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DETECTION OF ASYMPTOMATIC DENGUE INFECTION

HEALTH TECHNOLOGY ASSESSMENT SECTION
MEDICAL DEVELOPMENT DIVISION
MINISTRY OF HEALTH MALAYSIA

008/2018

DISCLAIMER

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DISCLOSURE

The author of this report has no competing interest in this subject and the preparation of this report is totally funded by the Ministry of Health, Malaysia.

EXECUTIVE SUMMARY

Background

Dengue fever is an important mosquito-borne human viral disease globally caused by the infections of four dengue virus serotypes (DENV 1-4). The incidence of dengue has increased 30-fold in the past 50 years. Presentations of dengue range from asymptomatic, mild febrile illness to life-threatening conditions including severe bleeding, organ dysfunction, and shock. It has been estimated that dengue resulted in 58.4 million symptomatic cases and was responsible for 1.14 million disability-adjusted life-years in 2013. The estimated population at risk of DENV infection worldwide is around 3.97 billion people. It has been a known fact that the majority of DENV infections are clinically inapparent. Transient dengue viremia can occur either in symptomatic cases before onset of clinical illness or in people with inapparent infection.

Detection of asymptomatic dengue infection is difficult and challenging. While symptomatic dengue can be clinically suspected and then the confirmatory laboratory diagnosis can provide definite diagnosis, there is no clinical clue for asymptomatic infection. Detection of asymptomatic dengue infection is therefore based on laboratory diagnosis. The currently used diagnostic test for dengue infection can be divided into virologic/ molecular based [virus Isolation (cell culture), nucleic acid hybridization, reverse transcriptase –polymerase reaction (RT-PCR)], antigen based (NS1) and serologic based [haemaglutination-inhibition (HAI), Enzyme linked immunosorbent immunoassay (ELISA) and plaque reduction neutralization test (PRNT)]. Since viremia occurs for only a short period (one to two days before onset of symptom and up to five to seven days after onset of symptom), the virologic/ molecular/ antigen based are applicable only in symptomatic infection. Therefore, serologic methods to detect rising in dengue antibody in asymptomatic persons are more convenient.

It is claimed that the burden of asymptomatic dengue infection is high and it may play a role in dengue transmission. Apart from that, methods for detecting asymptomatic infection [including the diagnostic tests used and blood sampling] depend on the objectives, budgets, the level of accuracy needed and the feasibility.

This technology review was requested by Head of Vector Borne Disease Sector, Disease Control Division, Ministry of Health to look into evidence on asymptomatic dengue infection in terms of its' burden, detection and transmission.

Objective/aim

The objective of this technology review was to evaluate the effectiveness, cost-implication, safety and organisational issues of detecting asymptomatic dengue infection.

Results and conclusions

A total of 143 records were identified through the Ovid interface and PubMed, and two were identified from other sources (references of retrieved articles). There were 19 full text articles included in this review comprised of two diagnostic accuracy studies, four cohort studies and 13 cross sectional studies. The studies were conducted in Vietnam, Thailand, China, Saudi Arabia, Pakistan, India, Malaysia, Taiwan and Singapore.

There was very limited diagnostic accuracy studies retrieved for detecting asymptomatic dengue infection. The accuracy of NS1 in detecting asymptomatic dengue infection could not be determined due to limited number of study (one study) with limited sample size (17 individuals). Indirect ELISA was reported to have accuracy of 83% in detecting symptomatic and asymptomatic dengue infection compared to gold standard test ($PRNT_{50}$). Serology test involving ELISA were frequently used in incidence and seroprevalence studies of asymptomatic dengue infection.

Generally, the incidence of asymptomatic dengue infection was found to be higher compared to symptomatic dengue infection. Inapparent to symptomatic (I:S) ratio ranged from 0.9:1 to 2.5:1. However, viral load or viraemia level in asymptomatic patients was found to be lower compared to symptomatic patients.

Very limited evidence retrieved to suggest that several factors such as symptomatic dengue incidence and dengue serotype circulation affects the incidence of inapparent and symptomatic dengue infection among school children.

There was also very limited evidence retrieved to suggest that asymptomatic and pre-symptomatic DENV-infected people were more infectious to mosquitoes compared to symptomatic people. However, there was no retrievable evidence on transmission of dengue virus from mosquitoes to human among asymptomatic infection.

There was no evidence retrieved on the cost-effectiveness, safety and organisational issues on detection of asymptomatic dengue infection.

Methods

Electronic databases were searched through the Ovid interface: Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Ovid MEDLINE® Daily and Ovid MEDLINE® 1946 to Present, EBM Reviews - Cochrane Central Register of Controlled Trials - December 2018, EBM Reviews - Cochrane Database of Systematic Reviews - 2005 to December 2018, EBM Reviews - Health Technology Assessment – 4th Quarter 2018, EBM Reviews – NHS Economic Evaluation Database 4th Quarter 2018. Searches were also run in PubMed database and U.S. Food and Drug Administration (USFDA) website. Google and Google Scholar was also used to search for additional web-based materials and

information. Additional articles were identified from reviewing the references of retrieved articles. Last search was conducted on 13th January 2019.

DETECTION OF ASYMPTOMATIC DENGUE INFECTION

1. BACKGROUND

Dengue fever is an important mosquito-borne human viral disease globally caused by the infections of four dengue virus serotypes (DENV 1-4). The incidence of dengue has increased 30-fold in the past 50 years. The clinical presentations of dengue range from asymptomatic, mild febrile illness to life-threatening conditions including severe bleeding, organ dysfunction, and shock. It has been estimated that dengue resulted in 58.4 million symptomatic cases and was responsible for 1.14 million disability-adjusted life-years in 2013. The estimated population at risk of DENV infection worldwide is around 3.97 billion people. It has been a known fact that the majority of DENV infections are clinically inapparent. Transient dengue viremia can occur either in symptomatic cases before onset of clinical illness or in people with inapparent infection. [1]

Since the year 2000, the dengue incidence in Malaysia continues to increase from 32 cases per 100,000 population to 260 cases per 100,000 population in 2017. Ministry of Health has already reported about 43,500 dengue cases throughout the first quarter of year 2019.[2]

In dengue infection, immunoglobulin M (IgM) is produce approximately five days after infection in both primary and secondary infections, while immunoglobulin G (IgG) is produce about two to four weeks after onset of primary infection and almost immediately after onset of a secondary infection. Seroconversion occurs approximately three to seven days following exposure and, therefore, testing of acute and convalescent sera may be necessary to make the diagnosis.[3]

People with inapparent dengue virus infections are generally considered dead-end host for transmission because they do not reach sufficiently high viraemia levels to infect mosquitoes. However, there is a concern that transmission of dengue virus to mosquitoes was occur. [4]

Detection of asymptomatic dengue infection is difficult and challenging. While symptomatic dengue can be clinically suspected and then the confirmatory laboratory diagnosis can provide definite diagnosis, there is no clinical clue for asymptomatic infection. Detection of asymptomatic dengue infection is therefore based on laboratory diagnosis. The currently used diagnostic test for dengue infection can be divided into virologic/ molecular based [virus Isolation (cell culture), nucleic acid hybridization, reverse transcriptase–polymerase chain reaction (RT-PCR)], antigen based (NS1) and serologic based [haemaglutination-inhibition (HAI), Enzyme linked immunosorbent immunoassay (ELISA) and plaque reduction neutralization test (PRNT)]. Since viremia occurs for only a

short period (one to two days before onset of symptoms and up to five to seven days after onset of symptoms), the virologic/ molecular/ antigen based are applicable only in symptomatic infection. Therefore, serologic methods to detect rising in dengue antibody in asymptomatic person are more convenient.[5]

It is claimed that the burden of asymptomatic dengue infection is high and it may play a role in dengue transmission. Apart from that, methods for detecting asymptomatic infection [including the diagnostic tests used and blood sampling] depend on the objectives, budgets, the level of accuracy needed and the feasibility.[5]

This technology review was requested by Head of Vector Borne Disease Sector, Disease Control Division, Ministry of Health to look into evidence on asymptomatic dengue infection in terms of its' burden, detection and transmission.

2. OBJECTIVE / AIM

The objective of this technology review was to evaluate the effectiveness, cost-implication, safety and organisational issues of detecting asymptomatic dengue infection.

3. TECHNICAL FEATURES

An inapparent infections occurs when a person is infected with a pathogen but remains asymptomatic. The proportion of inapparent infections among those infected with the disease is commonly called inapparent/ asymptomatic to symptomatic ratio (I:S) or vice versa (S:I). Knowing the I:S ratio for a disease allows researchers to turn observed disease incidence into estimates of actual incidence. This process can yield pos hoc estimates for how many individuals were infectious during (and immune after) an endemic. This information in turn can guide public health control measures and modelling disease spread.[6]

The asymptomatic dengue infection is difficult to diagnose without laboratory tests. Several types of asymptomatic dengue testing are available as below and illustrated as in figure 1, figure 2 and figure 3: [7,8]

i) PCR/RT-PCR

Using PCR test, virus can be isolated and sequenced for additional characterization. Furthermore, RT–PCR assays have been developed. Because antibodies are detected later, RT–PCR has become a primary tool to detect virus early in the course of illness. Current tests are between 80-90% sensitive, and more that 95% specific. A positive PCR result is a definite proof of current infection and it usually confirms the infecting serotype as well. However, a negative result is interpreted as "indeterminate". Patients receiving negative results before five days of

illness are usually asked to submit a second serum sample for serological confirmation after the fifth day of illness.

ii) IgM ELISA

IgM antibody capture ELISA (MAC-ELISA) format is most commonly employed in diagnostic laboratories and commercial available diagnostic kits. The assay is based on capturing human IgM antibodies on a microtiter plate using anti-human-IgM antibody followed by the addition of dengue virus specific antigen (DENV1-4). The antigens used for this assay are derived from the envelope protein of the virus. One of the limitation of this testing is the cross reactivity between other circulating flaviviruses. This limitation must be considered when working in regions where multiple flaviviruses co-circulate. IgM detection is not useful for dengue serotype determination due to cross-reactivity of the antibody and the IgM only appears within short period of time.

iii) IgG ELISA

The IgG ELISA used for the detection of a past dengue infection utilizes the same viral antigens as the IgM ELISA. In general IgG ELISA lacks specificity within the flavivirus serocomplex groups, cross-reacting with other flaviruses and not useful for dengue serotype identification. However they are useful for seroepidemiological studies. Samples with a negative IgG in the acute phase and a positive IgG in the convalescent phase of the infection are primary dengue infections. Samples with a positive IgG in the acute phase and a four-fold rise in IgG titer in the convalescent phase (with at least a seven days interval between the two samples) is a secondary dengue infection.

iv) NS1 ELISA

The non-structural protein 1 (NS1) of the dengue viral genome has been shown to be useful as a tool for the diagnosis of acute dengue infections. Dengue NS1 antigen is detected in the serum of DENV infected patients as early as 1 day post onset of symptoms (DPO), and up to 18 DPO. The NS1 ELISA based antigen assay is commercially available for DENV and many investigators have evaluated this assay for sensitivity and specificity. The NS1 assay may also be useful for differential diagnostics between flaviviruses because of the specificity of the assay.

v) PRNT

Plaque Reduction and Neutralization Test (PRNT) and the microneutralization PRNT can be used when a serological specific diagnostic is required, as this assay is the most specific serological tool for the determination of dengue antibodies (gold standard for serology testing). The PRNT test is used to determine the infecting serotype in convalescent sera. This assay measures the titer of the neutralizing antibodies in the serum of the infected individual and determines the level of protective antibodies this individual has towards the infecting virus. The

assay is a biological assay based on the principle of interaction of virus and antibody resulting in inactivation of virus such that it is no longer able to infect and replicate in cell culture. Some of the variability of this assay is differences in interpretation of the results because of the cell lines and virus seeds used as well as the dilution of the sera.

vi) HAI

The hemagglutination inhibition (HAI) is very useful for differentiating primary and secondary infections. The antibody titers of convalescent phase samples of a primary infection are usually below 1:640 while in a secondary or tertiary infection the titre is usually higher than 1:5120. However, it does not allow discrimination between infections of other flaviviruses.

v) Combination Assays

The combined use of antigen and antibody assays have overall resulted in increased ability for diagnosis. Using more than one marker has resulted in confirmation of cases especially where only one sample is obtained. These kits are increasingly being used especially the rapid tests which allow diagnosis within 20 minutes with as little as 100ul of whole blood.

