Published in final edited form as:

Curr Opin Virol. 2018 April ; 29: 51–61. doi:10.1016/j.coviro.2018.03.004.

The use of longitudinal cohorts for studies of dengue viral pathogenesis and protection

Leah C. Katzelnick and Eva Harris*

Division of Infectious Diseases and Vaccinology, School of Public Health, University of California, Berkeley, Li Ka Shing Center, 1951 Oxford Street, Berkeley, CA 94720-3370

Abstract

In this review, we describe how longitudinal prospective community-, school-, and household-based cohort studies contribute to improving our knowledge of viral disease, focusing specifically on contributions to understanding and preventing dengue. We describe how longitudinal cohorts enable measurement of essential disease parameters and risk factors; provide insights into biological correlates of protection and disease risk; enable rapid application of novel biological and statistical technologies; lead to development of new interventions and inform vaccine trial design; serve as sentinels in outbreak conditions and facilitate development of critical diagnostic assays; enable holistic studies on disease in the context of other infections, comorbidities, and environmental risk factors; and build research capacity that strengthens national and global public health response and disease surveillance.

Introduction

Although the most well-known prospective cohort studies have focused on predictors of chronic disease [1], cohort studies are also important for understanding infectious diseases. In this study design, individual-level baseline characteristics are measured in a healthy population followed over time as participants naturally acquire disease, thus enabling identification of factors associated with or protective against disease risk. For example, two key findings of such studies include identification of distinct transmission rates of influenza A and B viruses among humans [2] and differential gender-based HIV transmission rates in discordant couples [3]. Prospective community-, school-, and household-based cohort studies are particularly useful to study acute viral diseases such as dengue. Dengue virus is comprised of four serotypes, DENV1–4. Infection with one serotype provides long-term protection against disease upon re-infection with the same serotype. However, prior immunity can protect against or enhance disease during secondary heterotypic DENV infection, which is the greatest risk factor for severe dengue, Dengue Hemorrhagic Fever/Dengue Shock Syndrome (DHF/DSS). DHF/DSS is thought to be caused in part by

*Corresponding author: eharris@berkeley.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Conflicts of interest: The authors declare no conflicts of interest.

antibody-dependent enhancement (ADE): sub-neutralizing antibody titers enhance viremia [4] by enabling infection of monocytes and macrophages via Fc γ receptors [5,6]; this instigates pathologic immune cell activation and elevated NS1 secretion that result in vascular leak and shock [7]. Because immune history is critical for understanding subsequent disease risk and protection, cohort studies are invaluable for studying protection against and pathogenesis of dengue disease.

Here, we discuss the full value of longitudinal cohorts for: measuring basic determinants and immunological and virological characteristics of dengue disease in populations, estimating correlates of protection and disease risk, providing critical and timely information during outbreaks, enabling rapid development of new assays for diagnosis and surveillance, informing vaccine trial design, studying disease in a broader population context, building research capacity, and informing local and international policy-making (Table 1).

Review of dengue cohort studies

We used PubMed to download all articles with the term 'dengue' in the title and 'cohort' in either the title or abstract (n=283, January 4, 2018). Titles and abstracts were screened to identify prospective cohort studies of dengue in healthy populations (some reviewed previously in [8–10]; we do not review infant cohorts here). We identified 28 cohort studies from 1964 to the present (Table 2).

Incidence, burden, and risk factors

Incidence

Dengue cohort studies are used to estimate DENV infection and disease incidence in a given population. Symptomatic disease is measured by active surveillance for febrile illness and testing of acute and convalescent blood samples with molecular biological, virological and/or serological methods. Inapparent infections are measured by rise in antibody titers between pre- and post-epidemic or annual blood samples. Dengue disease incidence ranges from 0.3 to 4.6 per 100 person-years, exhibiting substantial heterogeneity by year and location. Cohort studies have shown that incidence of symptomatic dengue is higher in Asia than Latin America and that a larger fraction of dengue cases require hospitalization in Asia [11–13]. DENV-attributable incidence among febrile cases was measured in Thailand and accounted for 15% of DALYs attributable to febrile illness [14,15]. Estimates of dengue disease incidence in cohort studies have been compared to national-level surveillance data, enabling determination of expansion factors (e.g., from 4.7 to 22 cases identified by active surveillance for every 1 case identified by passive surveillance) [16–19] and estimation of national and global incidence, burden, and mortality [20–22]. DENV infection incidence ranges from 3 to 39.4 per 100 person-years [12,23–25], with the ratio of symptomatic to inapparent infections (S:I) varying dramatically in cohorts within epidemics, across years [12], and by geographic area (e.g., nearby schools) [13,26,27]. Analysis has shown that years with high S:I ratio (more symptomatic infections) are often followed by years with low S:I ratio (more inapparent infections) [12,28].

