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A prospective cohort study of dengue infection in schoolchildren in Long Xuyen, Viet Nam

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ABSTRACT

A dynamic school-based cohort of 2–15 year-olds was established in Long Xuyen, Viet Nam to provide epidemiological data for a dengue vaccine efficacy trial. Active surveillance of febrile episodes identified clinically-suspected dengue and acute and convalescent sera were collected. IgG seroconversion between annual seroprevalence surveys identified sub-clinical infections. In 2004, 2190 children were enrolled with 3239, 3146, and 3081 present each year from 2005 to 2007 consecutively. In all, 627 children had a total of 690 clinically-suspected dengue episodes (394 hospitalisations, 296 outpatients) with 284–310 (41.2–45.0%) laboratory-confirmed depending on testing. Dengue serotype 2 was predominant in 2004 and 2005, and serotype 1 in 2006 and 2007.

The acute dengue disease incidence rate per 1000 person-years ranged from 16.9 in 2005 to 40.4 in 2007. The average annual incidence of primary dengue infection (IgG seroconversion in previously naïve children) was 11.4% and the symptomatic to asymptomatic primary infection ratio ranged from 1:3–1:6. Study withdrawal rate, a feasibility indicator for conducting efficacy trials, was low: 4.2% per year when excluding children who changed schools. Our 2004–2007 results confirm the high transmission of dengue in children in Long Xuyen and demonstrate the suitability of this study site for a large scale efficacy trial.

1. Introduction

The mosquito-borne dengue virus (DENV) is endemic or hyperendemic (two or more serotypes co-circulating) in most of Southeast Asia, the Western Pacific, the Americas and Africa. Four virus serotypes (DENV 1–4) can infect humans and produce a wide spectrum of clinical illness, from non-specific viral illness, to classical dengue fever (DF) and severe life-threatening dengue, including dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS).[1] Infection can also be asymptomatic. Recent estimates put 3.61 billion people (55% of the world's population) at risk of dengue disease with 36 million DF cases, 2.1 million severe cases and 21 000 deaths annually.[2]

Dengue vaccines in development will need testing where incidence is high enough to measure vaccine efficacy in a reasonable timeframe and where support for

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vaccine introduction exists, for example where there is sufficient awareness of the disease burden and the need for national immunization policies.[3–5] Identifying appropriate sites requires in-depth research. Prospective epidemiological studies can be performed to identify and prepare suitable efficacy trials sites. The incidence of virologically-confirmed dengue cases as defined by the World Health Organization (WHO), together with data including age-specific incidence, serotype prevalence, infection rates, specific mortality are all invaluable for efficacy trial preparation.[6,7] Disease surveillance at potential trial sites must also be sufficient to identify febrile episodes early enough to allow dengue viraemia to be tested and confirmed. A standard case evaluation algorithm should be implemented together with procedures to identify suspected cases at any medical facility serving the population (for a detailed discussion of the prerequisites for dengue vaccine clinical trials in endemic areas, see [6,7]).

Viet Nam meets all of the above criteria with high disease incidence, circulation of all four serotypes and a national dengue surveillance system. Since 1963, DF and DHF cases have steadily increased to become leading causes of paediatric hospitalisations and deaths in Viet Nam, accounting for 70% of 552 088 cases reported from the Western Pacific region during 1993–1997.[8,9] Transmission is highest in the Mekong Delta region of southern Viet Nam where a widespread DHF epidemic occurred in 1998, with 434 cases/100 000 population and 342 deaths, the highest figures ever recorded by the WHO in one year.[10,11]

We present the first four years of an ongoing, prospective, dynamic cohort study initiated in December 2003 in this affected region. The primary objective is to estimate the incidence rate of laboratory-confirmed dengue disease in school-aged children living in this area. Secondary objectives are to estimate prevalence and incidence risk of dengue infection, identify circulating serotypes, and to assess its suitability for a vaccine efficacy trial.

2. Methods

2.1. Study site and population

The study was conducted in Long Xuyen, capital of An Giang province in the Mekong Delta in southern Viet Nam, 250 km from Ho Chi Minh City. In the 1999 census Long Xuyen had a population of 249 535 inhabitants, with 28% (68 937) younger than 15 years. Long Xuyen has 13 public nursery schools, 34 primary schools and 10 secondary schools. We selected three nursery schools, two primary schools and one secondary school based on their location and logistical reasons: chosen schools were close to each other, and also near to the provincial hospital where medical consultations are performed, thus favouring continuity of the cohort from year to year between each level of education and increasing the probability that cases would present at the study hospital. This was a dynamic cohort: children leaving prematurely were replaced by children of the same age and children in the lowest age group were recruited each year. Children could move from one school to another (between those included in the study), and remain in the study. This study was approved by the scientific committee of the Pasteur Institute Ho Chi Minh City and by the Ministry of Health of Viet Nam. Written informed consent was obtained from the parents or legal representatives of all participants prior to enrolment.