Figure 1: 96 well ELISA microtitre plate

Figure 2: Dengue NS1

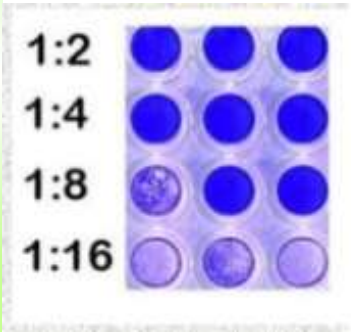

Figure 3: PRNT test

4. METHODS

4.1. Searching

Electronic databases were searched through the Ovid interface: Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Ovid MEDLINE® Daily and Ovid MEDLINE® 1946 to Present, EBM Reviews - Cochrane Central Register of Controlled Trials - December 2018, EBM Reviews - Cochrane Database of Systematic Reviews - 2005 to December 2018, EBM Reviews - Health Technology Assessment – 4th Quarter 2018, EBM Reviews – NHS Economic Evaluation Database 4th Quarter 2018. Searches were also run in PubMed database and U.S. Food and Drug Administration (USFDA) website. Google and Google Scholar was also used to search for additional web-based materials and information. Additional articles were identified from reviewing the

references of retrieved articles. Last search was conducted on 13th January 2019.

Appendix 1 showed the detailed search strategies.

4.2. Selection

A reviewer screened the titles and abstracts against the inclusion and exclusion criteria and then appraise the full text articles for final article selection.

The inclusion and exclusion criteria were:

Inclusion criteria

Population	Asymptomatic dengue person, inapparent dengue infection, healthy population
Interventions	NS1, ELISA, PRNT, HAI, and other methods in detecting asymptomatic dengue
Comparators	Gold standard (either for molecular or serologic test), current practice, no comparator
Outcomes	• Effectiveness: Sensitivity, specificity, negative predictive value (NPV), positive predictive value (PPV), correlation, agreement, bias, asymptomatic dengue incidence seroprevalance of dengue infection, vireamia level • Economic implication • Safety • Organisational issue
Study design	Diagnostic accuracy study, Systematic Review (SR), Health Technology Assessment (HTA), economic evaluation study, Randomised Controlled Trial (RCT) or non-randomised controlled trial, cohort study, pre- and post-intervention study , cross sectional study, case series, case report
	English full text articles

Exclusion criteria

Study design	Studies conducted in animals, narrative reviews
	Non English full text articles

Relevant articles were critically appraised using Critical Appraisal Skills Programme (CASP) and graded according to the NHS Centre for Reviews and Dissemination (CRD) University of York, Report Number 4 (2nd Edition) and US/Canadian preventive services task force (Appendix 2). Data were extracted and summarised in evidence table as in Appendix 3.

5. RESULTS AND DISCUSSION

A total of 143 records were identified through the Ovid interface and PubMed, and two were identified from other sources (references of retrieved articles). After removal of 38 duplicates, 107 records were screened and 76 were excluded. Of these, 31 relevant abstracts were retrieved in full text. After reading, appraising and applying the inclusion and exclusion criteria to the 31 full text articles, 19 full text articles were included and 12 full text articles were excluded. The articles were excluded due to irrelevant study design (n=7), irrelevant population (n=4), irrelevant outcome (n=1). Flow chart of study selection is shown in figure 3.

There were 19 full text articles which comprised of two diagnostic accuracy studies, four cohort studies and 13 cross sectional studies finally selected for this review. The studies were conducted in Vietnam, Thailand, China, Saudi Arabia, Pakistan, India, Malaysia, Taiwan and Singapore.

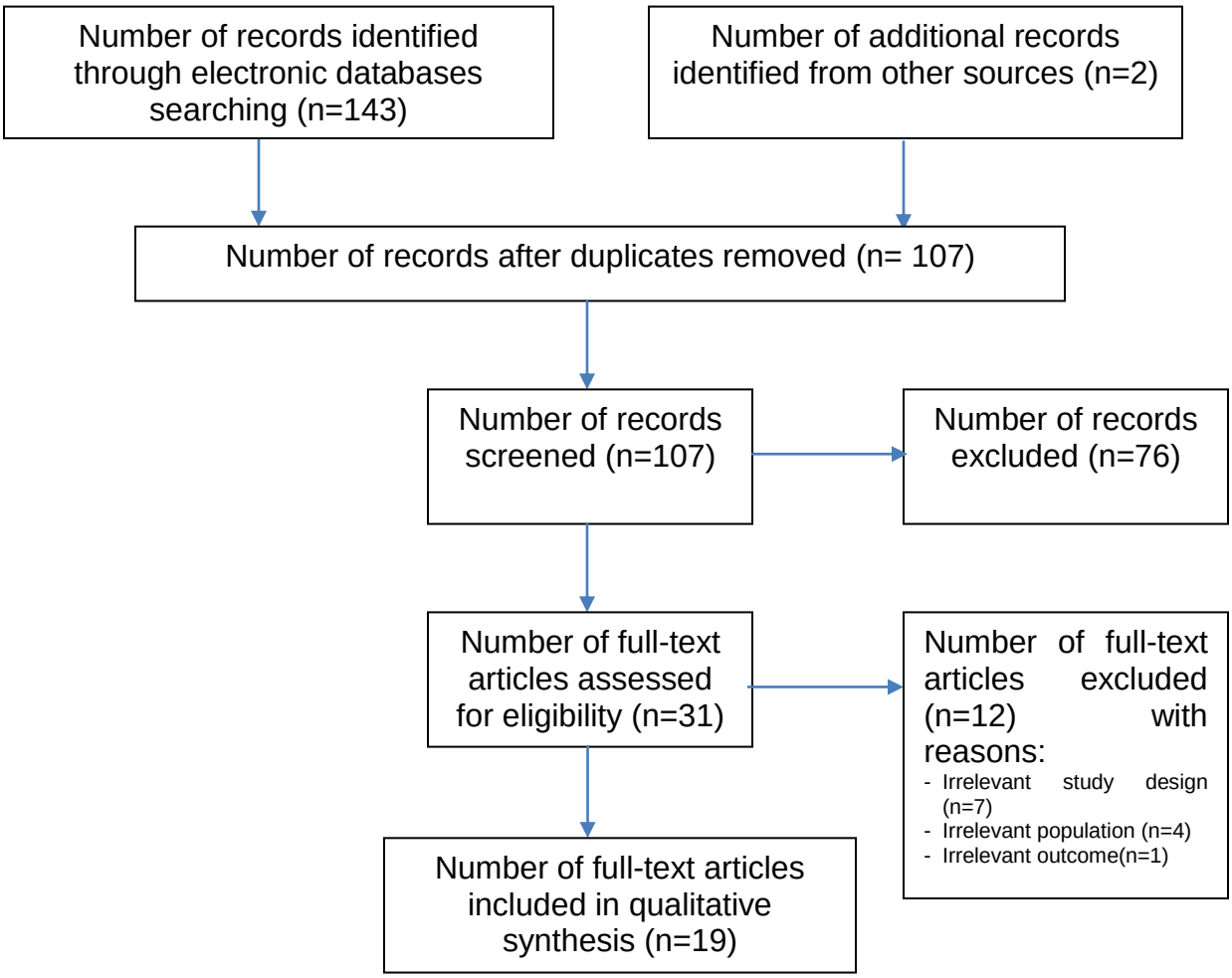

Figure 3: Flow chart of study selection

Risk of bias

One of the tools that are being used by MaHTAS to assess the risk of bias is the CASP checklist which consists of eight critical appraisal tools designed for SR, RCT, cohort studies, case control studies, economic evaluations, diagnostic accuracy studies, qualitative studies and clinical prediction rule. This is achieved by answering a pre-specified question of those criteria assessed and assigning a judgement relating to the risk of bias as either “Yes” indicates low risk of bias, “No” indicates high risk of bias, and “Can’t tell” indicates unclear or unknown risk of bias.

+	Indicates YES (low risk of bias)
?	indicates UNKNOWN risk of bias
-	Indicates NO (high risk of bias)

Figure 4 shows risk of bias of the two diagnostic accuracy studies included. Two studies were not clear regarding blinding.

Criteria assessed	Was there a comparison with an appropriate reference standard?	Did all patients get the diagnostic test and reference standard?	Could result of the test have been influenced by the results of the reference standard? - blinding	Disease status of tested population clearly described?	Methods performing the test described in sufficient detail? – Protocol followed
Duong V et al. 2011	+	+	?	+	+
Catchen S et al. 2016	+	+	?	+	+

Figure 4: Assessment of risk of bias of diagnostic study (CASP)

Figure 5 shows risk of bias of the four cohort studies included. One study was unclear regarding confounding factors involved.

Criteria assessed

	Selection of cohort	Exposure accurately measured	Outcome accurately measured	Confounding factors	Follow-up of subjects
Endy TP et al. 2002	+	+	+	+	+
Endy TP et al. 2011	+	+	+	+	+
Yoon IK et al. 2012	+	+	+	?	+

Mammen MP Jr et al. 2008	+	+	+	+	+

Figure 5: Assessment of risk of bias of cohort (CASP)

5.1. EFFECTIVENESS

5.1.1 DIAGNOSTIC ACCURACY

Duong V et al. (2011) conducted a diagnostic accuracy study to evaluate the clinical and virological factors influencing the performance of Platelia NS1 Ag kit (Biorad) and to assess the potential use of NS1 Ag as dengue viral load markers of dengue severity. Blood specimen was collected from patients hospitalised at the Kampong Cham hospitals during the 2006 and 2007 dengue endemics in Cambodia. Serum were tested for dengue using serology method [Anti-dengue virus (DENV)-specific IgM, Hemagglutination inhibition (HAI)], antigen detection method [NS1 antigen] and molecular methods DENV isolation, RT-PCR. However, the method how they carried out diagnostic accuracy study was not clearly mentioned. Family members of dengue-infected patients were visited the next day following laboratory confirmation of dengue infection (which usually took approximately 24 hours) to identify non-symptomatic cases. A non-symptomatic dengue case was defined as a household member who tested positive for dengue infection but did not display any of the symptoms. They reported that, among 15 dengue index cases (DIC), 214 household members were identified. Of the 214 household members, 17 (8%) did not experience any symptoms while 2 (1%) were symptomatic (the rest of the household members was not mentioned in the article). Sensitivity of NS1 test was significantly lower in asymptomatic individuals than that in DIC (35.3% versus 86.7%, p =0.003). Asymptomatic individuals also had lower incidence of secondary infection compared to DIC group (73% versus 100% respectively, p=0.063). Meanwhile, nested RT-PCR detection was reported to be significantly more sensitive than NS1 antigen-capture assay (76.5% versus 35.3%, p=0.015) in asymptomatic individuals. The author's concluded that, overall sensitivity of NS1 Ag detection kit varied widely across the various forms of dengue infection or disease. In asymptomatic patients, RT-PCR assay has proved to be more sensitive than NS1 antigen detection.[9,level 2]

Catchen S et al. (2016) developed indirect ELISA test based on monoclonal immunoglobulin against all four DENV types. They conducted a diagnostic accuracy study to evaluate the test for diagnosis of asymptomatic dengue infection in paired annual samples compared to PRNT (gold standard). Serum samples were obtained from the cohort epidemiology study of dengue infection in school children in Ratchaburi Province, Thailand during 2006 to 2009. The cohort recruited 3000 primary school children. There were 333 RT-PCR proved dengue episodes out of 5842 febrile episodes. Twenty two paired annual samples

from children who had symptomatic RT-PCR proven dengue infection were randomly selected from the whole cohort and tested for both PRNT and indirect ELISA. Meanwhile, for children who had no history of dengue infection, indirect ELISA test was performed on totally 5513 paired serum sample. Forty paired serum samples from who had risen in ELISA titre (ratio >1.5) and 32 paired serum samples from those who had no rise in ELISA titre (totally 72 paired sera) were randomly selected to test for $PRNT_{50}$ (concentration of serum to reduce the number of plaques by 50% compared to the serum free virus). Total annual serum samples from children aged four to 11 years old were tested to estimate the incidence of asymptomatic dengue. They reported that, correlation between indirect ELISA and $PRNT_{50}$ were $r=0.736$ ($p<0.001$). Sensitivity, specificity and accuracy for indirect ELISA (regardless asymptomatic or symptomatic infection) was 87.5%, 78.3% and 83.0% respectively. For the incidence of asymptomatic dengue, they reported that 8.9% (489/5513) of the population were asymptomatic dengue infection detected using the indirect ELISA test. The asymptomatic or inapparent: symptomatic (I:S) ratio in that period was 2.5:1.[10, level 2]

5.2 INCIDENCE

A cohort study was conducted by Endy TP et al. (2002) to study the epidemiology and immunology of inapparent to severe dengue disease and to identify risk factors for developing severe disease after acquiring a secondary dengue infection. However, the article focused only on epidemiology of inapparent and symptomatic dengue virus infections, and clinical presentation of dengue virus infection during the first three years of study. The study was conducted on 12 primary school children (n= 2119) in Kamphaeng Phet Province, Thailand. Children were recruited during January 1998 from grade one to five. Children were eligible to remain in the study until graduation (sixth grade). New first grade students were enrolled in the cohort each January subsequent year. Baseline demographic were evaluated three times a year (June 1, August 15 and November 15). Meanwhile, case surveillance were conducted for active illness which occurred during the dengue season from June 1 to November 15. Acute illness which due to dengue virus infection was identified on the basis of absence from school or visit to the school nurse. Blood samples were tested for dengue virus antigen and antibody using heamaglutination inhibition (HAI) assay and the IgM/ IgG immunoassay (EIA). Inapparent (subclinical) dengue virus infection was defined as fourfold rise in HAI antibody against any dengue virus serotype between two sequential sera samples obtained during the surveillance months (June, August, or November). Sera was tested concurrently for Japanese encephalitis to exclude the cross-reactivity infection.[11, level II-2]