Primary versus secondary infections

The first dengue cohort studies found that DHF/DSS cases were only observed in individuals who had anti-DENV antibodies in pre-infection samples [8,29]. Larger cohort studies proved that pre-existing immunity is a strong risk factor (odds ratio 6.5 in one study, relative risk >50 in another) for DHF/DSS, and DENV2 was most strongly associated with DSS [30–32]. Cohort studies also showed that the probability of symptomatic disease is lower during primary than secondary DENV infection, particularly when the secondary DENV infection occurred >1 year after primary infection [33].

Age and sex

Cohort studies have not consistently shown differences in DENV infection or symptomatic dengue by sex [12,13,24,25], although differences in DSS by sex have been observed [8]. Age is related to both the probability of exposure and disease incidence. First, younger children have more undifferentiated fever caused by DENV, possibly because they do not describe symptoms as easily as older children [34]. Second, older age is associated with probability of DENV infection, likely due to increased mobility [12,35] and body surface area [36,37]. Third, age of secondary DENV infection is associated with higher probability of severe disease [38], while age of acquisition of post-secondary infection immunity is associated with reduced probability of serologically detectable DENV infection given exposure [24,29]. Finally, older age is associated with greater probability of disease, even controlling for anti-DENV antibody titer and number of previous infections [39].

Spatial heterogeneity

Dengue cohort studies have revealed spatial heterogeneity of circulating serotypes and genetic diversity of viral strains circulating in a given population, including extensive gene flow from larger urban centers into more rural populations as well as between nearby schools [40–42]. Spread of a novel serotype, DENV3, in Iquitos, Peru, was correlated with high pre-existing community seroprevalence, suggesting certain areas had higher risk of transmission [27,43].

Force of infection

Cohort studies collect age-stratified seroprevalence data, enabling estimation of the force of infection – the rate at which naïve individuals become infected in a population. Age-stratified seroprevalence data from cohort studies have been used to estimate average historical and annual differences in the force of infection, and where serotype-specific neutralizing antibody titers were measured, serotype-specific force of infection [24,44–46].

Correlates of protection and disease risk

The value of cohort studies for measuring immune correlates

While hospital-based studies are critical for identifying prognostic indicators in acute-phase samples for progression to severe dengue [47] or viral determinants [48] associated with severe dengue outcomes, they are limited in that they can only examine individuals who are

already sick. Cohorts are essential for evaluating how *pre-existing* immunity correlates with infection and disease outcome.

Correlates of protection and risk

Most studies of correlates of protection and risk have examined neutralizing antibody or ADE titers in non-random subsets of dengue cohorts using classical serological assays such as the plaque reduction neutralization test (PRNT) or ADE assays, although some use newer tools [27,32,35,49,50]. In a Thai cohort, neutralizing antibodies distinguished non-severe dengue fever (DF) cases from DHF/DSS cases infected with DENV3, but not DENV2 [51]. In the same cohort, antibody-dependent cellular cytotoxicity was found to correlate with neutralizing antibody titers and IgG₁ levels as well as viremia in DENV3 patients, but not with disease severity [52]. Another Thai cohort confirmed that PRNT titer does not perfectly correlate with protection, as individuals with high PRNT titers could still acquire symptomatic DENV infections [53]. Other cohort studies have compared inapparent to symptomatic DENV infections and found that the magnitude [50,54] and breadth [35,55] of neutralizing antibody titers correlate with reduced probability of symptomatic disease. The Dengvaxia Phase 3 clinical trial data demonstrated that neutralizing antibody titers are associated with vaccine efficacy, although with differences by age and/or pre-vaccination immune status [56].

Efforts to relate ADE titers to severe dengue probability have been less successful in cohort studies, possibly due to issues with how ADE is measured *in vitro*. One early success measured enhancement capacity in primary human monocytes [57], but other studies using different cell lines were not able to find an association between ADE level *in vitro* and disease severity [58]. Recently, it has been shown that specific titers of pre-existing anti-DENV binding antibodies are associated with elevated risk of DHF/DSS, controlling for other covariates, in the Nicaraguan cohort [39].