2.2. Detection of febrile episodes

Schoolchildren were actively and passively monitored year-round for febrile episodes. During the nine months of school-term (1 September–31 May), daily absentees were recorded with the reason, if known. In the first year of the study (2004), the children had to be absent for two consecutive days before being documented. To improve case detection and increase the probability of detecting viraemia, this delay was reduced to one day for all subsequent years. Study nurses visited the homes of absentees to identify fever. Children with axillary temperature $\geq$38 °C were taken to the paediatric ward. Children who had already consulted or who were absent for non-medical reasons were not visited. During the three month school holiday (1 June–31 August), coinciding with the peak dengue season, all children were visited at home three times a week to check for febrile episodes. Children with temperature $\geq$38 °C were taken to the paediatric ward. In addition, passive detection of febrile episodes was facilitated by providing all participants with free access to a dedicated consultation room at the paediatric ward. All hospitalisations were documented, whatever the reason.

2.3. Definition of clinically-suspected dengue episodes

Clinically-suspected dengue was defined as a child presenting with a recent history of acute febrile illness (reported by the adult caretaker or confirmed by axillary temperature $\geq$38 °C) for $\geq$2 days and for which a diagnosis of dengue or viral infection (i.e. acute fever without clinical signs of focused infection) was suspected, irrespective of the presence of clinical signs of severity such as haemorrhagic tendencies or shock. When dengue was suspected, a detailed clinical examination was performed recording: general condition (weight; height; temperature), cardiac and respiratory rates; blood pressure, dengue associated clinical signs (shock, pleural effusion, rash, haemorrhage, positive tourniquet test, hepatomegaly) and other relevant clinical signs, especially signs associated with concomitant diseases (e.g. respiratory infection). Two blood samples were collected for laboratory confirmation: the first (acute sera) at consultation and the second (convalescent) either upon discharge of hospitalised cases or 10 days after outpatient consultation. Sera were shipped frozen to the Pasteur Institute arbovirus laboratory in Ho Chi Minh City for confirmation. Baseline haematological (i.e. white blood cells, platelet counts and haematocrit) tests were performed on the acute sample at the hospital. Other haematological tests were performed at the clinician's request. Consultations and/or hospitalisations by the same patient within 2–3 weeks were considered as part of the same dengue episode. A doctor and a laboratory technician were available for home-based medical consultations.

