to quantify seroconversion rates among susceptible groups during the transmission season. In India, serological surveys have provided valuable data on the point prevalence of antibodies against JE virus and other flaviviruses,<sup>a</sup> but information on inapparent infection rates and the ratio of inapparent to apparent infections is inadequate. We therefore carried out a 3-year prospective serological study of a cohort of primary-school children in an area in Tamil Nadu where JE is endemic. At the same time we monitored the JE virus infection status of sentinel pigs in the study area.

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Reprint No. 5592

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<sup>a</sup> *Japanese encephalitis in India*. Pune, National Institute of Virology, 1980 (information document).

## Materials and methods

### Study site

In 1981 an extensive epidemic of encephalitis in children was reported in the South Arcot District of Tamil Nadu, in which a serological diagnosis of JE was made for 61% of the patients examined (3). Subsequently, many cases of encephalitis have been reported each year, mainly in October and November, coinciding with the period of the north-east monsoon. Nallur Primary Health Centre, which covers about 120 000 people, was chosen for the study because it is located in one of the worst-affected areas. Between 50 and 200 pigs are reared in each village, in addition to other domestic animals. Details of cases and deaths due to encephalitis were obtained from the Tamil Nadu Public Health Service.

### Mosquito blood meal identification

A relatively high proportion of recognized vectors of JE virus in the area (*Culex tritaeniorhynchus*, *C. vishnui*, and *C. pseudovishnui*) feed on humans and pigs in addition to cattle. Methods of identifying mosquito blood meals have been described previously (4).

### Seroconversion in sentinel pigs

Ten locally procured piglets born during the non-transmission season (January–June) and aged 3–6 months were placed in each of four villages. Blood specimens were collected from them in heparinized vials at the time of placement and every fortnight thereafter from June to December. The animals were included in the study only when their maternal antibodies, if present, disappeared.

### Human infections

We carried out an exploratory investigation in eight villages in August and November 1988 in order to plan a subsequent seroconversion study. Samples of fingerprick blood (200–300 µl) were collected in heparinized vials from nursery and primary-school children aged 3–14 years, following the informed consent of their parents.

Fifteen villages were selected at random from those in which at least one case of encephalitis had occurred since 1986. In all the schools in these villages, primary-school children, mainly aged 5–7 years but with a small number aged 8–9 years, who were present on the day of the first visit, were recruited for the study, following informed consent. Only these children were followed up prospectively. None had received JE vaccine but they were all covered by the Expanded Programme on Immunization. From each child 200–300 µl of fingerprick blood was collected as described above. Paired surveys

were conducted before and after the JE transmission season, as follows: in August and December 1989, August 1990 and January 1991, and August 1991 and February 1992.

Blood specimens were transported on ice to the field laboratory for separation of plasma, which was then shipped on ice to Madurai and stored at –20 °C pending examination.

### Serological tests

The haemagglutination inhibition (HI) test (5) was performed on microtitration plates on acetone-extracted, goose-erythrocyte-absorbed plasma. Each specimen was tested against JE, West Nile (WN), and dengue (DEN-2) virus antigens. Paired specimens were tested simultaneously and known positive and negative controls were included in each day's tests. The diagnostic criteria used were as follows:

- *seronegative*, <1:10 HI titre for all three viruses;
- *seropositive*, ≥1:10 HI titre for at least one virus;
- *seroconversion*, pretransmission season specimen negative and post-transmission season specimen positive, or both specimens positive with a four-fold or greater rise in titre in the post-transmission season specimen;
- *seroreversion*, post-transmission season specimen positive and next pretransmission season specimen negative;
- *specific virus infection*, monospecific response or broadly reacting, with HI titres to one virus that were at least four times greater than those to others; and
- *unclassified*, less than fourfold differences in HI titres for more than one virus.

Virus-specific IgM antibodies were detected by IgM-capture enzyme-linked immunosorbent assay (ELISA) (6) using kits provided by the National Institute of Virology, Pune. The JE, WN, and DEN-2 virus antigens supplied in the kits were used to test each sample.

### Statistical analysis

The precision level for the seroconversion rates was determined using the Epi Info software package (Centers for Disease Control, Atlanta, GA, USA, and WHO, Geneva, Switzerland).