Flavivirus interactions

Cohort studies are ideal for estimating the effect of prior dengue immunity on Zika disease, and will ultimately enable measurement of the effect of ZIKV infection on dengue. The historical finding that anti-Japanese Encephalitis Virus neutralizing antibodies are associated with elevated probability of symptomatic DENV infection is potentially relevant to the current Zika epidemic and the impact it may have on future dengue disease risk [59].

Antibody longevity

Another major value of cohorts is longitudinal sampling that enables measurement of durability and decay of key antibody populations and biomarkers over long periods of time. Hospital-based studies reveal antibody decay out to 6–12 months post-infection, followed by stability or increases in titer at >1 year [60–62]. Studies of cohort samples also show stability or increases in breadth >1 year after primary DENV infection, with less clear trends following secondary infection [27,49,50,63]. Further analyses of the dynamics of antibodies for all individuals in cohorts will improve our understanding of the durability of antibodies following primary, secondary, and post-secondary DENV infection. Systems serology

approaches, as used for evaluation of HIV trial samples, are also promising, especially when high-throughput methods are available [64].

Full cohort analyses

Promising but under-utilized potential immune correlates include the antibody titers (often binding antibodies) measured regularly on all cohort participants. It has now been shown that anti-DENV binding antibodies correlate with protection from symptomatic dengue at high titer and with elevated risk of DHF/DSS at a specific range of low titers [39], suggesting these antibody measurements are more useful than previously thought. Because it is not feasible to run all assays on all cohort participants, a research priority is sampling cohort participants for in-depth analysis in a way that accounts for how well they represent the population as a whole to allow inference of cohort-level effects.

Infection histories

Measuring the rise in anti-DENV binding antibodies [12,13,24,55] and/or neutralizing antibodies [27,49,65], between paired samples has enabled identification of the sequence and number of previous DENV infections. However, cross-reactivity between serotypes, which can be assay-dependent, makes inferring the exact history of infections difficult, and serological responses resembling homologous re-exposures may have been erroneously called heterotypic infections [66,67]. There are now efforts to call infection histories with fewer assumptions and in a more data-driven way, accounting for potential homotypic infections [66,67] and for boosts [50,68] that do not reach the 4-fold rise antibody titer requirement for inapparent infections. For influenza, accounting for measurement error makes it possible to meaningfully interpret 2-fold rises in Hemagglutinin Inhibition titer [69], and a similar approach is in process for dengue (H.Salje, personal communication). Another approach is to estimate timing of infections based on differential antibody decay to distinct antigens, as has been done for malaria [70].

Viral antigenic diversity

Substantial antigenic diversity by genotype and clade exists, both within and between serotypes [71]. In Peru, prior DENV1 immunity differentially neutralized DENV2 genotypes, resulting in mild disease instead of the expected severe dengue epidemic when DENV2 was introduced for the first time [72,73]. A recent study in the same population suggests that immunity to one DENV2 genotype may not protect against another DENV2 genotype [67]. A key finding from the Nicaraguan cohort study was that a particular relationship existed between prior serotype-specific immunity and susceptibility to severe disease with different clades of the secondary infecting serotype [74]. Importantly, there is concern about the effect of genotypic variation on homologous protection – some type-specific antibodies may be so specific that they do not protect against other genotypes of the same serotype [67,75,76], which could affect vaccine efficacy [77].

Novel immunological and virological techniques

Antibodies

Natural infections are critical for understanding vaccine-mediated protection. Tools such as antibody depletions followed by neutralization tests [78,79] and chimeric viruses in which key epitopes are swapped between serotypes enable identification of particular antibody populations and epitopes associated with protection for each serotype [80–82]. A recent study used chimeric viruses and samples from hospital and cohort studies to show that variable proportions of DENV3 neutralizing activity in natural primary DENV3 infections were attributable to the quaternary type-specific epitope 5J7 across individuals, indicating that other DENV3 type-specific epitopes remain to be identified [63]. Investigating whether cross-neutralizing epitopes other than the potentially neutralizing multitypic EDE epitope [83,84] exist is also a major priority.