They reported that, for the three years of study, overall incidence of dengue infection was 5.8%. The incidence of inapparent and symptomatic

dengue infection was 3.1% and 2.7% respectively. The incidence of symptomatic non-hospitalized dengue, hospitalized dengue fever, and hospitalized dengue hemorrhagic fever was 2.1%, 0.2%, and 0.3%, respectively. There were no fatal cases of dengue. Of the symptomatic cases of dengue infection, 6/154 (3.9%) were primary dengue virus infections. Majority of inapparent dengue cases occurred during the June 1 to August 15 interval in 1998, 1999 and 2000. The ratio of inapparent to symptomatic (I:S ratio) dengue virus infection was 1.2 in 1998, 0.9 in 1999, and 1.8 in 2000. The I:S ratio for dengue infection was not correlated with total dengue incidence nor to hospitalization rates ($r = -0.03$, $p = 0.9$, Pearson's correlation, two tailed and $r = 0.3$, $p = 0.08$, Pearson's correlation, two tailed, respectively). Other factors that did not differ among children with inapparent or more severe dengue infections were mean age, sex, and history of Japanese encephalitis vaccination (data was not shown). The authors' concluded that the study illustrate the spatial and temporal diversity of dengue virus infection and the burden of dengue disease in school children in Thailand.[11, level II-2]

Endy TP et al. (2011) conducted another cohort study to report spatial and temporal trends of the I:S ratio in the dengue infection in order to determine epidemiologic factors associated with the variations. A five year (1998 to 2002) longitudinal cohort and geographic cluster study was undertaken in rural Thailand. Cohort of school children underwent pre- and post-season serology and active school absence-based surveillance to detect inapparent and symptomatic dengue. The laboratory assays used to detect acute dengue infection was PCR, HAI and anti-dengue IgM/IgG EIA. Inapparent (subclinical) dengue infection was defined as a four-fold rise in HAI antibody against any virus serotype between two sequential sera obtained during the surveillance month (June, August or November). Only symptomatic and inapparent dengue infections that were detected between June and November each year were included in the analysis. A total 2044 students were enrolled in 1998, 1915 students in 1999, 2203 students in 2000, 2011 students in 2001 and 1759 students in 2002. They reported that, a total of 1024 dengue virus infection was detected in over five years study (909 children experienced at least one dengue infection and 115 experienced second infection). However, when analyses was restricted to 615 infections detected during active surveillance period (June to November), 66% (406) of them were inapparent and 34% (209) were symptomatic. Overall, one-year incidence (from 1998 to 2002) of symptomatic dengue infection ranged from 5.2% to 17.5% (with 58.0% to 81.3% of those infection detected during the active surveillance period were inapparent). There was a significant annual variation of the Symptomatic to Inapparent (S:I) ratio ($p<0.0001$ by Pearson chi-square) with a relatively severe year noted in 2001 reported.[12, level II-2]

Yoon IK et al. (2012) conducted a cohort study to provide detail analysis of the full four years prospective study which include transition in the predominant circulating DENV serotype in rural Thailand. A total of 10487 person-seasons cohort children underwent pre- and post-season serology and active school absence–based surveillance to detect inapparent and symptomatic dengue. Cluster investigations were triggered by cohort dengue and non-dengue febrile illnesses (positive and negative clusters, respectively). Inapparent DENV infection was defined by paired pre- and post-season blood samples that showed a rise in dengue HAI and PRNT titers but was not associated with a symptomatic DENV infection between May and January. They reported that, there were a total of 189 confirmed symptomatic DENV infections with average incidence of 2.3% per season. Meanwhile, there were a total of 346 (3.3%) inapparent dengue infections and 20 (0.2%) clinically unclassified infections in the cohort with average incidence of 6.9% per season. Inapparent to symptomatic (I:S) ratio was 1.8:1 for the entire study ranging from 1.1:1 to 2.9:1.[13,level II-3]

Another cohort study was conducted by Mammen MP Jr et al. (2008) comparing the geographic and temporal characteristics within Thai villages where DENV were and were not being actively transmitted. The objective was to test the hypothesis that DENV transmission is spatially and temporally focal. Cluster investigations were conducted within 100 m of homes where febrile index children with (positive clusters) and without (negative clusters) acute dengue lived during two seasons of peak DENV transmission. One of the outcome measure was burden of inapparent and symptomatic infections. A total of 2215 children were enrolled in a school cohort and followed using the active based surveillance (conducted in June and November of 2004 and 2005) for febrile illness. Positive clusters were triggered by index cases with laboratory-confirmed dengue viremia determined within 24 hours of fever presentation while negative clusters by a febrile child without dengue viremia (and subsequent evidence of lack of DENV seroconversion). Dengue virus infections were identified by a dengue IgM/IgG ELISA. Dengue viremia and serotype were determined by RT-PCR/nested PCR. Dengue cases were classified as inapparent (lack of subjective and objective fever, defined as ≥ 38°C) or symptomatic dengue. They reported that, of the 1,204 febrile children (506 in 2004 and 698 in 2005) who provided blood specimens, 48 (28 in 2004 and 20 in 2005) had detectable DENV viremia. Among the 556 village enrollees (217 in positive and 339 in negative clusters), 27 DENV infections were detected during the 15 days follow-up period. Of the 27 DENV infections among village enrolees (14 inapparent and 13 symptomatic). Inapparent infections were more likely with primary [five out of six (83.3%)] than secondary [seven out of 19 (36.8%)] DENV infections ($p = 0.05$; Pearson's Chi2 test). Proportion of primary infection to secondary infection was 2.26:1. [14,level II-2]

Wang T et al. (2015) conducted a cross sectional study to better understand the dengue virus infection spectrum and to estimate the I:S ratio during an outbreak in Southern China in 2013. During the outbreak, an investigation of 887 index cases contacts was conducted to evaluate inapparent and symptomatic DENV infections. Samples collected from individuals who reported no dengue-like symptoms were tested for IgM and IgG ELISA. During post-outbreak, an additional 817 subjects from four towns and 350 subjects from two towns (with and without reported indigenous DENV transmission respectively) were evaluated for serological IgG antibodies. From the study they reported that, of 887 case contacts enrolled during the outbreak, 13 (1.5%) exhibited symptomatic DENV infection, while 28 (3.2%) were inapparent. The overall I:S ratio was 2.2:1 (95% CI:1.1,4.2:1). Meanwhile, post-outbreak serological data showed that the proportion of DENV IgG antibody detection from four towns with and the two towns without reported DENV transmission was 2.7% (95% CI: 1.6%, 3.8%) and 0.6% (95% CI: 0, 1.4%) respectively. The authors concluded that, there was a high I:S ratio during a documented outbreak in Zhongshan, Southern China.[15, level II-3]

5.3 SEROPREVALENCE

Vikram K et al. (2016) conducted a cross sectional study to investigate and assess the epidemiology of dengue infection and to estimate the proportion of both asymptomatic and symptomatic dengue infections in Delhi. A total of 2125 individuals as household and neighbourhood contacts (from 50 confirmed dengue cases) with or without dengue febrile illness were finger pricked and serologically tested for NS1, IgM and IgG dengue using SD duo Bioline rapid diagnostic test (SD, Korea). They reported that, out of 2125 individuals, 768 (36.1%) individuals showed positive dengue test with past (25.5%), primary (1.88%) or secondary (8.8%) dengue infections. Higher percentage of IgG was found in age groups 15 to 24 years and 25 to 50 years (36% each). Infants (<1 year) presented higher incidence of new infections (22% of NS1 + IgM positives) as compared to adults. Further analysis revealed that out of the 226 newly infected cases (including NS1 and IgM positives), 142 (63%) [NS1 positive = 13 person, IgM positive =19 person, IgM + IgG positive = 105 person, NS1 + IgG positive = 1 person and NS1 + IgM + IgG = 4 person] were asymptomatic and 84 (37%) were symptomatic. They concluded that, on the basis of the results, it may be hypothesised that there are large number of asymptomatic dengue infections in the community as compared to reported symptomatic cases in Delhi. For the effective control of dengue transmission in such community like Delhi where dengue epidemics have frequently been encountered, it is essential to ascertain the proportion of asymptomatic dengue infections which may act as a reservoir for dengue transmission, as well as threat for developing dengue haemorrhagic fever (DHF).[16, level II-3]

Janjoom GA et al. (2016) conducted a cross sectional study to measure seroprevalence of past dengue virus infection in healthy Saudi nationals from different areas in the city of Jeddah and to investigate demographic and environmental factors that may increase exposure to infection. Serum was collected from 1984 subjects attending primary healthcare centres in six districts of Jeddah. General patients of various ages seeking for routine vaccination, antenatal care or treatment of different illness were included in the study. Patients with fever or suspected dengue were excluded. A number of blood donors were also tested. Serum sample was tested by enzyme immunoassay (EIA) for IgG antibodies to dengue viruses 1,2,3,4. Questionnaire was completed for each patient recording various anthropometric data and factors that may indicate possible risk of exposure to mosquito bites and dengue infection. They reported that the overall prevalence of dengue virus infection as measured by anti-dengue IgG antibodies from asymptomatic in residents of Jeddah was 47.8% (927/1938) and in blood donors was 37% (68/184). Anti-dengue seropositivity increased with age and was higher in males than females [odd ratio (OR) male versus female was 1.374 (95%CI: 1.139, 1.658), p=0.001] and in residents of communal housing and multi-storey building than villas (p=0.004). One of the six districts (Al-Aziziah District) showed significant increase in exposure rate compared to others [OR was 1.326 (95% CI: 1.093, 1.607), p=0.004). Availability of public sewage was associated with lower infection was inconclusive [OR was 1.210 (95% CI: 0.996, 1.471), p=0.052]. However, infection was not related to history of travelling [OR for subjects who did not travel outside country versus who had travelled was 1.869 (95% CI: 1.092, 3.198), p=0.022]. The authors concluded that there was a relatively high exposure of Jeddah residents to infection and remained asymptomatic.[17, level II-3]

Ashshi AM et al. (2017) conducted a cross sectional study to estimate seroprevalence of asymptomatic DENV infection and antibodies among eligible Saudi blood donors. Serum samples from 910 healthy/eligible adult male Saudi blood donors, who reside in Holy Makkah were collected between March 2015 and August 2016. The samples were screened for the DENV non-sctructural protein 1 (NS1) antigen and anti DENV IgM and IgG antibodies using commercial ELISA kit (Panbio, Brisbane). They found that, there were 48 (5.3%) seropositive for DENV-NS1 antigen, 50 (5.5%) seropositive for IgM antibodies and 354 (38.9%) seropositive for IgG antibodies among the tested donors. Seropositivity for DENV-NS1 antigen and/or anti-DENV IgM antibody among the tested donors reflects their ongoing asymptomatic vireamic infectious stage with DENV during their donation time, whereas high prevalence of anti-DENV IgG seropositivity reflects the high endemicity of dengue disease in this region of Saudi Arabia. The authors concluded that, there was a high prevalence of asymptomatic DENV infection and its antibodies among Saudi blood donors.[18, level II-3]

Mahmood S et al. (2013) conducted a cross-sectional study to determine seroprevalence of anti-dengue IgG in healthy adult population of Lahore. A total of 274 individuals aged 15 years old and above were randomly selected using multistage sampling technique. The individuals were interviewed between July to September 2012 using semistructured questionnaire and followed by drawing 3mL of venous blood for dengue IgG test (Nova Tech ELISA kit). They reported that 67.2% (184/274) were found to be positive for dengue IgG antibodies. Seroprevalence was higher among individuals with poor awareness about potential breeding sites for dengue mosquito (63.6%), followed by subjects who had poor knowledge about dengue signs/symptoms and complications (52.2% and 68.5% respectively). The authors concluded that about two-third of healthy population of Lahore was also seropositive for anti-dengue IgG during July to September 2012, indicating a considerable burden of subclinical dengue infection in the city.[19, level II-3]

Rafique I et al. (2017) conducted a cross sectional study in five major cities of Pakistan (Islamabad, Karachi, Lahore, Multan and Peshawar) to determine the asymptomatic dengue infection in adults of Pakistani population. A total of 5230 adults aged 18 years and above without a history of dengue fever at any point in their life were enrolled from participating laboratories. Those who were confirmed for dengue previously were excluded. Participants were briefed about the objectives of the study, a written consent was obtained to perform dengue IgG test using ELISA. The brief information related to age, gender and area was also taken from profoma. They reported that, overall 32.3% (n=1691) was having asymptomatic dengue infection which was 67.5% (n=756) in Karachi followed by 39.1% (n=391) in Islamabad, 29.9% (n=316) in Lahore and 21 % (n=228) in Peshawar and none from Multan. More males (34.5%) were affected with asymptomatic dengue infection than females (31%).However, the different was not significant (X^2= 4.405, p=0.353). Meanwhile, asymptomatic infection was significantly higher in different cities (X^2=243.81, p<0.001). The authors concluded that, asymptomatic dengue infection is higher in cities i.e. Karachi, Islamabad and Lahore which are at risk of developing secondary dengue infections. There is a need of awareness among the public about secondary dengue infection.[20, level II-3]