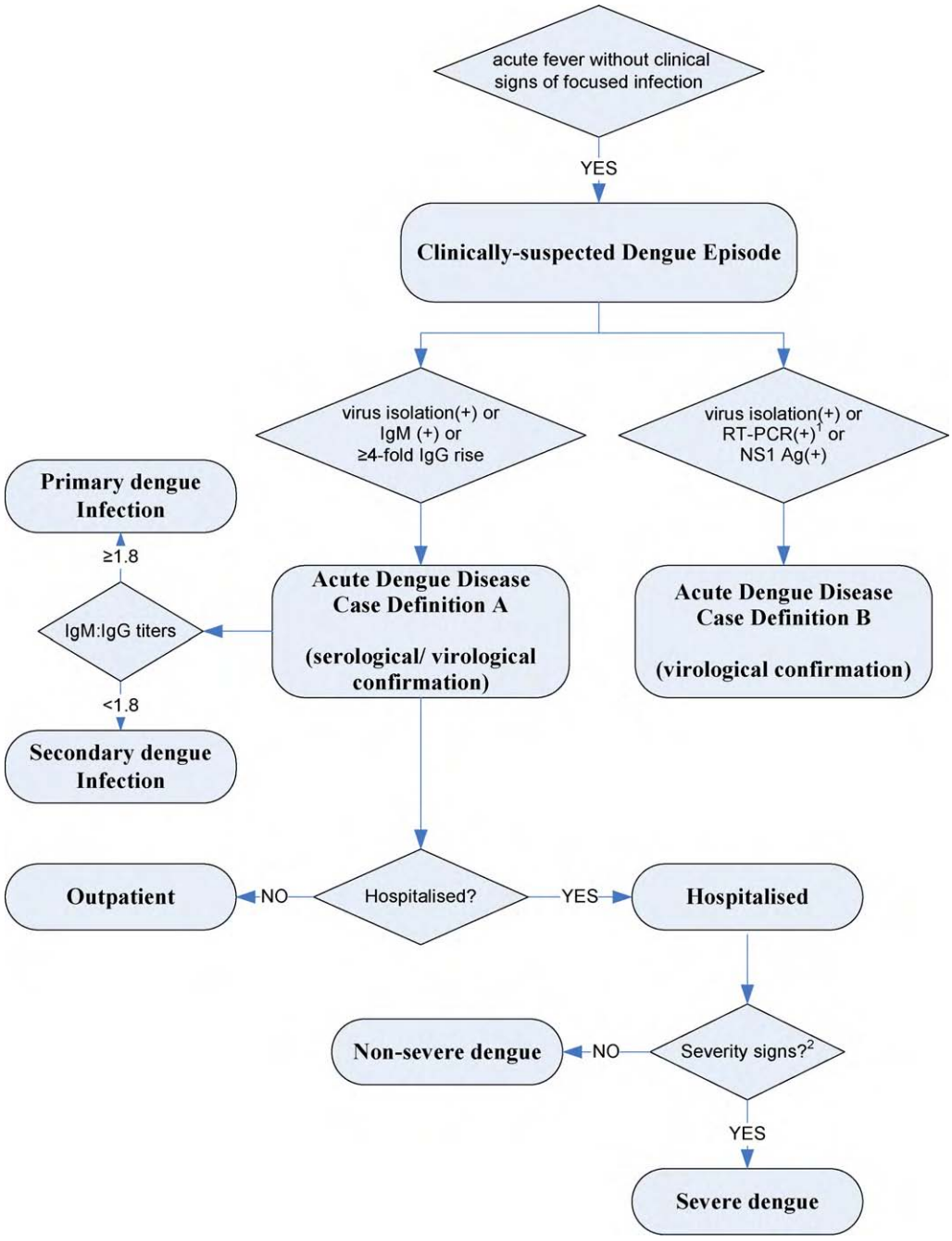

Figure 1. Case definitions and classification of dengue episodes used in the study. [1]RT-PCR confirmation was performed from 2006 only. [2]Severity signs for hospitalised cases were (i) haemorrhagic manifestations other than tourniquet test or petechiae, (ii) shock or undetectable blood pressure, (iii) pleural effusion or signs of plasma leakage or haematocrit ≥44% at any time during the hospitalisation or variations in haematocrit ≥20%, or (iv) thrombocytopenia: platelets ≤100,000 mm3 at any time during hospitalisation.

2.4. Annual seroprevalence surveys

A seroprevalence survey was performed each December, outside of the rainy season (i.e. outside of the peak transmission period) to determine the prevalence and incidence risk of primary dengue infection. Blood samples were collected to assess dengue virus-specific antibodies. Height, weight and temperature were also measured. Positive IgG titres indicated previous exposure to dengue virus. It was postulated that children with no detectable IgG antibodies were dengue-naïve.

2.5. Laboratory assays

The dengue-specific antibody response was tested in acute and convalescent samples from each clinically-

Table 1
Cohort characteristics at beginning of each year of surveillance

	2004 *n* (%)	2005 *n* (%)	2006 *n* (%)	2007 *n* (%)
Number of enrolees	2190	1645	722	652
Size of the cohort in January	2190	3239	3146	3081
Sex				
Male	1103 (50.4)	1593 (49.2)	1559 (49.6)	1551 (50.3)
Female	1087 (49.6)	1646 (50.8)	1587 (50.4)	1530 (49.7)
Age (years)				
Mean ± SD	6.89 ± 2.11	8.45 ± 2.78	8.53 ± 2.87	8.55 ± 2.91
Range	3–10	3–14	2–15	3–15
Median	7.00	9.00	9.00	9.00
Age group				
2–5	688 (31.4)	659 (20.3)	621 (19.7)	568 (18.4)
6–10	1502 (68.6)	1732 (53.5)	1602 (50.9)	1560 (50.6)
11–15	ND	848 (26.2)	923 (29.3)	953 (30.9)
Dropouts				
Total:	596 (27.2)	813 (25.1)	717 (22.8)	730 (23.7)
Voluntary withdrawal	39 (1.8)	73 (2.3)	24 (0.8)	54 (1.8)
Moving to another school	493 (22.5)	631 (19.5)	640 (20.3)	604 (19.6)
Moving to another place of residence	40 (1.8)	94 (2.9)	41 (1.3)	38 (1.2)
Lost-to follow-up	24 (1.1)	15 (0.5)	12 (0.4)	34 (1.1)