## Results

### Seroconversion in sentinel pigs

Of 124 animals examined, 95 (76.6%) seroconverted during the transmission season. In individual villages

the seroconversion rates were as follows: 60–100% in 1989, 75–100% in 1990, and 58–66% in 1991. Seroconversions occurred in all the months when tests were carried out, i.e., June–December, the peak being in November (Fig. 1). Of the 95 animals that seroconverted, 24 (25%) had HI antibodies to JE virus alone; and 42 (44%) had HI antibody titres against JE virus that were at least four times greater than the titres against WN/DEN-2. JE virus infection was therefore confirmed in 69% of the animals that seroconverted; the remaining 29 animals (31%) were unclassified.

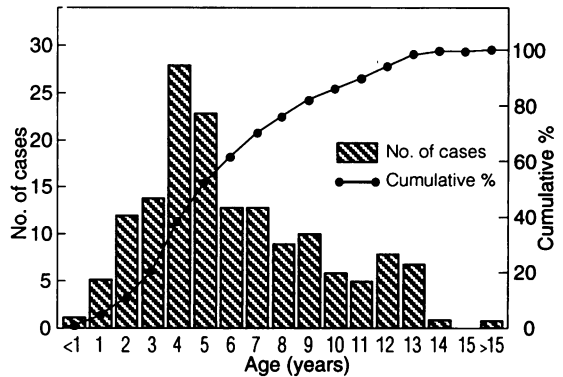
### Human cases of encephalitis

Between 1981 and 1990, a total of 229 patients with encephalitis were reported at the primary health centre, the numbers corresponding to the successive years being as follows: 24, 5, 18, 11, 15, 49, 39, 9, 30, and 29. Data for 1991 were inadequate and were therefore not included in the analysis. As shown in Fig. 2, about 99% of patients were under the age of 15 years and there was a distinct peak in the incidence among 4–5-year-olds. The male:female ratio was 1:0.8, and 80% of cases occurred in October and November.

### Human seroepidemiology

In an exploratory study, blood specimens from 793 children aged 3–14 years were examined for HI antibodies to flaviviruses, and 271 (34.2%) proved positive against one or more antigens. Of these seropositive children, 221 (81.5%) were positive for JE, 131 (48.3%) for WN, and 112 (41.3%) for DEN-2, alone or in combination. The age-specific prevalence of HI antibodies showed a significant linear regression of percentage positivity on age ( $y = 1.25 + 4.72x$ ;  $r =$

Fig. 2. Age distribution of human cases of encephalitis recorded at Nallur Primary Health Centre, 1986–90.



WHO 94867

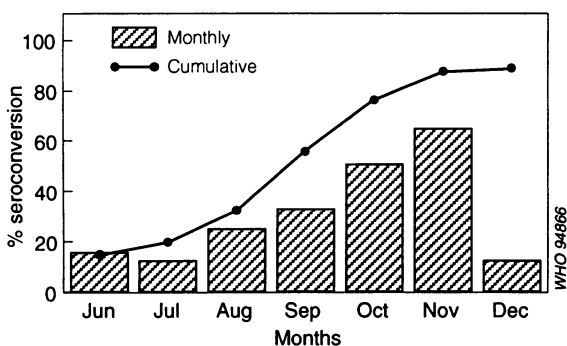
0.9159,  $P < 0.005$ ). The value of the regression coefficient suggested that the annual increment in seropositivity was about 5%, and on this basis it was considered that a sample size of 450 children would be required for a precision of  $\pm 2\%$  in estimating this increment. We therefore recruited about 1000 children for the prospective study, in the expectation that about 50% would drop out during the 3-year study period.

Our assumption that the rate of increase in cohort positivity was about 5% per year proved to be incorrect. The precision levels achieved, as calculated from actual sample sizes and rates of increase, varied from 2.9% in the first year to 4% in the third, when blood samples were obtained in only nine of the original 15 villages because of lack of cooperation (Table 1).

### Prospective cohort study

Among children from whom paired blood specimens were taken in 1989, 15.8% possessed antibodies to flaviviruses before and 53.3% after the transmission season, an increase of 37.5 percentage points (Table 1). In 1990, 33.7% of children were positive before and 75.8% after the transmission season, an increase of 42.1 percentage points. Before the 1991 transmission season, 48.2% of children were positive; after this season, 73.2% were positive, an increase of 25 percentage points. Table 1 shows that in the non-transmission season, i.e., the period between the post-transmission survey and the following year's pretransmission survey, there was a considerable decline in seropositivity (19.6 and 27.6 percentage points, in 1990 and 1991, respectively). Thus, the net annual increases in cohort positivity to HI antibodies, as determined by comparison of pretransmis-

Fig. 1. Seroconversions in sentinel pigs in the area covered by Nallur Primary Health Centre, by month, 1989–91.