Dhanoa A et al. (2018) conducted a cross sectional seroprevalence study to establish DENV seroprevalence amongst healthy adults in a rural district in Southern Malaysia, and to identify influencing factors. The study was conducted between April and May 2015. A total of 277 adult participants were recruited from households across three localities in the Sungai Segamat subdistrict in Segamat district. Sera were tested for immunoglobulin G (IgG) (Panbio® Dengue Indirect IgG ELISA/high-titre

capture) and immunoglobulin M (IgM) (Panbio®) antibodies. The plaque reduction neutralization test (PRNT) was conducted on random samples of IgG positive sera for further confirmation. Medical history and a recall of previous history of dengue were collected through interviews, whereas sociodemographic information was obtained from an existing database. They reported that, overall seroprevalence for DENV infection was 86.6% (240/277) (95% CI: 83, 91%). Serological evidence of recent infection (IgM/high-titre capture IgG) was noted in 11.2% (31/277) of participants, whereas there was evidence of past infection in 75.5% (209/277) of participants (indirect IgG minus recent infections). For the recall of dengue history, only 12.9% (31/240) of participants recalled having dengue. Amongst participants who had serological evidence suggesting past DENV infections, only 9.6% (20/209) reported having dengue previously. Meanwhile, amongst those with recent DENV infections, 35.5% (11/31) recalled having dengue. [21, level II-3]

The multivariate analysis showed that the older age group was significantly associated with past DENV infections. Seropositivity increased with age (48.5% in the age group of <25 years to more than 85% in age group of >45 years, $p < 0.001$). There was no associations with occupation, study site housing type, comorbidity, educational level, and marital status were observed, although the latter two were statistically significant in the univariate analysis. None of the studied factors were significantly associated with recent DENV infections in the multivariate analysis, although there was a pattern suggestive of recent outbreak in two study sites populated predominately by Chinese people. The authors concluded that the pilot study provide preliminary evidence that people of the Segamat rural community in Southern Malaysia had a very high previous exposure to DENV. Despite that, below 13% of participants recalled having dengue in the past, suggesting a potentially large reservoir of subclinical infection with unrecognized transmission lurking in the community, undetected by the official surveillance system.[21, level II-3]

Another cross sectional study conducted in Costa Rica by Iturrino-Monge R et al. (2006) to compare the presence of antibodies in children who live in a coastal region where dengue is endemic and in inland area where dengue is not endemic. An ELISA was used to test the serum for dengue virus IgG antibodies. None of the children had a prior history of dengue, fever, immunosuppressive therapy or underlying disease. They reported that, during the period from July 2002 to July 2003, a total of 103 children were recruited from each area. Seroprevalence of IgG for coastal and inland region was 36.9% (95% CI: 27.55%, 46.15%) and 2.9% (95% CI: 0.00%, 6.21%) respectively. The authors concluded that there was a substantial number of asymptomatic dengue in Costa Rican children. There increases the risk of dengue haemorrhagic fever or dengue shock syndrome in the children, in whom previous dengue had gone undetected.

Preventive efforts should be targeted at the coastal region due to higher prevalence in the area.[22, level II-3]

Yew YW et al. (2009) conducted a retrospective cross sectional study to determine the seroepidemiology of dengue virus infection in a representative sample of the adult resident population aged 18 years old to 74 years old in Singapore and to estimate the proportion of asymptomatic dengue infection during the 2004 epidemic. The study was based on 4152 stored blood samples collected between September and December 2004 from participants aged 18 years old to 74 years old during the 2004 National Health Survey. Sera were tested for IgG and IgM antibodies using a commercial test kit (PanBio Capture/Indirect ELISA). They found that, 2449 (59.0%) of the study population were seropositive for IgG, indicating that they had past dengue infection (95%CI: 57.5%, 60.5%).[23, level II-3]

Tsai JJ et al. (2018) conducted a cross sectional study to determine the prevalence of asymptomatic dengue virus-infected blood donors during the largest dengue outbreak in Taiwan history which occurred in 2015. They examined the evidence of DENV infection by detection of DENV RNA genome using real-time RT-PCR, DENV NS1 antigen using rapid diagnosis test and anti-dengue IgG/IgM ELISA. A total 8000 serum samples were obtained from blood donation centre, Taiwan Blood Service Foundation (TBSF). They reported that, only one (0.013%) sample was positive for DENV RNA detection using real-time RT-PCR. The virus serotype was DENV-2 determined by serotype-specific real-time RT-PCR and sequencing. The recipient of the blood did not develop any dengue fever symptom on follow-up. Although none of the samples was NS1 reactive but 17 (0.21%) IgM-positive samples were identified (probable of DENV infection). They concluded that there was a low prevalence of asymptomatic confirmed or probable DENV-infected blood donors in their study (0.013% and 0.21% respectively). [24,level II-3]

Yap G et al. 2013 conducted a cross sectional study during the dengue epidemic in Singapore, 2007 involving seven outbreak areas. The objective of the study was to estimate the dengue attack rate and the rate of inapparent dengue. A total of 3939 blood samples were collected and tested using IgM ELISA and indirect IgG ELISA. A reverse transcription polymerase chain reaction (RT-PCR) was performed on 400 randomly selected samples by using an in-house assay. Seroprevalence was calculated by adding the number of samples that were positive for IgG and those positive for IgM and dividing by total number of samples. The presence of dengue IgM indicates recent infection. Persons with no recollection of symptoms in the previous three months but positive for dengue IgM were considered as having inapparent dengue infection. They reported that, overall seroprevalence of dengue infection was 65.9%

(range=57.7%, 81.4%). The dengue incidence rate was 6.8% (6803.8 cases/100,000 populations) in the outbreak areas during three months prior to the study. Among person with recent infection, 78.0% had no recollection of any fever or dengue symptoms in the preceding three months. [25,level II-3]

Another cross sectional study was conducted by Muhammad Azami et al. (2011) to determine the actual magnitude of dengue endemicity in the Malaysian population. A total of 1000 subjects were randomly recruited from The Malaysian Cohort (TMC) population (13725 participants recruited into the TMC from 1 January 2008 until 31 December 2008). In the TMC, all subjects were healthy during the time of sample collection and consented towards the storage and usage of their samples for medical and epidemiological research. Serum samples were measured for the presence of dengue virus-specific IgG antibodies using the Dengue IgG indirect ELISA (PanBio). They reported that, 916 (91.60%) were positive for dengue IgG. Of the dengue seropositive result, 541 (90.17%) were females and 375 (93.75%) were males. The authors concluded that High dengue IgG seropositivity found in the population is an indication that dengue might be endemic in Malaysia for a long time into the future. [26,level II-3]

5.4 VIREAMIA

Duong V et al. (2011) in their diagnostic accuracy study reported that levels of viremia (log^{10} cDNA equivalents/mL) was significantly lower in asymptomatic than in DIC group (2.72, SD: 2.72, n =13 versus 4.96, SD: 2.37, n= 15; p=0.043). However, the difference was not significant if compared with the level of viremia in all dengue confirmed cases (3.79, SD: 3.06, n= 176; p=0.145). [9,level 2]

Yap G et al. 2013 in their cross sectional study reported that only one person who did not report any symptoms when blood sample was obtained, was positive for dengue by RT-PCR (dengue serotype 2) and had viral load of 100 plaque-forming units/mL. Of persons with recent symptoms of dengue, only five (8.2%) consulted a physician, and only one person was given a diagnosis of dengue. [25,level II-3]

Another cross sectional study was conducted by Duong V et al. in 2015. The objective was to document variation in DENV infectiousness of naturally infected humans across the spectrum of disease manifestations (including fully asymptomatic infections) and to verify the assumption that people with inapparent infections were not infectious to mosquitoes. The study was carried out in two part. First part was capturing DENV-infected people across the continuum of disease manifestations while they were viremic using a comprehensive catchment system combining passive, hospital-based surveillance and cluster investigations in and around the

households of hospitalized index cases. Detection method used was NS1 antigen commercial rapid diagnostic test followed by quantitative RT-PCR (qRT-PCR). Second part was mosquito feeding [wild-type but laboratory reared Ae. aegypti mosquitoes were allowed to feed on the blood of study participants] through direct (biting the person) and indirect (artificial feeder) methods. They included 181 participants who were either viremic at the time of mosquito feeding (n = 176) or were viremic at the time of inclusion, but had already reached an undetectable level of viremia at the time of experimental mosquito exposure and were nevertheless subsequently found to be infectious to mosquitoes (n = 5). They found that, magnitude of viremia was not associated with age (p=0.204), gender (p=0.702), DENV serotype (p=0.257), or immune status [primary, secondary and intermediate, (p=0.257)] but significantly different with disease category [asymptomatic, pre-symptomatic and symptomatic, (p=0.008)]. The viremia level measured in people with asymptomatic infections (mean ± SE: 4.75 ± 0.39 log10 cDNA copies per mL) was lower on average than in people with pre-symptomatic (mean ± SE: 6.74 ± 0.25 log10 cDNA copies per mL) or symptomatic (mean ± SE: 6.12 ± 0.17 log10 cDNA copies per mL) infections. [4,level II-3]

5.5 TRANSMISSION

For the mosquitoes feeding experiment by Duong V et al. 2015, most participants (n = 156; 86.2%) underwent both direct and indirect mosquito feedings, (4.4 %; n=8) participated in only direct and (9.4%; n = 17) only in indirect mosquito feedings. Direct mosquito feedings were only performed with children above four years of age. A total of 3,163 individual Ae. Aegypti were assayed [1,645 were fed directly on the infected person (mean per participant, 10.0; median, 9; IQR, 5–14) and 1,518 indirectly on viremic blood (mean per participant, 8.8; median, 7; IQR, 5–11)]. Following direct feeding, they reported that, mean viral loads (±SE) of infected mosquitoes expressed in log10 cDNA copies per milliliter were higher for mosquitoes that fed on asymptomatic people (6.52 ± 0.26) and pre-symptomatic people (5.29 ± 0.16) than on symptomatic patients in the zero to two days (4.91 ± 0.18), three to four days (5.20 ± 0.13), and five to eight days of illness (5.14 ± 0.16). Following indirect feeding, the mean viral loads (±SE) of infected mosquitoes were also higher after feeding on the blood drawn from asymptomatic people (6.06 ± 0.32) and pre-symptomatic people (5.06 ± 0.22) than on blood from symptomatic patients in the zero to two days (4.33 ± 0.25), three to four days (4.86 ± 0.18), and five to eight days of illness (3.43 ± 0.21). A significant effect of transmission for serotype DENV-1 was detected for only indirect mosquito feeding method [OR =3.66 (95%CI: 1.76, 7.63), p<0.001]. [4,level II-3]

5.6 DETERMINANTS OF INAPPARENT AND SYMPTOMATIC DENGUE INFECTION AMONG SCHOOL CHILDREN

Endy TP et al. (2011) in their cohort study among school children also found that there were several factors associated with inapparent and symptomatic dengue infection such as dengue incidence [higher incidence of infection at a given school, for a given year, was associated with lower proportion of inapparent infections during that epidemic season (OR = 0.62, 95% CI; 0.53,0.74.)], serotype circulation in present years [higher number of serotypes in circulation for a given year was associated with decreased likelihood of inapparent infections (OR= 0.78, 95% CI; 0.62, 0.99)], and also the influence from the previous year [the higher the incidence in previous year, the greater the probability a child experienced an inapparent infection the following year (OR= 1.34, 95% CI: 1.11, 1.61), p<0.01]. There was also serotype-specific effects reported which is a higher proportion of DENV-3 circulating at a given school being associated with a lower likelihood of inapparent infections (OR = 0.82, 95% CI: 0.68–0.99) and a higher proportion of DENV-2 being weakly associated with a higher likelihood of inapparent infections (OR= 1.12, 95% CI: (0.98–1.28). [12], level II-3

5.7. SAFETY

There was no retrievable evidence on the adverse events of detecting asymptomatic dengue infection. Various methods in detecting asymptomatic dengue infection had received 510k approval from USFDA.[27]

5.8. COST-EFFECTIVENESS

There was no retrievable evidence on the cost-effectiveness of asymptomatic dengue infection detection. However, estimated price for dengue IgG/IgM and NS1 antigen rapid test ranged from [redacted] per test. [28,29] Meanwhile, price for dengue IgG/IgM ELISA ranged from [redacted] per kit of 96 well.[29]

5.9. ORGANISATIONAL ISSUE

There was no retrievable evidence on the organisational issue of asymptomatic dengue infection detection.