ND: Not done

suspected dengue case and in the annual seroprevalence survey samples as follows. Anti-dengue IgM and IgG antibodies were assessed by in-house ELISA assays at the Pasteur Institute laboratory, according to techniques developed at the Center for Disease Control (CDC) Colorado USA: the dengue IgM capture ELISA (MAC-ELISA) was performed with monoclonal antibody SLE 6B6C-1/HRP conjugate, and the IgG ELISA was performed with DEN-2 (NGC) Mab 4G2.[12,13] Samples with optical density readings (OD) above the defined cut-off value were considered seropositive. All acute samples, irrespective of the results of serological analysis, collected from suspected cases within five days of fever onset were tested with up to three separate virological assays:

(1) **Virus isolation** (all samples): undiluted serum (0.05 mL per tube) was inoculated into duplicated tubes of C6/36 (*Aedes albopictus*) cells and incubated at 28 °C for 7 to 14 days at the Pasteur Institute's arbovirus laboratory. Cells were then harvested and dengue viruses were identified and typed using indirect immunofluorescence assay with serotype-specific monoclonal antibodies supplied by CDC, Colorado, USA.[14–16]
(2) **NS1 antigen assay** (retrospectively on all samples collected from 2004–2005, then prospectively from 2006): a commercially available Platelia Dengue NS1 Antigen assay (BIO-RAD, Marnes-la-Coquette, France) was used at Pasteur Institute's arbovirus laboratory.
(3) **Quantitative real-time polymerase chain reaction** (qRT-PCR; retrospectively on all samples collected from 2006–2007)**:** Using a previously described qRT-PCR assay, dengue serotype-specific RNA was quantified at the research laboratories of Sanofi Pasteur, France.[17]

2.6. Definition of acute dengue disease

Acute dengue disease was defined as a clinically-suspected dengue episode with virological or serological confirmation. Two definitions of laboratory confirmation were used (Figure 1): *Case definition A, serological or virological confirmation:* (1) positive dengue virus isolation test result or (2) IgM anti-dengue antibodies detected in acute or convalescent serum or (3) an increase in anti-dengue IgG titre of at least four-fold between acute and convalescent sera. *Case definition B, virological confirmation:* positive test result by either virus isolation or NS1 antigen or qRT-PCR assays.

Acute dengue cases defined using case definition A were further classified as outpatient or hospitalised, as severe (presence of at least one severity criterion; Figure 1) or non-severe, and as primary (IgM/IgG titre ratio ≥1.8) or secondary infection (IgM/IgG titre ratio <1.8).[18]

2.7. Incidence risk of primary dengue infection and incidence rate of acute dengue disease

The annual incidence risk of primary dengue infection was defined as the proportion of children seroconverting (i.e., negative to positive IgG) between paired samples obtained in consecutive seroprevalence surveys. If the child had had an acute dengue episode (as defined above) during the year, then primary dengue infection was classified as symptomatic otherwise it was classified as asymptomatic.

For each year of surveillance, the number of person-years at risk was calculated from the 1 January (entry date) until 31 December (exit date). For children with an outcome of interest, the exit date was the onset date, and for a child with multiple outcomes of interest only the first was used.

Table 2
Number of acute dengue disease cases according to the laboratory method

	2004	2005	2006	2007	TOTAL
Positive virus isolation	28	18	42	63	151
Positive RT-PCR	ND	ND	73	100	173
Positive NS1	53	36	58	97	244
Positive IgM	66	50	72	102	290
4-fold increase in IgG	6	6	4	3	19
Total Case definition A	73	51	77	109	310
Total Case definition B	54	38	78	114	284

ND: Not done
Case definition A: either positive virus isolation in acute sample or positive IgM in acute or convalescent sample or 4-fold increase in IgG between acute and convalescent samples.
Case definition B: either positive virus isolation or positive RT-PCR or positive NS1 in acute sample.

For children who withdrew from the study, the exit date was the date of withdrawal. Incidence rate was calculated for three outcomes of interest: acute dengue disease using case definition A, acute dengue disease using case definition B and severe dengue cases (definition A). Incidence rates were calculated by dividing the number of outcomes of interest by the number of person years at risk.