WHO 94866

Table 1: Haemagglutination inhibition antibody against flaviviruses among the study children, 1989–91

	No. examined	No. positive	Increase (percentage points)	Precision level (%) <sup>a</sup>	Net annual increase in positivity (percentage points)
<b>1989</b>					
Preseason	1 102	174 (15.8) <sup>b</sup>			
Postseason	1 102	587 (53.3)	37.5	2.9	
<b>1990</b>					
Preseason	650	219 (33.7)			17.9
Postseason	650	493 (75.8)	42.1	3.8	
<b>1991</b>					
Preseason	440	212 (48.2)			14.5
Postseason	440	322 (73.2)	25.0	4.0	

<sup>a</sup> 95% confidence interval.

<sup>b</sup> Figures in parentheses are percentages.

sion samples in successive years, were 17.9 percentage points in 1989–90 and 14.5 percentage points in 1990–91. The geometric mean titres of JE virus antibodies of all the sera titrated in the post-transmission seasons did not reveal differences between age groups or between successive years, varying from 12.3 to 17.1 in December 1989, from 10.8 to 14.5 in January 1991, and from 10.6 to 12.2 in February 1992.

Seroconversion rates were studied on pre- and post-transmission season samples. The rates among children who were negative before the transmission season were 46.2%, 76.6% and 71.9%, respectively, in 1989, 1990, and 1991 (Table 2). The rates among children who were positive before the transmission season were 32.7%, 27.8%, and 7.4%, respectively. Seroreversion rates were studied during the nontransmission period. Among positive children, 49.7% seroreverted in 1989–90, and 50.5% in 1990–91. The probability of seroreversion was inversely related to

the initial JE virus antibody titres at the end of the transmission season. Table 3 shows that 56.9% of children with an initial titre of  $\leq 1:20$  seroreverted, while 33.3% did so among those with an initial titre of  $\geq 1:40$ . The difference was significant ( $\chi^2$  test = 28.92,  $P < 0.05$ ).

The postseason percentage point increases in seropositivity were similar for males and females (36 and 38 in 1989, 40 and 42 in 1990, and 23 and 27 in 1991;  $\chi^2$  test, not significant). However, statistically significant variations were observed between villages, with the percentage point increases ranging from 23 to 50 in 1989, and from 26 to 58 in 1990 ( $P < 0.05$ ). In 1991 only nine villages were followed up; in five of them seropositivity increased (range: 4–69 percentage points); in one village there was a marginal increase (1 percentage point); and in three villages there was a decrease (range: 43–4). For three villages, data on the blood-feeding rates of vectors were available. In Erappavur, where the cattle popu-

Table 2: Seroconversion and seroreversion rates against flaviviruses in a cohort of school-children covered by the Nallur Primary Health Centre, 1989–91

Year	Seroconversion: <sup>a</sup>						
	<i>n</i>	Preseason HI <sup>b</sup> antibody status:				Seroreversion <sup>a</sup>	
		Negative <sup>a</sup>		Positive <sup>a</sup>			
<i>n</i>	No. seroconverting	<i>n</i>	No. seroconverting	<i>n</i>	No. seroreverting		
1989	928	429 (46.2) <sup>c</sup>	158	51 (32.7)	493	245 (49.7)	
1990	431	330 (76.6)	97	27 (27.8)	279	141 (50.5)	
1991	228	164 (71.9)	135	10 (7.4)			

<sup>a</sup> See text for definitions.

<sup>b</sup> Haemagglutination inhibition.

<sup>c</sup> Figures in parentheses are percentages.

Table 3: Seroreversion rate in relation to Japanese encephalitis virus-haemagglutination inhibition antibody titre among the study children

Post-transmission JE-HI titre <sup>a</sup>	No. examined	No. seroreverting
1:10	281	163 (58.0) <sup>b</sup>
1:20	90	48 (53.3)
1:40	94	36 (38.3)
1:80	75	21 (28.0)
1:160	32	10 (31.3)
Total	572	278 (48.6)
≤1:20	371	211 (56.9)
≥1:40	201	67 (33.3)

<sup>a</sup> JE-HI = Japanese encephalitis virus-haemagglutination inhibition.