5.10. LIMITATIONS

This technology review has several limitations. The selection of studies was done by one reviewer. Although there was no restriction in language during the search but only English full text articles were included in this review.

6. CONCLUSION

There was very limited diagnostic accuracy studies retrieved for detecting asymptomatic dengue infection. The accuracy of NS1 in detecting

asymptomatic dengue infection could not be determined due to limited number of study (one study) with limited sample size (17 individuals). Indirect ELISA was reported to have accuracy of 83% in detecting symptomatic and asymptomatic dengue infection compared to gold standard test ($PRNT_{50}$). Serology test involving ELISA were frequently used in incidence and seroprevalence studies of asymptomatic dengue infection.

Generally, the incidence of asymptomatic dengue infection was found to be higher compared to symptomatic dengue infection. Inapparent to symptomatic (I:S) ratio ranged from 0.9:1 to 2.5:1. However, viral load or viraemia level in asymptomatic patients was found to be lower compared to symptomatic patients.

Very limited evidence retrieved to suggest that several factors such as symptomatic dengue incidence and dengue serotype circulation affects the incidence of inapparent and symptomatic dengue infection among school children.

There was also very limited evidence retrieved to suggest that asymptomatic and pre-symptomatic DENV-infected people were more infectious to mosquitoes compared to symptomatic people. However, there was no retrievable evidence on transmission of dengue virus from mosquitoes to human among asymptomatic infection.

There was no evidence retrieved on the cost-effectiveness, safety and organisational issues on detection of asymptomatic dengue infection.

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8. APPENDIX

8.1. Appendix 1: LITERATURE SEARCH STRATEGY

Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Ovid MEDLINE®Daily and Ovid MEDLINE®1946 to Present

1 ASYMPTOMATIC INFECTIONS/ (860)
2 asymptomatic infection*.tw. (2454)
3 subclinical infection*.tw. (1677)
4 DENGUE VIRUS/ (7365)
5 breakbone fever virus*.tw. (0)
6 dengue virus*.tw. (8072)
7 ((break bone or break-bone or breakbone) adj fever).tw. (21)
8 classical dengue*.tw. (71)
9 classical dengue fever*.tw. (44)
10 dengue.tw. (17791)
11 1 or 2 or 3 (4771)
12 4 or 5 or 6 or 7 or 8 or 9 or 10 (18073)
13 11 and 12 (103)
14 Asymptomatic dengue.tw. (27)
15 (Asymptomatic adj dengue).tw. (27)
16 (Dengue virus adj carrier).tw. (0)
17 Dengue virus carrier.tw. (0)
18 Dengue contact.tw. (1)
19 Inapparent dengue fever.tw. (0)
20 (Inapparent adj dengue fever).tw. (0)
21 13 or 14 or 15 or 16 or 17 or 18 or 19 or 20 (121)
22 ANTIBODIES, VIRAL/ (70696)
23 viral antibodies.tw. (451)
24 IMMUNOGLOBULIN M/ (49106)
25 igm.tw. (61346)
26 immunoglobulin m.tw. (5757)
27 IMMUNOGLOBULIN G/ (120941)
28 igg.tw. (126681)
29 immunoglobulin G.tw. (19787)
30 ENZYME-LINKED IMMUNOSORBENT ASSAY/ (140587)
31 Elisa.tw. (144896)
32 enzyme linked immunosorbent assay.tw. (70240)
33 enzyme-linked immunosorbent assay*.tw. (77019)
34 ANTIGENS, VIRAL/ (38675)
35 viral antigens.tw. (4321)
36 REVERSE TRANSCRIPTASE POLYMERASE CHAIN REACTION/ (149115)
37 reverse transcriptase pcr.tw. (5431)
38 reverse transcriptase polymerase chain reaction.tw. (22495)

39 polymerase chain reaction.tw. (211394)
40 ANTIBODIES, VIRAL/ (70696)
41 DENGUE VIRUS/ (7365)
42 Dengue serology.tw. (73)
43 HEMAGGLUTINATION TESTS/ (20590)
44 hemagglutination test*.tw. (2053)
45 Plaque reduction neutralization test.tw. (481)
46 Polymerase chain reaction/ (233305)
47 (pcr adj2 (inverse or anchored or nested)).tw. (10786)
48 (anchored or inverse or nested).tw. (147353)
49 PHYSICAL EXAMINATION/ (38290)
50 physical examination*.tw. (53537)
51 (physical examination* adj diagnos*).tw. (164)
52 22 or 23 or 24 or 25 or 26 or 27 or 28 or 29 or 30 or 31 or 32 or 33 or 34 or 35 or 36 or 37 or 38 or 39 or 40 or 41 or 42 or 43 or 44 or 45 or 46 or 47 or 48 or 49 or 50 or 51 (1199985)
53 21 and 52 (79)

OTHER DATABASES	
EBM Reviews - Cochrane Central Register of Controlled Trials	Same MeSH, keywords, limits used as per MEDLINE search
EBM Reviews - Cochrane database of systematic reviews	
EBM Reviews - Health Technology Assessment	
EBM Reviews – NHS Economic Evaluation Database	
EBM Reviews – Database of Abstract of Review of Effects	

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VIRAL[Title/Abstract]) OR viral antibodies[Title/Abstract]) OR IMMUNOGLOBULIN M[Title/Abstract]) OR igm[Title/Abstract]) OR immunoglobulin m[Title/Abstract]) OR IMMUNOGLOBULIN G[Title/Abstract]) OR igg[Title/Abstract]) OR immunoglobulin G[Title/Abstract]) OR ENZYME-LINKED IMMUNOSORBENT ASSAY[Title/Abstract]) OR Elisa[Title/Abstract]) OR enzyme linked immunosorbent assay[Title/Abstract]) OR enzyme-linked immunosorbent assay*[Title/Abstract]) OR ANTIGENS, VIRAL[Title/Abstract]) OR viral antigens[Title/Abstract]) OR REVERSE TRANSCRIPTASE POLYMERASE CHAIN REACTION[Title/Abstract]) OR reverse transcriptase pcr[Title/Abstract]) OR reverse transcriptase polymerase chain reaction[Title/Abstract]) OR polymerase chain reaction[Title/Abstract]) OR ANTIBODIES, VIRAL[Title/Abstract]) OR DENGUE VIRUS[Title/Abstract]) OR Dengue serology[Title/Abstract]) OR HEMAGGLUTINATION TESTS[Title/Abstract]) OR hemagglutination test*[Title/Abstract]) OR Plaque reduction neutralization test[Title/Abstract]) OR Polymerase chain reaction[Title/Abstract]) OR PHYSICAL EXAMINATION[Title/Abstract]) OR physical examination*[Title/Abstract])

8.2. Appendix 2

HIERARCHY OF EVIDENCE FOR TEST ACCURACY STUDIES

Level	Description	
1.	A blind comparison with reference standard among an appropriate sample of consecutive patients	
2.	Any one of the following	Narrow population spectrum
3.	Any two of the following	Differential use of reference standard
4.	Any three or more of the following	Reference standard not blind Case control study
5.	Expert opinion with no explicit critical appraisal, based on physiology, bench research or first principles	

SOURCE: NHS Centre for Reviews and Dissemination (CRD) University of York, Report Number 4 (2nd Edition)

DESIGNATION OF LEVELS OF EVIDENCE

I Evidence obtained from at least one properly designed randomized controlled trial.

II-I Evidence obtained from well-designed controlled trials without randomization.

II-2 Evidence obtained from well-designed cohort or case-control analytic studies, preferably from more than one centre or research group.

II-3 Evidence obtained from multiple time series with or without the intervention. Dramatic results in uncontrolled experiments (such as the results of the introduction of penicillin treatment in the 1940s) could also be regarded as this type of evidence.

III Opinions or respected authorities, based on clinical experience; descriptive studies and case reports; or reports of expert committees.

SOURCE: US/CANADIAN PREVENTIVE SERVICES TASK FORCE (Harris 2001)

8.3. Appendix 3
Evidence Table : Diagnostic accuracy of asymptomatic dengue infection detection
Question : What is the diagnostic accuracy of asymptomatic dengue infection?

Bibliographic citation	Study Type/Methods	LE	Number of Patients & Patient Characteristic	Intervention	Comparison	Length of Follow Up (If Applicable)	Outcome Measures/Effect Size	General Comments
1.Duong V, Ly S, Try PL et al. Clinical and Virological Factors Influencing the Performance of a NS1 Antigen-Capture Assay and Potential Use as a Marker of Dengue Disease Severity. 2011;7: e1244	**Study design** Diagnostic accuracy study **Objective** To assess the potential use of NS1 antigen and dengue viral loads as markers of dengue severity **Methods** Patients in the paediatric ward of Kampong Cham hospital were enrolled during 2 consecutive dengue epidemic (May and October in 2006 and 2007) Patients diagnosed for other infections beside dengue and patients hospitalized with a non-infectious disease (e.g., cranial trauma, etc.) were recruited as control group Sera were tested for dengue using serology and molecular methods Family members of dengue-infected patients were visited the next day following (after confirmation) to identify	2	339 symptomatic patients 214 asymptomatic household members	1.Anti-dengue virus (DENV)-specific IgM 2.Hemagglutination inhibition (HI) 3.NS1 antigen 4. DENV isolation 5.RT-PCR (gold standard)	Nil		**Dengue infection** Dengue infection was confirmed in 243/339 symptomatic patients and in 17/214 asymptomatic individuals of household members tested **Performance of NS1 among dengue confirmed cases:** • sensitivity and specificity of the NS1-capture assay were 57.7% (95% CI: 51.4–63.8%) and 100% respectively • NS1 antigen kit combined with MAC-ELISA detected a significantly higher number of acute dengue cases than NS1 antigen kit alone (overall sensitivity: 85.7% vs. 57.7%; p<0.001) • Overall sensitivity of the NS1 detection kit was significantly higher in DF (72.3%; 95% CI: 63.5–81%) than in DHF/DSS (40.2; 95% CI: 29.85–51.3). However, only significant for samples collected after day 3 of fever and not for specimen obtained	

Bibliographic citation	Study Type/Methods	LE	Number of Patients & Patient Characteristic	Intervention	Comparison	Length of Follow Up (If Applicable)	Outcome Measures/Effect Size	General Comments
	non-symptomatic cases Body temperature was followed (7 days). Blood samples were taken at the first and 7th day of follow-up and if a family member developed fever. A non-symptomatic dengue case was defined as a household member who tested positive for dengue infection but did not display any of the symptoms of the inclusion criteria. A confirmed dengue infection ("gold standard algorithm") was defined by the detection of: • Anti-dengue virus (DENV)-specific IgM (MAC- ELISA) **OR** • A 4 fold increase of hemagglutination inhibition (HI) titer in the pair of sera collected with an interval of minimum 7 days **AND** detection of NS1 antigen in serum by the NS1 Platelia test						during the very early phase of the disease • Sensitivity of the NS1-capture assay was significantly higher in primary dengue infection (87.5%; 95% CI: 70.0–96.5) than in secondary infection (53.5%; 95% CI: 46.1–60.7) (p<0.001) • Sensitivity of the test also varied with the virus serotype **Performance of NS1 among asymptomatic individual:** Sensitivity: • sensitivity of NS1 test was significantly lower in asymptomatic individuals than that in DIC (35.3% versus 86.7%,p =0.003) • however, limit of significance was lower compared to sensitivity in all symptomatic cases (59.3%, p=0.053) Incidence of secondary infection: • 73% (8/11) of the asymptomatic individuals experienced secondary infection which was lower than in DIC (100%,	

Bibliographic citation	Study Type/Methods	LE	Number of Patients & Patient Characteristic	Intervention	Comparison	Length of Follow Up (If Applicable)	Outcome Measures/Effect Size	General Comments
	(BioRad, Hercules, CA) **AND/OR** • The isolation of DENV after inoculation into mosquito cell lines **AND/OR** • The detection DENV RNA by RT-PCR or real time RT-PCR assay						p=0.063) Levels of viremia (log10 cDNA equivalents/mL): • Significantly lower in asymptomatic than in DIC (2.72, SD: 2.72, n =13 vs. 4.96, SD: 2.37, n= 15; p=0.043) • difference was not significant if compared with the level of viremia in all dengue confirmed cases (3.79, SD: 3.06, n= 176; p=0.145) Nested RT-PCR detection was significantly more sensitive than NS1 antigen-capture assay (76.5% vs. 35.3%, p=0.015) in asymptomatic individuals **Authors' conclusion** Overall sensitivity of NS1 Ag detection kit varied widely across the various forms of dengue infection or disease. In asymptomatic patients, RT-PCR assay has proved to be more sensitive than NS1 antigen detection.	

Evidence Table : Diagnostic accuracy of asymptomatic dengue infection detection
Question : What is the diagnostic accuracy of asymptomatic dengue infection?