2.8. Statistical methods

Analyses were performed using SAS v8.02 for Windows (SAS Institute, Carry, NC, USA) with AdClin 3.2.6 (AdClin SA, Paris, France) to format tables and listings.

3. Results

3.1. Study cohort and baseline characteristics

In December 2003, 2190 children aged 2–10 years were recruited. A further 1645, 722 and 652 children aged 2–15 years were recruited in December 2004, 2005 and 2006 respectively. The size of the cohort was 2190 in January 2004, 3239 in January 2005, 3146 in January 2006 and 3081 in January 2007. Baseline characteristics are summarised in Table 1.

Some 2856 children withdrew during the first four years of the study, the main reason for which was moving to a non-participating school upon graduation. Excluding these withdrawals, the lost-to-follow-up rate was 4.2%.

3.2. Acute febrile illness

There were 6667 outpatient consultations and 561 hospitalisations from 2004–2007. Dengue was clinically-suspected for 362 (5.4%) consultations and 397 (70.8%) hospitalisations. Most outpatient consultations (4998, 75%) were for upper respiratory disease: pharyngitis (41.3%), upper respiratory infection (15.9%), cold (7.0%), tonsillitis (6.7%) and influenza-like illness (4.0%).

Overall 627 children experienced 690 clinically-suspected dengue episodes (296 outpatients consultations and 394 hospitalisations), 310 (45.0%) and 284 (41.2%) of which were acute dengue disease according to case definitions A and B, respectively (Table 2).

The specificity of dengue diagnosis increased with disease severity, and the positivity rate (case definition A) was higher in hospitalised cases (238/394; 60.4%) than in outpatients (72/296; 24.3%) ($p < 0.001$). As a consequence, the ratio of hospitalised to outpatient cases was 1.3:1 for clinically-suspected dengue episodes and 3.3:1 for laboratory-confirmed case definition A.

The median duration of hospitalisation for hospitalised dengue cases was 5 days (range: 2–17), median age was 9.8 years (range: 4–14). The median interval between the onset of fever and hospital admission was 4 days (range: 1–6) and patient presented with a mean axillary temperature of 37.9 °C ± 0.8 (range: 37.0- 40.2). Of these 238, 133 (51.9%) presented at least one severity criterion; 11 cases (4.6%) were DSS. There were no deaths.

Dengue cases treated as outpatients presented with a median duration of 4 days (range: 1–9) after fever onset with a mean axillary temperature of 37.4 °C ± 0.8 (range 36.0–39.5).

Of the 690 clinically-suspected dengue episodes, 284 (41.2%) were virologically-confirmed. The dengue serotype was identified by virus isolation or RT-PCR for 223 episodes: dengue serotype 1, 2, 3 or 4 was identified in 122, 62, 17, and 22 samples respectively (Figure 2). RT-PCR identified four dengue co-infections: three with serotypes 1 and 3 (serotype 1 displayed the highest titres in two of these three co-infections), and one with serotypes 4 and 1 (serotype 4 displayed the highest titre). Dengue serotype 2 was predominant in 2004-2005, whereas serotype 1 was predominant in 2006-2007. No child had two virologically-confirmed episodes during the follow-up.

Of the 310 acute dengue cases definition A, 123 (39.7%) were primary infections and 187 (60.3%) were secondary or subsequent infections. The hospitalisation rate was sim-

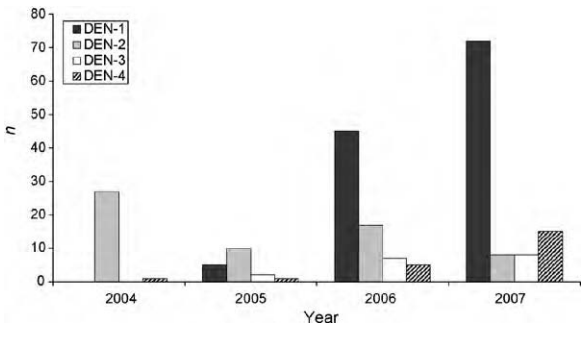

Figure 2. DENV serotype distribution per year.