B cells

Multiple studies have shown significantly more plasmablasts during DENV infection than other febrile illnesses [85], in some cases associated with secondary dengue and disease severity [86]. B cells were found to be type-specific early after primary infection but became more cross-reactive later (6 months), while cross-reactive B cell responses were seen both early and late after secondary infection, often with highest specificity to a serotype other than the secondary infecting serotype [86,87]. A sequencing study of peripheral blood cells from dengue cases across time revealed a convergent antibody signature in the B cell complementarity-determining region 3 enriched in acute-phase samples [88]. Ongoing work is measuring the specificity of B cells to all four DENV serotype plus ZIKV at a single-cell level in the context of a cohort study where prior DENV infection history is known [89,90].

T cells

Early studies suggested that during secondary infection, DENV-specific CD4⁺ and CD8⁺ T cells were mostly heterotypic and became activated, leading to a “cytokine storm”, and then underwent programmed cell death, potentiating severe dengue disease [91,92]. However, more recent studies point to a protective role for T cells. A nested case-control in the Kampheng Phet cohort found higher levels of TNF α -, IFN γ -, and IL-2-producing T cells in those who developed inapparent as compared with symptomatic DENV infection [93]. Other studies used population-based estimates of T cell responses and associated these with previously identified HLA alleles found to be protective in case-control hospital studies [94]. Further T cell profiling of pre-infection samples in cohorts is needed [95], as it is difficult to establish the direction of causality of T cell functionality in acute-phase samples.

Immune profiling

Comprehensive immune profiling of individuals with severe versus non-severe dengue versus inapparent DENV infection is an important area of future research. A recent study identified viremic but asymptomatic individuals using an index cluster design and found that asymptomatic individuals have feedback mechanisms that regulate activation of the adaptive

immune response that facilitates viral clearance as compared to those with symptomatic or severe dengue [96].

Informing vaccine trial design and evaluation

Estimates of DENV infection and dengue incidence from cohort studies enabled sample size and power calculations for Phase 3 dengue vaccine efficacy trials, while an understanding of the patterns of serotype circulation allowed vaccine trials to be designed in locations to ensure sufficient representation of serotypes for estimating serotype-specific vaccine efficacy [10].

Additionally, four critical observations from cohort studies were borne out in recent Phase 3 clinical trials of a dengue vaccine. First, decent vaccine efficacy was estimated up to 12 months after final vaccine dose (25 months post-first dose) [97,98], but elevated risk of hospitalized dengue was observed thereafter in young vaccine recipients [99]. Cohort studies have observed heterotypic protection against symptomatic secondary dengue <2 years after a primary infection but elevated risk of symptomatic and severe dengue 2 years after primary infection [49,100,101]. Second, the observation of dramatically different vaccine efficacy against symptomatic dengue between individuals with prior dengue immunity and individuals who were dengue-naïve [97,98] is consistent with the observation of differential risk of dengue following primary versus secondary DENV infection [32] as well as differences in the composition of neutralizing antibodies after primary versus secondary infections [79]. Third, serotype-specific vaccine efficacy correlated with prevalence of type-specific neutralizing antibodies to that serotype [78], consistent with natural infection studies showing quaternary type-specific potentially neutralizing antibodies after primary infection [79]. However, heterotypic neutralizing antibodies at sufficiently high titers can protect against symptomatic DENV infection in both natural infections and vaccine trials [50,56]. Finally, elevated incidence of severe dengue was observed for seronegative vaccinees compared to placebo controls and seropositive vaccinees >12 months after vaccination [99,102], an effect strongly suggestive of classical ADE [4,103], as observed in cohort studies [39].

Added value of longitudinal cohort studies

Sentinels in outbreak conditions

Cohort studies have proven highly valuable during outbreaks of emerging pathogens, pivoting quickly to address new emerging disease threats. Cohort study personnel, especially in coordination with national health systems, serve as critical human resources for identifying and characterizing emerging pathogens as well as measuring key determinants of novel infectious diseases. Such real-time high-quality data are very useful for public health decision-making by national authorities and international agencies, as well as for the scientific community. For example, the Nicaraguan cohort captured the first pandemic H1N1 case in 2009 in Nicaragua [104], and chikungunya [105] and Zika were added to the cohort study as the respective epidemics emerged. Because the Nicaraguan cohort study is embedded within the Ministry of Health, this enabled analyses of Zika in Nicaragua that furnished actionable data in real time, including studies of age-stratified seroprevalence,

spatial risk factors, the effect of prior DENV immunity on Zika, and outcomes of ZIKV infection in pregnancy.