Bibliographic citation	Study Type/Methods	LE	Number of Patients & Patient Characteristic	Intervention	Comparison	Length of Follow Up (If Applicable)	Outcome Measures/Effect Size	General Comments
2.Catchen S, Limkittikul K, Sriburin P et al. Monoclonal Antibody-Based Enzyme Immunoassay for Epidemiological Studies of Asymptomatic Dengue Infection. Adv Infectious Disease. 2016;6: 113-119 Thailand	**Study design** Diagnostic accuracy study **Objective** To evaluate the developed indirect ELISA in diagnosing asymptomatic dengue infection in paired annual serum samples **Methods** Serum sample were taken from cohort epidemiology study of dengue infection in school children in Ratchiburi province, Thailand (2006-2009) The cohort: -Recruited 3000 primary school children -333 RT-PCR proved dengue episode out of 5842 febrile episode 22 paired annual sera from children who had symptomatic RT-PCR proven dengue infection were randomly selected and tested for PRNT50 and indirect ELISA(DENV 1 =9 cases, DENV2=7 cases, DENV3=6 cases)	2	22 paired RT-PCR dengue samples 72 samples of no dengue history	Indirect ELISA	$PRNT_{50}$	-	51.4% of children with no history of symptomatic dengue infection were non-dengue infection 48.6% of children with no history of symptomatic dengue infection were asymptomatic dengue infection Indirect ELISA Sensitivity:87.5% Specificity:78.3% Correlation (r): 0.736 8.9% (489/5513) of the population were asymptomatic dengue infection detected using the indirect ELISA test. The asymptomatic or inapparent: symptomatic (I:S) ratio in that period was 2.5:1 **Authors conclusion** Indirect ELISA has shown to be comparable to PRNT as serodiagnosis of asymptomatic dengue infection	

Bibliographic citation	Study Type/Methods	LE	Number of Patients & Patient Characteristic	Intervention	Comparison	Length of Follow Up (If Applicable)	Outcome Measures/Effect Size	General Comments
	Indirect ELISA was performed on sample of children with no history of dengue infection (5513 pairs) 42 paired samples with rise in ELISA titer (ratio 1.5) and 32 paired sample with no rise in ELISA titer were randomly selected to test for PRNT50 Rising ratio was calculated by fold of rised titer between annual serum samples							

Evidence Table : Incidence of asymptomatic dengue infection
Question : What is the incidence of asymptomatic dengue infection?

Bibliographic citation	Study Type/Methods	LE	Number of Patients & Patient Characteristic	Detection method	Comparison	Length of Follow Up (If Applicable)	Outcome Measures/Effect Size	General Comments
3.Endy TP, Chunsuttiwat S, Nisalak A. Epidemiology of Inapparent and Symptomatic Acute Dengue Virus Infection: A Prospective Study of Primary School Children in Kamphaeng Phet, Thailand. Am J Epidemiol. 2002;156:40–51.	**Study design** Prospective Cohort **Objective** To study the epidemiology and immunology of inapparent to severe dengue disease and to identify risk factors for developing severe dengue disease after acquiring a secondary dengue infection. **Methods** The study is being conducted in subdistrict Muang, Kamphaeng Phet Province, Thailand Children were recruited during January 1998 from grades 1–5 and they were eligible to remain in the study until graduation from sixth grade Baseline demographic information, height and weight, and a blood sample were obtained every January Height, weight, blood sample for dengue	II-2	2,119 elementary school children (717,106 person-school days)	HAI EIA IgM/IgG		3 years	**Inapparent and symptomatic dengue virus infection** • Overall incidence of dengue virus infection was 5.8% • The incidence of inapparent dengue virus infection and symptomatic dengue was 3.1 percent and 2.7 percent, respectively • The ratio of inapparent to symptomatic dengue virus infection was 1.2 in 1998, 0.9 in 1999, and 1.8 in 2000 • The ratio of inapparent to symptomatic dengue infection was not correlated to total dengue incidence nor to hospitalization rates (r = –0.03, p = 0.9, Pearson's correlation, two tailed and r = 0.3, p = 0.08, Pearson's correlation, two tailed, respectively)	

Bibliographic citation	Study Type/Methods	LE	Number of Patients & Patient Characteristic	Detection method	Comparison	Length of Follow Up (If Applicable)	Outcome Measures/Effect Size	General Comments
	serology) were measured three times during the surveillance period (June 1, August 15, and November 15) of each year Hemagglutination inhibition (HAI) assay and immunoglobulin (Ig)M/IgG enzyme immunoassay (EIA) were used for serology testing **Active case surveillance:** Acute illness due to dengue virus infection was identified on the basis of absence from school or a visit to the school nurse **Hospital/clinic surveillance:** Throughout June–November, and including weekends and holidays, clinical research nurses tracked children who reported to the public health clinic with an illness or were admitted to the hospital. **Inapparent dengue virus infection:** -The condition was defined as a fourfold rise in HAI antibody against any							

Bibliographic citation	Study Type/Methods	LE	Number of Patients & Patient Characteristic	Detection method	Comparison	Length of Follow Up (If Applicable)	Outcome Measures/Effect Size	General Comments
	dengue virus serotype between two sequential sera samples obtained during the surveillance months (June, August, or November), without a febrile illness identified during active surveillance in the time period in which seroconversion occurred. Sera were tested concurrently for Japanese encephalitis- specific HAI antibody to exclude Japanese encephalitis infection and antibody cross-reactivity as a cause for a fourfold rise in dengue antibody.							

Evidence Table : Incidence of asymptomatic dengue infection
Question : What is the incidence of asymptomatic dengue infection?

Bibliographic citation	Study Type/Methods	LE	Number of Patients & Patient Characteristic	Detection method	Comparison	Length of Follow Up (If Applicable)	Outcome Measures/Effect Size	General Comments
4.Endy TP, Anderson KB, Nisalak A et al. Determinants of Inapparent and Symptomatic Dengue Infection in a Prospective Study of Primary School Children in Kamphaeng Phet, Thailand. PLoS Negl Trop. 2011; 5(3): e975. doi:10.1371/journal.pntd.0000975	**Study design** Cohort **Objective** To report the spatial and temporal trends of the S:I ratio in dengue infection in a five-year **Methods** A five years (1998 to 2002) longitudinal cohort and geographic cluster study was undertaken in rural Thailand. Cohort children underwent pre- and post-season serology and active school absence-based surveillance to detect inapparent and symptomatic dengue. The laboratory assays used to detect acute dengue infection was PCR, HAI and anti-dengue IgM/IgG EIA. Inapparent (subclinical) dengue infection was defined as a four-fold rise in HAI antibody against any virus serotype between two	II-2	9932 primary school students Grade 1 to grade 6	PCR HAI EIA IgM/IgG		5 years	**Description** • 1,024 dengue virus infections were detected in total over the 5 years of the study • 909 children experienced at least one dengue infection during the study period • 115 experienced a second infection • Restricting analyses to the 615 infections detected during the active surveillance period (June 1– November 1), 66%(406) were inapparent and 34% (209) were symptomatic **Incidence** • The one-year incidence of total dengue infection and its constituents, symptomatic and inapparent infection was: for 1998 16.2% (of which 68.1% of those infection detected during the active surveillance period were inapparent); for 1999 13.6% (66.7% inapparent); for 2000 5.2% (81.3% inapparent) for 2001 17.5 (58.0% inapparent); and for 2002 7.1% (66.2% inapparent) **Spatial and temporal variation** • Higher incidence of infection in	

Bibliographic citation	Study Type/Methods	LE	Number of Patients & Patient Characteristic	Detection method	Comparison	Length of Follow Up (If Applicable)	Outcome Measures/Effect Size	General Comments
	sequential sera obtained during the surveillance month (June, August or November). Only symptomatic and inapparent dengue infections that were detected between June and November each year were included in the analysis.						a given year, was associated with lower proportion of inapparent infections (i.e., a higher S:I ratio) during that epidemic season (OR = 0.62, 95% CI; 0.53,0.74.)] • Higher number of serotypes in circulation for a given year was associated with decreased likelihood of inapparent infections (OR= 0.78, 95% CI; 0.62, 0.99)] • The higher the incidence in previous year, the greater the probability a child experienced an inapparent infection the following year (OR= 1.34, 95% CI: 1.11, 1.61)	

Evidence Table : Incidence of asymptomatic dengue infection
Question : What is the incidence of asymptomatic dengue infection?

Bibliographic citation	Study Type/Methods	LE	Number of Patients & Patient Characteristic	Detection method	Comparison	Length of Follow Up (If Applicable)	Outcome Measures/Effect Size	General Comments
5.Yoon IK, Rothman AL, Tannitisupawong D et al. Under recognized Mildly Symptomatic Viremic Dengue Virus Infections in Rural Thai Schools and Villages. J Infect Dis.2012;206:389-398 Thailand	**Study design** Cohort study **Objective** To provide detail analysis of the full 4 years prospective study which include transition in the predominant circulating DENV serotype **Methods** A 4-year (2004-2007) longitudinal cohort and geographic cluster study was undertaken in rural Thailand Cohort children underwent pre-/post-season serology and active school absence–based surveillance to detect inapparent and symptomatic dengue Cluster investigations were triggered by cohort dengue and non-dengue febrile illnesses (positive and negative clusters, respectively) Cohort subjects who were dengue PCR positive from an acute blood sample drawn within 3 days of illness onset served as an “index” case for a positive cluster investigation	II-3	Cohort of 10487 person-seasons Age ranging from 4 to 15 years old **Cluster investigation** 50 positive cluster (805 contact) 53 negative cluster (794 contact)	HAI PRNT PCR		-	**Incidence and clinical spectrum:** • Total of 189 EIA confirmed DENV infection (average incidence 2.3%) • Total of 346 inapparent dengue infection (average incidence 6.9%) • Inapparent to symptomatic (I:S) ratio was 1.8:1 for the entire study ranging from 1.1:1 in 2006 to 2.9:1 in 2005 **Viraemia** Dengue viremia was detected by PCR and culture in 9 of the 16 febrile DENV-infected children in the positive clusters (12 HAI positive, 4 HAI negative) who did not miss school (4 DENV-1, 5 DENV-4)	

Bibliographic citation	Study Type/Methods	LE	Number of Patients & Patient Characteristic	Detection method	Comparison	Length of Follow Up (If Applicable)	Outcome Measures/Effect Size	General Comments
	around the subject's house. Cohort subjects who were dengue PCR negative served as an "index" case for a negative cluster investigation Inapparent DENV infection was defined by paired pre- and post-season blood samples that showed a rise in dengue HAI and PRNT titers but was not associated with a symptomatic DENV infection between May and January							

Evidence Table : Seroprevalance of asymptomatic dengue infection
Question : What is the incidence of asymptomatic dengue infection?

Bibliographic citation	Study Type/Methods	LE	Number of Patients & Patient Characteristic	Detection method	Comparison	Length of Follow Up (If Applicable)	Outcome Measures/Effect Size	General Comments
6.Mammen MP, Pimgate C, Koenraadt CJ et al. Spatial and temporal clustering of dengue virus transmission in Thai villages. PLoS Med. 2008; 5(11): e205. doi:10.1371/journal.pmed.0050205	**Study design** Cohort study **Objective** To test the hypothesis that DENV transmission is spatially and temporally focal. **Methods** Cluster investigations were conducted within 100 m of homes where febrile index children with (positive clusters) and without (negative clusters) acute dengue lived during two seasons of peak DENV transmission One of the outcome measure was burden of inapparent and symptomatic infections A total of 2215 children were enrolled in a school cohort and followed using the active based surveillance (conducted in June and November of 2004 and 2005) for febrile illness Positive clusters were	II-2	2215 primary school children tested for febrile	IgM/IgG ELISA RT-PCR/nested PCR			• Of the 1,204 febrile children (506 in 2004 and 698 in 2005) who provided blood specimens, 48 (28 in 2004 and 20 in 2005) had detectable DENV viremia • Among the 556 village enrollees (217 in positive and 339 in negative clusters), 27 DENV infections were detected during the 15 days follow-up period. • Of the 27 DENV infections among village enrolees (14 inapparent and 13 symptomatic) • Inapparent infections were more likely with primary (five out of six) than secondary (seven out of 19) DENV infections (p = 0.05; Pearson's Chi2 test)	

Bibliographic citation	Study Type/Methods	LE	Number of Patients & Patient Characteristic	Detection method	Comparison	Length of Follow Up (If Applicable)	Outcome Measures/Effect Size	General Comments
	triggered by index cases with laboratory-confirmed dengue viremia determined within 24 hours of fever presentation while negative clusters by a febrile child without dengue viremia (and subsequent evidence of lack of DENV seroconversion) Dengue virus infections were identified by a dengue IgM/IgG ELISA Dengue viremia and serotype were determined by RT-PCR/nested PCR Dengue cases were classified as inapparent (lack of subjective and objective fever, defined as ≥ 38°C) or symptomatic dengue.							

Evidence Table : Seroprevalance of asymptomatic dengue infection
Question : What is the seroprevalance of asymptomatic dengue infection?