Table 3
Yearly incidence rates of acute dengue cases

	2004			2005			2006			2007		
	P-years	No cases	IR/1000 p-years (95% CI)	P-years	No cases	IR/1000 p-years (95% CI)	P-years	No cases	IR/1000 p-years (95% CI)	P-years	No cases	IR/1000 p-years (95% CI)
Sero/virologically confirmed dengue (Case Definition A)												
All cases	2006	73	36.4 (28.9–45.8)	3027	51	16.9 (12.8–22.2)	2902	77	26.5 (21.2–33.1)	2826	109	38.6 (32.0–46.6)
Sex												
Male	1009	35	34.7 (24.9–48.3)	1487	31	20.9 (14.7–29.7)	1434	43	30.0 (22.2–405.5)	1432	49	34.2 (25.8–45.3)
Female	997	38	38.1 (27.7–52.4)	1540	20	13.0 (8.4–20.2)	1468	34	23.2 (16.6–32.5)	1394	60	43.0 (33.4–55.4)
Age group												
2–5	619	18	29.1 (18.3–46.2)	604	7	11.6 (5.5–24.3)	553	9	16.3 (8.5–31.3)	513	7	13.6 (6.5–28.5)
6–10	1386	55	39.7 (30.5–51.7)	1622	36	22.3 (16.1–30.9)	1521	45	29.6 (22.1–39.6)	1478	62	41.9 (32.7–53.7)
11-15	ND	ND	ND	800	8	10.0 (5.0–20.0)	828	23	27.8 (18.5–41.8)	834	40	47.9 (35.1–65.3)
Severe cases	2026	19	9.4 (6.0–14.7)	3039	21	6.9 (4.5–10.6)	2919	31	10.6 (7.5–15.1)	2848	62	21.8 (17.0–28.0)
Virologically confirmed dengue (Case definition B)	2013	54	26.8 (20.5–35.0)	3032	38	12.5 (9.1–17.2)	2902	78	26.9 (21.5–33.6)	2822	114	40.4 (33.6–48.5)

P-years: Person-years; IR: Incidence rate
ND: No data

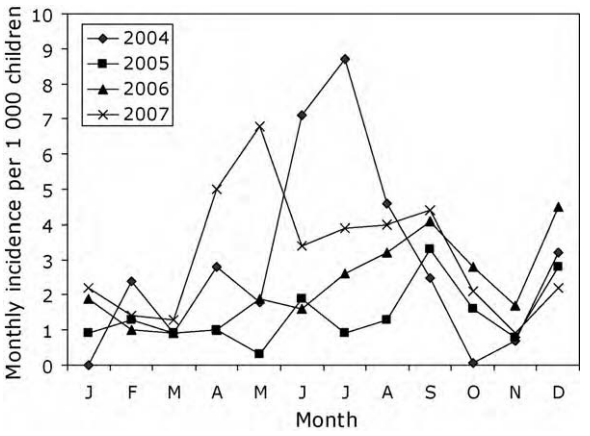

Figure 3. Monthly incidence of acute serologically or virologically confirmed dengue disease (case definition A).

ilar for primary and secondary infections (78.0% vs. 76.0%, $P = 0.77$) and the proportion of severe cases was similar in primary and secondary hospitalised cases (42.2% vs. 44.0%, $P = 0.86$).

3.3. Incidence rates of acute dengue disease

Incidence rates of acute dengue disease are shown in Table 3. There were no significant gender-specific differences in incidence rates. No significant difference was observed between 2–5 and 6–10 years age groups in 2004 ($P = 0.25$). There were no statistically significant differences in incidence rates between the 2–5, 6–10 and 11–15 year age groups in either 2005 or 2006, nevertheless the highest incidence rate was consistently observed in the 6–10 year-old group (39.7, 22.3 and 29.6 per 1000 in 2004, 2005 and 2006 respectively). In 2007, the highest incidence rate was observed in 11–15 year-olds (47.9 per 1000), which was 3.5 times higher than that observed in children aged 2–5 years ($P = 0.002$). The highest incidence rate for severe dengue disease was seen in 6–10 year-olds in 2007 (25.5 per 1000; 95% CI:18.6–35.0; data not shown). Acute dengue disease occurred year-round. 2004 and 2007 were epidemic years with peaks occurring in July and May respectively (Figure 3).

3.4. Annual prevalence and incidence risk of primary dengue infection

In December 2003, 20.9% (95% CI: 19.2–22.6) of the children had dengue virus specific IgG. During the following annual surveys, the proportion of children with dengue antibodies was stable: 28.7%, 27.2%, 19.6% and 26.3% in 2004, 2005, 2006 and 2007 respectively. Figure 4 shows the increasing prevalence of dengue antibodies with age.