<sup>b</sup> Figures in parentheses are percentages.

lation was high relative to that of humans, the percentage of JE vectors feeding on cattle was greater and the seroconversion rate was lower than in two other villages where the cattle:humans ratio and the percentage of feeding on cattle were relatively low (Table 4). The humans:pigs ratio and the corresponding feeding rates were approximately the same in all three villages.

Among those children who seroconverted, virus specificity was established in about a third, while the rest remained unclassified (Table 5). JE-virus-specific seroconversions occurred in 29.6%, 37.5%, and 23.6% over successive years; the corresponding values for WN virus were 3.8%, 0.3%, and 0. DEN-2-

Table 4: Seroconversion rates, animal and human population ratios, and blood-feeding rates of Japanese encephalitis vectors in three villages covered by Nallur Primary Health Centre

	Village:		
	Erappavur	Sepakkam	Kodikkalam
% Seroconversion in children <sup>a</sup>	26.5	32.5	48.5
Pigs:cattle:humans	1:12:25	1:4:20	1:4:29
<i>Culex tritaeniorhynchus</i>			
% Feeding on:			
Cattle	92.3	85.8	85.2
Humans	1.6	3.8	5.9
Pigs	1.6	5.9	5.4
<i>C. vishnui</i>			
% Feeding on:			
Cattle	93.5	86.3	83.3
Humans	2.4	5.1	11.5
Pigs	0.6	11.5	7.9

<sup>a</sup> Mean for 1989 and 1990.

Table 5: Virus-specific seroconversions among school-children covered by Nallur Primary Health Centre, 1989-91

Seroconversion to: <sup>a</sup>	1989	1990	1991	Total
JE	142 (29.6) <sup>b</sup>	134 (37.5)	41 (23.6)	317 (31.4)
WN	18 (3.8)	1 (0.28)	0	19 (1.9)
DEN	0	0	27 (15.5)	27 (2.7)
Unclassified	320 (66.7)	222 (62.2)	106 (60.9)	648 (64)
Total	480	357	174	1 011

<sup>a</sup> JE = Japanese encephalitis virus; WN = West Nile virus; and DEN = dengue virus.

<sup>b</sup> Figures in parentheses are percentages.

virus-specific seroconversions were seen only in 1991, when they accounted for 15.5% of seroconverters.

JE-virus-specific IgM antibodies were demonstrated in 10 of 134 postseason samples from children who seroconverted in 1989, but in none of the 60 similar samples tested in 1990. No WN- or DEN-2-virus-specific IgM was detected in either year.

#### Apparent:inapparent infection ratio

In the first 2 years of the study, seroconversions were mainly due to JE virus, and it was therefore assumed for the purposes of calculation that the post-transmission rise in positivity was due to JE virus infections. On this basis we estimated the number of cases of inapparent infection among the 10 400 children aged 5-9 years who were covered by the primary health centre and compared this with the number of clinically diagnosed cases of encephalitis in this age group, also presumably due mainly to JE virus (Table 6); the ratio was 1:282 in 1989 and 1:257 in 1990, with an average of 14.9 cases per 10 000 population. No calculations were attempted for 1991, since DEN-2 virus was also active and data on encephalitis cases were inadequate.

## Discussion

The present study followed the temporal changes in transmission rates over 3 years in an area where JE was endemic, unlike previous investigations that have covered only one transmission season (7-9). Schoolchildren aged 5-9 years were selected so that there would be an adequate number of susceptible individuals and sufficient participation. Of the 10 400 children aged 5-9 years covered by the primary health centre, the study sample size ranged from 10% in 1989 to 4.2% in 1991. The HI test has several advantages making it particularly appropriate

**Table 6: Apparent:inapparent infection ratios for children aged 5–9 years in the area covered by Nallur Primary Health Centre (population 10 400), 1989–90**

	Proportion seroconverted	Estimated number of inapparent infections	Observed number of cases of encephalitis	Apparent:inapparent infection ratio
1989	0.38	3 952	14	1:282
1990	0.42	4 368	17	1:257

for following the same cohort through more than one transmission season. It is a sensitive and accurate indicator of subclinical infection with JE virus in the early postinfection phase (10), needing only a small quantity of serum or plasma, easily obtainable from fingerprick blood specimens. HI antibodies develop faster than neutralizing antibodies and previously infected persons may show measurable HI antibody responses (7, 11).