Bibliographic citation	Study Type/Methods	LE	Number of Patients & Patient Characteristic	Detection method	Comparison	Length of Follow Up (If Applicable)	Outcome Measures/Effect Size	General Comments
7.Wang T, Wang M, Shu B et al. Evaluation of Inapparent Dengue Infections During an Outbreak in Southern China. PLoS Negl Trop Dis 9(3): 1-11	**Study design** Cross sectional study **Objective** To better understand the dengue virus infection spectrum and to estimate the I:S ratio during the 2013 DENV outbreak in Zhongshan, Guangdong **Methods** Study methods were reviewed and approved by the Zhongshan Center for Disease Control and Prevention Institutional Review Board. All study participants provided informed consent The study was divided into 2 phases: **During outbreak** - 887 index case contacts was conducted to evaluate inapparent and symptomatic DENV infections -Samples collected from individuals who reported no dengue-like symptoms	II-3	**Phase 1** 887 index case contact **Phase 2** 1,167 subjects from 6 towns	ELISA IgM and IgG		-	**Phase 1** Out of 887 index case contact: • 41 (4.62%) positive DENV (95% CI: 3.24%-6.00%) • 13 (1.5%) symptomatic infection • 28 (3.2%) inapparent infection • I:S ratio 2.2:1 (95%CI: 1.1:1,4.2:1) **Phase 2** Overall post-outbreak DENV IgG antibody positive was 24/1167 (2.1%): • None reported having dengue prior to 2013. • 4 had symptomatic infection in 2013 • 14 had inapparent infection in 2013 • 6 had infection prior to 2013 Proportion of DENV IgG antibody detection by town was positively correlated with the reported incidence rate ($r = 0.88$, $p = 0.02$) The overall proportion of DENV IgG antibodies among subjects from : • 4 towns with reported dengue circulation during 2013 was 2.7% (95% CI: 1.6%-3.8%) • 2 towns without reported DENV transmission during 2013 was 0.6% (95% CI: 0–1.4%).	

Bibliographic citation	Study Type/Methods	L E	Number of Patients & Patient Characteristic	Detection method	Comparison	Length of Follow Up (If Applicable)	Outcome Measures/Effect Size	General Comments
	were then tested for IgM and IgG antibodies against DENV by ELISA **Post-outbreak** (817 - subjects from 4 towns with, and 350 subjects from 2 towns without reported autochthonous DENV transmission, as determined by clinical diagnosis, were evaluated for serological evidence of dengue IgG antibodies) -blood sample was collected and tested for IgM and IgG antibodies against DENV using an ELISA kit **Analysis** Data for post-outbreak DENV IgG seroprevalence among towns, gender, age groups, and the reported incidence rate was analyzed						Estimated dengue infection I:S ratio during the outbreak in: • Huangpu, Guzhen and Xiaolan, where DENV-1 circulated, was 11.0:1 (95% CI: 3.7-1:1) • Dongfeng where DENV-3 circulated was 1.0:1 (95% CI: 0.5-1:1)	

Evidence Table : Seroprevalance of asymptomatic dengue infection
Question : What is the seroprevalance of asymptomatic dengue infection?

Bibliographic citation	Study Type/Methods	L E	Number of Patients & Patient Characteristic	Detection method	Comparison	Length of Follow Up (If Applicable)	Outcome Measures/Effect Size	General Comments
8. Vikram IK, Nagpal BN, Pande V et al. An epidemiological study of dengue in Delhi, India. Acta Tropica. 2016; 153: 21–27 India	**Study design** Cross sectional study **Objective** To investigate and assess the epidemiology of dengue infection and to estimate the proportion of asymptomatic and symptomatic dengue infections in Delhi **Methods** A community based descriptive study was conducted in the identified localities during the period June– December, 2013 Fifty confirmed cases of dengue reported by these sentinel hospitals, from the identified 18 localities, covering maximum zones of Delhi, were investigated Household contacts i.e. the index case and co-habiting family members and neighbourhood contacts i.e. those residents living in close vicinity (within 200 m radius of the index case) were included in the study	II-3	2125 individuals (household and neighbourhood contact)	NS1 ELISA IgM ELISA IgG			• 748/2125 (36.14%) were DENV positive • 542/2125 (25.5% cases were IgG positive without any symptom • 40/2125 (1.88%) were primary infection • 186/2125 (8.8%) were secondary infection • Of 226 (primary + secondary infection), 63% were asymptomatic and 37% were symptomatic The Odds of getting asymptomatic infection was: • Greater in low income group (LIG) than to medium income group (MIG) [OR=1.85, 95% CI: -1.01,3.41] • Greater in High income group than to MIG [OR=1.17, 95% CI:-0.55,-2.50] • Greater in LIG than HIG [OR=0.63, 95% CI: -0.30, 1.33] Authors conclusion Out of the total population screened, 10.6% dengue infection was either primary or secondary. On the basis of the results, it may be hypothesized that there are large number of asymptomatic dengue infections in the community as	

Bibliographic citation	Study Type/Methods	L E	Number of Patients & Patient Characteristic	Detection method	Comparison	Length of Follow Up (If Applicable)	Outcome Measures/Effect Size	General Comments
	They were serologically diagnosed as dengue positive or negative by using SD BIOLINE Dengue Duo combo device (Standard Diagnostic Inc., Korea) Symptomatic DENV infection was defined as fever with at least two symptoms of dengue (myalgia, headache, retro-ocular pain, arthralgia and rash) as per WHO guidelines (World Health Organization, 2009) Asymptomatic DENV infection—no clinical signs or symptoms of disease as mentioned above in symptomatic infection (World Health Organization, 2009) Group comparison for prevalence of IgG and IgM and other clinical symptoms was done using ANOVA. Odds ratio was calculated to ascertain the odds of getting asymptomatic patients in three income groups						compared to reported symptomatic cases in Delhi.	

Evidence Table : Seroprevalance of asymptomatic dengue infection
Question : What is the seroprevalance of asymptomatic dengue infection?

Bibliographic citation	Study Type/Methods	LE	Number of Patients & Patient Characteristic	Detection method	Comparison	Length of Follow Up (If Applicable)	Outcome Measures/Effect Size	General Comments
9. Jamjoom GA, Azhar EI, Kao MA. Seroepidemiology of Asymptomatic Dengue Virus Infection in Jeddah, Saudi Arabia. Virology: Research and Treatment 2016; 7:1-7	**Study design** Cross sectional study **Objective** To measure the seroprevalence of past dengue virus infection in healthy Saudi nationals from different areas in the city of Jeddah and to investigate demographic and environmental factors that may increase exposure to infection. **Methods** Subjects recruited from six primary care center Visited for primary care or for illness other than dengue fever or similar illness Serum samples were tested by enzyme immunoassay (EIA) for IgG antibodies to dengue viruses 1, 2, 3, 4 1958 questionnaires were completed by patients	II-3	1984 subjects Mean age : 38.6 years (range 3-80 years ±14.7)	EIA IgG		-	47.8% of subject had positive IgG Prevalence was higher in male (53.0%) compared to female (45.1%) p<0.001, odd ratio 1.374 (95% CI: 1.139, 1.658) Among 184 blood donors, 68 (36.95%) were tested positive for dengue IgG 111/221 (50.2%) who did not travel were tested Positive for dengue IgG, compared to 27/77 (35%) who travelled outside country [1.869 OR (95% CI: 1.092, 3.198), p=0.022]	

Bibliographic citation	Study Type/Methods	L E	Number of Patients & Patient Characteristic	Detection method	Comparison	Length of Follow Up (If Applicable)	Outcome Measures/Effect Size	General Comments
	Addition 189 sample were collected from 189 without questionnaire							

Evidence Table : Seroprevalance of asymptomatic dengue infection
Question : What is the seroprevalance of asymptomatic dengue infection?

Bibliographic citation	Study Type/Methods	LE	Number of Patients & Patient Characteristic	Detection method	Comparison	Length of Follow Up (If Applicable)	Outcome Measures/Effect Size	General Comments
10.Ashshi AM, Alghamdi S, El-Shemi A et al. Seroprevalence of Asymptomatic Dengue Virus Infection and Its Antibodies Among Healthy/Eligible Saudi Blood Donors: Findings From Holy Makkah City. Virology: Research and Treatment. 2017; 8 :1-5	**Study design** Cross sectional study **Objective** To estimate the seroprevalence of asymptomatic DENV infection and its antibodies among eligible Saudi blood donors. **Methods** Ethical approval obtained Blood sample collected after informed consent obtained All participant donors were negative for infection with human immunodeficiency virus (HIV), hepatitis B virus (HBV), and hepatitis C virus (HCV); did not suffer from fever or any sign of dengue infection as per WHO guidelines; and were accepted for blood donation according to the policy set up by the Kingdom of Saudi Arabia Health Ministry The tests were conducted using commercially	II-3	910 healthy male blood donors Age: 25 to 55 years Mean age: 37.13 ± 7.45 years	NS1 ELISA IgM ELISA IgG		-	48 donors (5.3%) seropositive for DENV-NS1 antigen • 7 (0.8%) monopositivity to DENV-NS1 alone • 2 (0.2%) dual positivity to DENV-NS1 plus anti-DENV IgM antibodies • 26 (2.9%) dual positivity to DENV-NS1 plus anti-DENV IgG antibodies • 13 (1.4%) triple positivity to DENV-NS1 plus anti-DENV IgM and IgG antibodies 50 (5.5%) seropositive anti-DENV IgM antibodies 354 (38.9%) seropositive anti-DENV IgG antibodies	

Bibliographic citation	Study Type/Methods	LE	Number of Patients & Patient Characteristic	Detection method	Comparison	Length of Follow Up (If Applicable)	Outcome Measures/Effect Size	General Comments
	available DENV Pan-E NS1 early enzyme-linked immunosorbent assay (ELISA), DENV IgM capture ELISA, and DENV IgG capture ELISA, respectively (all from Panbio, Brisbane, QLD, Australia) All samples were processed in duplicate on a fully automated ELISA system (Human Diagnostics, Wiesbaden, Germany) **Result interpretation** Index values of 9 to 11 PU: • DENV-NS1 antigen equivocal • anti-DENV IgM equivocal Index value >11 Panbio Units (PU): • DENV-NS1 antigen +ve • anti-DENV IgM +ve Index value 18 to 22 PU • anti-DENV IgG equivocal Index value >22 PU • anti-DENV IgG +ve							

Evidence Table : Seroprevalance of asymptomatic dengue infection
Question : What is the seroprevalance of asymptomatic dengue infection?

Bibliographic citation	Study Type/Methods	LE	Number of Patients & Patient Characteristic	Detection method	Comparison	Length of Follow Up (If Applicable)	Outcome Measures/Effect Size	General Comments
11.Mahmood S, Nabeel H, Hafeez S et al. Seroprevalance of Dengue IgG Antobodies among Healthy Adult population in Lahore, Pakistan. ISRN Tropical Med. 2013;1 :1–6 Pakistan	**Study design** Cross sectional study **Objective** To determine seroprevalence of dengue IgG in adult population of Lahore as well as describe risk factors related to seropositivity **Methods** Study conducted from July-September 2012 A total of 274 individuals aged 15 years and above were included in this study using multistage sampling technique After the interview about dengue awareness, 3 ml of venous blood was drawn by trained phlebotomist The samples were tested for serological IgG antibodies (Nova Tech ELISA) Samples were considered positive if the absorbance value is higher than 10% over the cut-off	II-3	274 healthy adult Aged ≥15 years old	ELISA IgG		-	184 (67.2%) participants positive dengue IgG Seroprevalance: • males = 68.9%, female=63.7% • 15-24 years=59.2%, 35-44 years=75.6% • High socioeconomic status=73%, low economic status 59.6% Age was associated with dengue anti-IgG seropositivity, adjusted odd ratio (OR) = 1.98; 95% CI. 0.81–4.83; p = 0.12 for age group of 35–44 years No statistical association in socioeconomic status [adjusted OR 0.21 (95% C: 0.02,1.54); p = 0.08] No statistically significant difference was observed in relation to good, satisfactory, or poor level of awareness about dengue transmission (P = 0.56), complications (P = 0.53), potential breeding sites (P = 0.30), and preventive measures (P = 0.64).	