Paired annual samples were available for 2061 children for 2003/2004, 3016 for 2004/2005, 3005 for 2005/2006, and 2951 for 2006/2007. During 2004, 2005, 2006 and 2007, respectively 194, 259, 175 and 325 initially seronegative children seroconverted, indicating primary infection, and the annual incidence risk of primary infection was 11.9%, 12.2%, 7.9%, and 13.6%. Of these primary infections, 31, 39, 46 and 73 were associated with acute dengue cases (definition A) respectively, and the incidence risk of primary symptomatic dengue infection was 1.9%, 1.8%, 2.1% and 3.1% respectively. The ratio of symptomatic to asymptomatic primary dengue infection was 1:5, 1:6, 1:3 and 1:3 for each year of surveillance.

4. Discussion

To our knowledge this is the first prospective cohort study to describe the incidence rate of laboratory-confirmed acute dengue disease in a population of healthy schoolchildren in southern Viet Nam. Our results confirm that high and sustained levels of dengue transmission occurred in Long Xuyen from 2004–2007. Indeed the study took place in the Mekong Delta region where dengue transmission is reportedly higher than in northern Vietnamese regions and where the National Dengue Control Program began in 1999, after a large outbreak hit the 19 provinces of southern Viet Nam in 1998.[11] Initially only hospitalised DHF and DSS cases were reported, with DF added from 2005. Passive surveillance systems rely on voluntary reporting which usually leads to an underestimation of the number of cases, making accurate estimation of

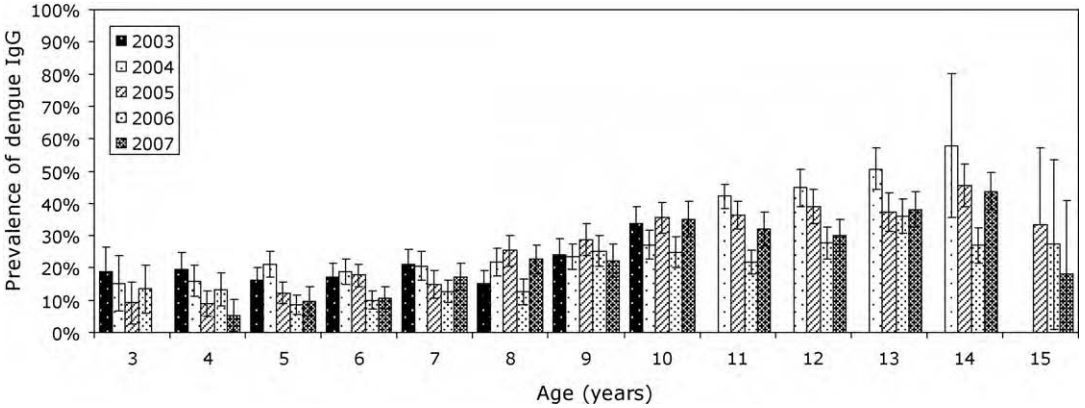

Figure 4. Age-specific seroprevalence of anti–dengue virus IgG antibodies.

disease incidence difficult.[19] Only a subset of clinically suspected cases are tested for laboratory confirmation which permits the surveillance of circulating serotypes but again does not provide accurate estimation of the dengue disease incidence that is essential in the preparation of field-sites for large-scale vaccine efficacy trials.[20,21]

In our cohort the average annual incidence rate was 28.8 acute virologically or serologically-confirmed dengue cases per 1000 person-years and 12.3/1000 severe virologically or serologically-confirmed dengue cases. These rates were compared with data obtained via the national surveillance system. The annual number of clinically-suspected DF and DHF cases reported to the surveillance system per 1000 children younger than 15 years living in southern Viet Nam (approximately 28% of the Vietnamese population, according to the 1999 census data) was 5.8 in 2004, 3.4 in 2005, 4.5 in 2006 and 6.2 in 2007. Our estimation of the incidence of acute dengue disease based on the active surveillance of febrile illness was approximately six times higher than the incidence of suspected dengue reported via the passive surveillance system in southern Viet Nam, although this comparison has limitations as different case definitions were used (laboratory-confirmed for active surveillance and clinically-suspected for passive surveillance) and the age groups differed (0–15 year-olds for passive surveillance and 2–15 year-olds for active surveillance). We have also found that the degree of under-reporting varies with disease severity (Luong Chan et al., unpublished data). Under-reporting is much higher for milder cases due to the non-specific clinical presentation which can mean that cases are not correctly diagnosed as dengue. Similar comparisons between prospective cohort data and surveillance data in Thailand and Cambodia have found that the surveillance systems in place underestimate the incidence of symptomatic dengue cases by a mean factor of 8.7 (Thailand) to 9.6 (Cambodia).[22] The rate of under-reporting (or under-detection) is highly dependant on the case definitions used, the surveillance system structure, and the local management of dengue cases, which can vary widely even within the same country.