There was intense flavivirus activity in all 3 years, and the annual seasonal increase in HI seropositivity ranged from 42.1 to 25 percentage points. This was considered to be caused predominantly by JE virus activity in 1989 and 1990, when the majority of the seroconversions diagnosed serologically were attributable to this virus. The fact that only JE-virus-specific IgM antibodies were identified in a sample of children provided supporting evidence; furthermore, 69% of the sentinel pigs in the area, all primary responders, exhibited mainly monospecific JE virus seroconversions during the season. WN virus activity was either absent (in 1991) or present at a low level (in 1989 and 1990), whereas in a post-epidemic survey in South Arcot District in 1982 the prevalence of WN neutralizing antibodies was significantly higher than that of JE (12). This indicates shifting patterns of virus activity in the area. Thus DEN-2 virus, which was not active in 1989 and 1990, exhibited relatively high activity in 1991, when 16% of seroconversions in children were due to it.

Previous studies in southern India have shown that, among cases of serologically confirmed JE, HI antibody titres began to fall about 3–4 months after onset (13). In the present study, for about half the children who suffered inapparent infections during the transmission season HI antibody titres had declined to undetectable levels 6–8 months later, before the start of the next transmission season; also, there was an inverse relationship between seroreversion rates and initial antibody titres. Similarly, in Chiangmai Valley, Thailand, 21% of people who had monospecific JE virus titres of 1:20 as a result of subclinical infection had reverted to <1:20 within 12 months, while none of those with titres of  $\geq$ 1:40 reverted (11). The high seroreversion rate in the present study arose at least partly because only small

children were investigated. It is interesting that the only other record of high seroreversion (28%) was among American servicemen in Korea, all of whom were nonimmune before the transmission season, who lost detectable HI antibodies in 7–9 months (10).

Notwithstanding the high seroreversion rates, the net annual increases in HI positivity (on average 16.2 percentage points, were much higher than those reported for populations in other areas of endemicity, although the data are not strictly comparable because of differences in the study designs and the age groups observed. Among Japanese schoolchildren aged 6–12 years, annual rates of increase of 5% (7) and 9–10% (8) have been reported. In the Chiangmai Valley, annual increments were in the range 2.5–11% in individual villages, while among urban schoolchildren the increment was 4.3% (11), and for under-40-year-olds in Sarawak the overall estimate was 6% (14). However, 50% of a group of susceptible American servicemen at an airbase in Korea developed antibodies in a single season (15).

Very high seroconversion rates were observed in children who did not possess HI antibodies before the transmission season (46.2%, 76.6% and 71.9% in successive years). Each seroconversion was presumably the result of at least one infective mosquito bite. The minimum probability of a child receiving an infective bite during the transmission season lay in the range 0.47–0.77. Studies are in progress to detect virus in wild-caught vectors in order to confirm whether such intense transmission can take place. Presumably the children who were already HI-positive at the beginning of the season received the same number of infective bites, but they did not all exhibit a detectable response. In successive years, with increasing acquisition of immunity, the seroconversion rate in the group of positive children decreased.

Statistically significant differences were observed between seroconversion rates in individual villages. At least, in part, these differences were possibly caused by variations in cattle populations, since a low seroconversion rate in children was associated with a high cattle:humans ratio. Vector abundance was approximately the same in these villages (Centre for Research in Medical Entomology, Madurai, unpublished data).

The overall incidence of JE cases in the Nallur Primary Health Centre was 6.0 per 10 000 population in the age group 1–19 years, higher than in Chiangmai Valley (Thailand) where it was 3.8 per 10 000 for the same group (16). However, in Nallur the incidence among 1–9-year-olds was strikingly higher (9.4 per 10 000) than that among 10–19-year-olds (2.3 per 10 000), whereas in Chiangmai Valley the incidence for the younger age group was lower than that for the older (3.1 and 4.6 per 10 000, respectively). This suggests that immunity develops early in Nallur as a result of repeated exposure to intense transmission. The ratio of overt:inapparent JE infections (1:270) for children aged 5–9 years in the present study was similar to the ratios observed in Chiangmai Valley (1:300) (11), Sarawak and China (Province of Taiwan) (1:250–500) (11), Japan (1:590) (8), and West Bengal (1:400) (17).