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12.Rafique I, Saqib MAN, Munir MA et al. Asymptomatic dengue infection in adults of major cities of Pakistan. Asian Pac J Trop Med.2017; 10 (10):1002-1006 Pakistan	**Study design** Cross sectional **Objective** To determine the asymptomatic dengue infection in adult of Pakistani population **Methods** Conducted in five major cities (Islamabad, Karachi, Peshawar, Multan) The participants were enrolled from selected laboratories in each city using convenient sample. Patients were asked about history of fever or any other related symptoms Those who confirmed any dengue symptom were excluded Blood were taken for IgG testing (DENV IgG ELISA kit, Cortez Diagnostic USA) <0.9 negative DENV IgG 0.9–1.1 borderline positive >1.1 positive for DENV IgG.	II-3	N=5230 subjects • 998 Islamabad • 1120 Karachi • 1087 Peshawar • 969 Multan Male (n=3276, 62.6%) Female (n=1954, 37.4%)	ELISA IgG		-	1691 (32.3%) have asymptomatic dengue • 756 (67.5%) from Karachi • 391 (39.2%) from Islamabad • 316 (29.9%) from Lahore • 228 (21%) from Peshawar • 0 (0%) from Multan Overall, asymptomatic dengue infection was more (34.5%) in females as compared to males (31%) No significant difference observed between genders (X^2 = 4.405, P = 0.353)	

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	Those who had positive dengue IgG were defined as having asymptomatic dengue infection							

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13.Dhanoa A, Hassan SS, Jahan NK et al. Seroprevalence of dengue among healthy adults in a rural community in Southern Malaysia: a pilot study. Infectious Diseases of Poverty. 2018; 7 (1): 1-13 (Malaysia)	**Study design** Cross sectional study **Objective** To establish DENV seroprevalence amongst healthy adults in a rural district in Southern Malaysia, and to identify influencing factors. **Methods** Participants were recruited from households across three localities in the Sungai Segamat subdistrict (highest number of reported dengue cases over the last five years) Exclusion criteria: Presence of fever (infrared forehead thermometer ≥37.5 °C at time of recruitment), history of yellow fever and Japanese encephalitis (JE) vaccination/ disease, pregnant women, and patients with terminal illness, organ failure, mental illness, cancer, and/or underlying bleeding disorders	II-3	277 healthy adults Aged ≥18 years	-ELISA IgM -ELISA IgG -PRNT on random sample for confirmation		-	Positive dengue anti-IgG indirect ELISA identified in the serum samples of 240 (86.6%) participants (95% CI: 83, 91%) 11.2% (31/277) of participants (95% CI: 7, 15%) had serological evidence of recent DENV infection 75.5% (209/277) of participants (95% CI: 70, 81%) had evidence of a past DENV infection 12.9% (31/240) of participants recalled having had dengue Amongst participants who had serological evidence suggesting past DENV infections, 9.6% (20/209) reported having dengue previously Amongst those with recent DENV infections, 35.5% (11/31) recalled having dengue **Authors conclusion** Despite this, below 13% of participants recalled having dengue in the past, suggesting a potentially large reservoir of subclinical infection with unrecognized transmission lurking in this community, undetected by the official surveillance system	

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	Sera were tested for immunoglobulin G (IgG) (Panbio® Dengue Indirect IgG ELISA/high-titer capture) and immunoglobulin M (IgM) (Panbio®) antibodies Plaque reduction neutralization test (PRNT) was conducted on random samples of IgG positive sera for further confirmation Medical history and a recall of previous history of dengue were collected through interviews Sociodemographic information was obtained from an existing database							

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14.Ituro-Monge R, Avila-Agüero ML, Avila-Agüero CR et al. Seroprevalence of dengue virus antibodies in asymptomatic Costa Rican children, 2002–2003: a pilot study. Pan Am J Public Health. 2006; 20(1):39-43	**Study design** Cross sectional study **Objective** To document the seroprevalence, measured as the presence of IgG antibodies, of dengue virus in asymptomatic children from two different geographical areas. **Methods** Serum samples were collected during a 12-month period between July 2002 and July 2003 at two national hospitals Patients were classified according to age into 10 groups Inclusion criteria were age 1 year to 10 years 11 months, permanent residence in the coastal or inland region, absence of fever for at least 72 hours prior to inclusion Exclusion criteria were history of yellow fever vaccination or disease, history of dengue disease	II-3	206 children (1 year to 10 years 11 months old)	ELISA IgG		-	**Coastal region** Antibodies for dengue virus IgG: 36.9% (38/103) children (95% CI: 27.55%, 46.15%) Median score: 30.7 PU (range 16–49) Secondary dengue infection: 7 cases (6.8%) **Inland region** Antibodies for dengue virus IgG: 2.9% (3/103) children (95% CI: 0.00%, 6.21%) Median score: 26.3 PU Secondary dengue infection: No	

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	with serological confirmation, known immunodeficiency, immunosuppressive treatment during the previous month (including corticosteroids for more than 4 weeks), and fever (axillary temperature ≥ 37.5 °C) at the time of recruitment The PanBio Dengue IgG enzyme-linked immunosorbent assay (ELISA) (PanBio, Brisbane, Australia) was used to detect dengue virus IgG antibodies Test results were interpreted according to the manufacturer's guidelines as Panbio units (PU) of absorbance in the sample, as follows: negative < 9 PU; indeterminate 9–11 PU; positive > 11 PU Values >40 PU are associated with secondary dengue							

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15.Yew YW, Ye T, Ang LW et al. Seroepidemiology of Dengue Virus Infection Among Adults in Singapore. Ann Acad Med Singapore. 2009;38: 667-675. Singapore	**Study design** Retrospective cross sectional study **Objective** 1)To determine the seroepidemiology of dengue virus infection in a representative sample of the adult resident population aged 18 years old to 74 years old in Singapore 2) To estimate the proportion of asymptomatic dengue infection during the 2004 epidemic **Methods** Study was conducted based on the 4152 stored blood sample of 2004 National Health Survey (collected between September and December 2004) Samples were analysed for dengue IgG and IgM antibodies to determine past and recent exposure (Panbio Dengue IgM Capture ELISA and Panbio Dengue IgG Capture/ Indirect ELISA systems	II-3	4125 stored blood	ELISA IgG and IgM	Nil	-	**IgG/IgM seroprevalence** • 2449 (59.0%) of the study population were seropositive for IgG, indicating that they had past dengue infection (95% CI: 57.5%-60.5%) • Recent infection [IgM/high-titre IgG] was 2.6% (95% CI: 2.1%-3.1%) **Population characterictic** • No significant difference in seropositivity between male and female participants was detected • Indians had the highest seropositivity (69.3%) as compared to that of Chinese (58.2%) and Malays (57.1%) (P <0.0005) • Lowest age-specific seropositivity of 17.2% was found in the 18 years to 24 years age group and the highest seroprevalence of 88.9% was found in the 55 years to 74 years age group	

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	(Panbio, Australia)							

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16. Tsai JJ, Lin PC, Tsai CY et al. Low frequency of asymptomatic dengue virus-infected donors in blood donor centers during the largest dengue outbreak in Taiwan. PLoS ONE.2018; 13(10): e0205248. https://doi.org/10.1371/journal.pone.0205248	**Study design** Cross sectional study **Objective** To determine the prevalence of asymptomatic dengue virus-infected blood donors during the largest dengue outbreak in Taiwan history occurred in 2015 **Methods** A total 8000 serum samples were obtained from blood donation centre, Taiwan Blood Service Foundation (TBSF) They examined the evidence of DENV infection by detection of DENV RNA genome using real-time RT-PCR, DENV NS1 antigen using rapid diagnosis test and anti-dengue IgG/IgM ELISA DENV NS1 and anti-dengue IgM/IgG in serum samples were detected using SD BIOLINE Dengue Duo Anti-dengue IgM and IgG of	II-3	8000 blood donation samples (randomly selected)	real-time RT-PCR DENV NS1 IgM/IgG ELISA			• Only one sample was positive for DENV RNA detection using real-time RT-PCR • The virus serotype was DENV-2 determined by serotype-specific real-time RT-PCR and sequencing • The recipient of the blood did not develop any dengue fever symptom on follow-up. • None of the samples was NS1 reactive • 17 (0.21%) IgM-positive samples were identified (probable of DENV infection) • In these cases, 13 cases were IgG-reactive (0.16%).	

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	all serum samples were tested at 1:100 dilution by capture ELISA							

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17.Yap G, Li C, Mutalib A et al. Short Report: High Rates of Inapparent Dengue in Older Adults in Singapore. Am. J. Trop. Med. Hyg. 2013; 88(6): 1065-1069	**Study design** Cross sectional study **Objective** To estimate the dengue attack rate and the rate of inapparent dengue **Methods** Study was conducted during the dengue epidemic in Singapore, 2007 involving seven outbreak areas A total of 3939 blood samples were collected and tested using IgM ELISA and indirect IgG ELISA A reverse transcription polymerase chain reaction (RT-PCR) was performed on 400 randomly selected samples by using an in-house assay Seroprevalence was calculated by adding the number of samples that were positive for IgG and those positive for IgM and dividing by total number of samples	II-3	3939 blood samples Children and adults (Age 7 to 85 years old)	IgM ELISA indirect IgG ELISA RT-PCR (random)			**Seroprevalence** • Overall seroprevalence of dengue infection was 65.9% (range=57.7%, 81.4%). • Among person with recent infection, 78.0% had no recollection of any fever or dengue symptoms in the preceding three months **Viral load** • Only one person who did not report any symptoms when blood sample was obtained, was positive for dengue by RT-PCR (dengue serotype 2) and had viral load of 100 plaque-forming units/mL	

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	The presence of dengue IgM indicates recent infection Persons with no recollection of symptoms in the previous three months but positive for dengue IgM were considered as having inapparent dengue infection							

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18.Muhammad Azami, Salleh SA, Neoh HM et al. Dengue epidemic in Malaysia: Not a predominantly urban disease anymore. BMC Res Notes. 2011; 4: 216. doi: 10.1186/1756-0500-4-216.	**Study design** Cross sectional study **Objective** To determine the actual magnitude of dengue endemicity in the Malaysian population **Methods** The Malaysian Cohort (TMC) population (13725 participants recruited into the TMC from 1 January 2008 until 31 December 2008). A total of 1000 subjects were randomly recruited In the TMC, all subjects were healthy during the time of sample collection and consented towards the storage and usage of their samples for medical and epidemiological research. Serum samples were measured for the presence of dengue virus-specific IgG antibodies using the Dengue IgG indirect ELISA (PanBio).	II-3	1000 randomly selected from TMC Healthy adult 35-74 (years old)	Dengue IgG indirect ELISA (PanBio)			• 91.6% subjects were found to be dengue seropositive • 541 (90.17%) were females • 375 (93.75%) were males	

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18. Duong V, Louis Lambrechts L, Paul RE et al. Asymptomatic humans transmit dengue virus to mosquitoes. Proc Natl Acad Sci U S A. 2015;112(47):14688-14693. doi: 10.1073/pnas.1508114112.	**Study design** Cross sectional study **Objective** 1.To document variation in DENV infectiousness of naturally infected humans across the spectrum of disease manifestations (including fully asymptomatic infections) 2.To verify the assumption that people with inapparent infections were not infectious to mosquitoes **Methods** First part was capturing DENV-infected people across the continuum of disease manifestations while they were viremic using a comprehensive catchment system combining passive, hospital-based surveillance and cluster investigations in and around the households of hospitalized index cases. DENV infection of hospitalized patients was confirmed by NS1 antigen detection using a	II-3	**First Part** 181 participants; Viremic at the time of mosquito feeding or either were viremic at the time of inclusion (n = 176) Nevertheless subsequently found to be infectious to mosquitoes (n = 5) **Second part** 3,163 individual *Ae. Aegypti* 1,645 direct feeding 1,518 indirect feeding	NS1 qRT-PCR			**Viraemia** Viremia level measured in people with asymptomatic infections (mean ± SE: 4.75 ± 0.39 log10 cDNA copies per mL) was lower on average than in people with pre-symptomatic (mean ± SE: 6.74 ± 0.25 log10 cDNA copies per mL) or symptomatic (mean ± SE: 6.12 ± 0.17 log10 cDNA copies per mL) infections **Transmission to mosquitoes** Direct feeding [mean viral loads (±SE) of infected mosquitoes expressed in log10 cDNA copies per milliliter]; • Asymptomatic people (6.52 ± 0.26) • Pre-symptomatic people (5.29 ± 0.16) • Symptomatic patients in the zero to two days (4.91 ± 0.18), three to four days (5.20 ± 0.13), and five to eight days of illness (5.14 ± 0.16). Indirect feeding [mean viral loads (±SE) of infected mosquitoes expressed in log10 cDNA copies per milliliter]; • Asymptomatic people (6.06 ± 0.32) and • Pre-symptomatic people (5.06 ± 0.22)	

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	commercial rapid diagnostic test (for details, see Dengue Diagnosis and Classification below) followed by qRT-PCR on the plasma sample obtained during the acute febrile phase of disease Second part was mosquito feeding [wild-type but laboratory reared Ae. aegypti mosquitoes were allowed to feed on the blood of study participants] through direct (biting the person) and indirect (artificial feeder) methods Legs and wings of blood-engorged mosquitoes that survived a 2-wk extrinsic incubation period (EIP) were tested by serotype-specific, quantitative reverse transcription–PCR (qRT-PCR) to detect and quantify DENV RNA Mosquitoes with DENV-positive wings and legs indicated that virus had disseminated from their midguts, which is a recognized proxy for their mosquito-to-human transmission potential						• Symptomatic patients in the zero to two days (4.33 ± 0.25), three to four days (4.86 ± 0.18), and five to eight days of illness (3.43 ± 0.21).	