The 6–10 year-old age group appeared to be most at-risk during the first three years of our study, and remained the second most at risk (after 11–15 year-olds) in 2007. In the report from Thailand, very young children were at lower risk and infection occurred primarily in older children due to exposure outside of the home, in particular during school time.[23] In Viet Nam primary school children (6–10 years) might similarly be expected to be at increased risk.

Most (76.8%) of the laboratory-confirmed acute dengue disease cases detected during the four years were hospitalised, although not all (43%) presented severity criteria. This may reflect a tendency to rapidly admit a child with suspected dengue into hospital for surveillance, as required in the country. It is also possible that we missed some short-lived febrile episodes not requiring medical care, or those seen at private practice, and tended to see more of the severe episodes. Indeed, despite the intense follow-up implemented in our study, children came later than expected to consultation and hospitalisation (on average four days after onset of fever). It is not possible to estimate the size of these effects. Finally, as convalescent samples were collected at discharge (i.e., an average of five days after admission and acute sample collection), it is possible that in some cases this was too soon to enable serological confirmation.

Dengue was the most common cause of hospitalisation with 42% of the hospitalisations due to laboratory-confirmed dengue. Dengue was clinically suspected in 5.4% of all consultations. When considering consultations only for acute undifferentiated fever in children <15 years old, dengue was suspected more than twice as often (12.9%) than in a similar study in southern Viet Nam in 2001–2002.[24]

We found all four serotypes to be circulating in Long Xuyen, with dengue serotype 2 predominant in 2004 and 2005 and serotype 1 in 2006 and 2007. The highest incidence rate of severe dengue was observed in 2007, which could be explained by the emergence of serotype 1 the previous year.

The annual prevalence rates of anti-dengue IgG antibodies were surprisingly low (from 19.6% to 28.7%) for an area of such high dengue transmission and appeared to contradict our incidence data, indicating a possible low sensitivity of the IgG ELISA. In contrast, antibody prevalence in 961 primary schoolchildren of another southern Vietnamese province was 65.7% in 2003 increasing from 53.0% to 88.2% with age.[25] The average annual crude incidence risk of primary dengue infection for the first four years was 11.4%, similar to the 17.3% rate reported by Thai et al. over 23 months.[26]

We found that there were three to six times as many asymptomatic dengue infections than there were dengue cases, which contrasts with a report from another school-based cohort in northern Thailand where there were no marked differences between asymptomatic and symptomatic dengue incidence.[27] In addition to the age structure that differed between the two studies (7–11 years in the Thai cohort), it is likely that we captured fewer mild, short-lived episodes than in the Thai study where cases were investigated on the first day of absenteeism. Comparisons between studies are also complicated by the fact that infecting serotype and prior infection history can influence the symptom profile.

The success of a clinical trial, in particular that of long term vaccine efficacy trials, depends, among other factors, on the stability of the study population, and how representative the study population is of the wider population. We therefore considered the low lost-to-follow-up rate as an important indication of the feasibility of conducting such a trial in this population. While a substantial number of subjects left this study to move to another, non-participating, school, a dengue vaccine efficacy trial will probably need to include more schools in the same area, thus avoiding this loss. According to Unicef data Viet Nam has a primary school attendance rate of 95%, which means that school-based cohort studies, such as ours, reliably capture the paediatric population.

These results confirm the high transmission of dengue in school children in Long Xuyen, with a variability of incidence over the years and a switch in circulating serotypes. Despite some limitations, the active surveillance of febrile

illness lead to a much higher incidence of dengue disease than reported by passive surveillance even in years of low transmission and all four serotypes were isolated, although the distribution changed. With a dengue vaccine becoming an increasingly real prospect, sites such as Long Xuyen demonstrate their suitability for a large scale efficacy trial.

Authors' contributions: CL, NTKT, JL and LPG designed the study; NTKT, NTT, VTQH, PVB, NNR and T-AW carried out the data collection; CL, LPG examined, analysed and interpreted the data; LPG and CL drafted the manuscript. All authors read, revised and approved the final manuscript. LPG and CL are guarantors of the manuscript.

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Conflicts of interest: CL, LPG, T-AW and JL are employees of Sanofi Pasteur.

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