The present study has revealed that young children in an area of endemicity in southern India are gravely at risk of developing JE during the transmission season because of the high probability of receiving an infective mosquito bite. However, only about 1 in 270 will develop the disease and others will suffer a latent infection resulting in high HI seroconversion rates, followed by high rates of seroreversion during the nontransmission season. Further studies are required to elucidate the implications of these findings for the development of protective immunity in the population.

### Acknowledgements

We are very grateful to all the children who donated blood samples and thank their teachers as well as the doctors and health workers of the Tamil Nadu Public Health Department for their invaluable help. Dr P.S.S. Sundar Rao, Professor and Head, Department of Biostatistics, Christian Medical College, Vellore, Tamil Nadu, is thanked for his help in designing the study. The excellent technical assistance by the staff of the Centre for Research in Medical Entomology, Madurai, and its field station at Vridhachalam is gratefully acknowledged. The study would not have been possible without the generous donation of flavivirus antigens, positive and negative sera, and ELISA kits by the National Institute of Virology, Pune. Mr R. Ilanthirayan was involved in a part of this study at the Centre for Research in Medical Entomology.

### Résumé

#### Etude dans la communauté des infections infracliniques à flavivirus chez l'enfant dans une région du Tamil Nadu (Inde) où l'encéphalite japonaise est endémique

Les infections infracliniques par le virus de l'encéphalite japonaise (JE) dépassent de très loin les

encéphalites déclarées. La notification des seuls cas déclarés sous-estime donc le niveau général de transmission du virus, dont la détermination quantitative est indispensable pour l'élaboration de stratégies de lutte. Nous avons réalisé une enquête sérologique prospective sur la période 1989–1991, sur une cohorte de 1102 écoliers âgés de 5 à 9 ans, sélectionnés dans 15 villages couverts par le centre de santé primaire de Nallur, au Tamil Nadu, où l'encéphalite japonaise est endémique. Pour chaque enfant, on a déterminé les titres d'anticorps par inhibition de l'hémagglutination (HI), à l'égard du virus de l'encéphalite japonaise (JE), du virus West Nile (WN), et du virus de la dengue type 2 (DEN-2), avant et après la saison de transmission. On a également étudié, parallèlement, les séroconversions chez des porcs sentinelles.

Chez l'enfant, l'augmentation post-saisonnière du pourcentage de positivité en HI était, pour les trois années successives, de 37,5, 42,1 et 25 points. Chez environ un tiers des enfants ayant opéré une séroconversion, un diagnostic viral spécifique a été établi. La séroconversion était due au virus JE chez 29,6%, 37,5% et 23,6% des enfants pour chacune des trois années, alors que pour le virus WN les valeurs correspondantes étaient de 3,8%, 0,3% et 0. Une activité du virus DEN-2 n'a été observée qu'en 1991, au cours de laquelle 15,5% des enfants étaient atteints. La prédominance du virus JE dans la région a été prouvée par la mise en évidence d'IgM anti-virus JE chez 7,5% des enfants ayant opéré une séroconversion, alors qu'il n'a pas été détecté d'anticorps dirigés contre les virus WN et DEN-2. Le taux de séroconversion chez les porcs était de 76,7%, dont 69,9% du fait du virus JE. Chez près de la moitié des enfants ayant fait une séroconversion, les titres d'anticorps avaient baissé jusqu'à n'être plus décelables lors de nouvelles analyses effectuées 6 à 8 mois après la saison de transmission. Il y avait, par conséquent, une augmentation annuelle nette moyenne du pourcentage de séropositivité de 16,2 points dans la cohorte. Les taux de séroconversion variaient de façon statistiquement significative d'un village à l'autre, et dans trois d'entre eux, ces variations étaient liées à des différences au niveau du rapport bovin:homme:porc.

Chez les enfants négatifs pour les anticorps en HI avant la saison de transmission, des taux de séroconversion très élevés ont été observés (46,5% à 76,6%), montrant l'existence d'une intense transmission du virus dans la région. L'incidence des cas de JE diagnostiqués d'après l'examen clinique était de 14,9 pour 10 000 chez

les enfants de 5 à 9 ans. En supposant que la plus grande partie de la séroconversion observée au cours des deux premières années de l'étude était due au virus JE, nous avons estimé que le rapport entre l'infection déclarée et l'infection inapparente était de 1:270.

Il est nécessaire de déterminer plus avant la signification des forts taux de séroconversion, qui peuvent être attribués à des taux élevés d'inoculation par les moustiques, en ce qui concerne l'acquisition d'une immunité protectrice.

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