Diagnostic assay development

Well-characterized samples are an essential resource for development of new diagnostic assays for public sector laboratories, academic studies, and companies. For example, when ZIKV arrived in the Nicaraguan cohort, existing pre-exposure DENV samples and post-exposure ZIKV samples enabled extensive diagnostic assay development and adaptation of existing methods to Zika, including multiplex realtime RT-PCR [106], NS1-based BOB ELISA [107], and Zika IgM-capture and inhibition ELISAs [108]. This enabled the Nicaraguan Ministry of Health to rapidly implement Zika diagnostics and disease surveillance during the epidemic. Samples were also shared widely, enabling multiple novel assays to be developed by other groups [109–112].

Use for other pathogens and risk factors

Long-running cohort studies can be expanded into "multiple use" cohorts, as the infrastructure and design for monitoring one acute viral disease is amenable to studies of other pathogens (e.g., dual dengue-influenza cohort studies; cohorts to study multiple arboviruses) [113,114]. Additionally, a broader conception of health and disease can link upstream determinants (e.g., household and environmental risk factors, socioeconomic status, geospatial data [115–117]) with individual biosignatures (e.g., microbiome, novel antibody repertoire profiling, metabolomics, GWAS, eQTL, viral sequencing) and reveal stable features that are associated with documented outcomes of specific infectious and chronic diseases and overall health patterns. These analyses in turn should suggest actionable interventions between the upstream determinants, the stable biosignatures, and downstream health outcomes.

Research capacity-building

Longitudinal cohort studies are also models of international collaborations that combine local expertise on disease, assay development, and surveillance with international scientific expertise, new technologies, and support [118,119]. Such programs require trust built over time as groups work side-by-side on research that benefits both the local population and the international scientific community. Such collaborations incorporate training opportunities for local students and health professionals [118] and technology transfer (biological as well as information technologies) to enable national and global public health departments to conduct the highest quality infectious disease research and surveillance. Robust involvement and communication with local Ministries of Health are critical so that key information about the diseases in question can be used for public health decision-making and evidence-based policy.

9. Concluding thoughts

Overall, longitudinal cohort studies have provided critical insights into dengue epidemiology, immune correlates, and pathogenesis. Importantly, there are many possibilities for digging deeper into understanding immunity and disease in populations and

impacting public health more broadly, by leveraging and expanding long-running cohorts studying endemic and emerging viral pathogens.

Acknowledgments

We thank past and present members of the study team based at the Centro de Salud Sócrates Flores Vivas, Hospital Infantil Manuel de Jesús Rivera, the Laboratorio Nacional de Virología in the Centro Nacional de Diagnóstico y Referencia de the Ministry of Health, and the Sustainable Sciences Institute in Nicaragua, and the Harris laboratory at the University of California, Berkeley, for their dedication to the Nicaraguan Pediatric Dengue Cohort Study for the last 14 years. We also thank all those who have conducted dengue cohort studies at other sites around the world for their contributions to understanding the epidemiology of dengue.

Funding: This work was supported by National Institutes of Health grants P01AI106695. The funders had no involvement in the writing of this manuscript.

References

1. Mahmood SS, Levy D, Vasan RS, Wang TJ. The Framingham Heart Study and the epidemiology of cardiovascular disease: a historical perspective. *Lancet*. 2014; 383:999–1008. [PubMed: 24084292]
2. Hall CE, Cooney MK, Fox JP. Comparative epidemiologic observations of infections with influenza-A and influenza-B viruses, 1965–1969, in families with young children. *Am J Epidemiol*. 1973; 98:365–380. [PubMed: 4746025]
3. Fauci AS, Marston HD. Ending the HIV–AIDS Pandemic — Follow the Science. *N Engl J Med*. 2015; 373:2197–2199. [PubMed: 26624554]
4. Halstead SB. In vivo enhancement of dengue virus infection in rhesus monkeys by passively transferred antibody. *J Infect Dis*. 1979; 140:527–533. [PubMed: 117061]
5. Halstead SB, O'Rourke EJ. Antibody-enhanced dengue virus infection in primate leukocytes. *Nature*. 1977; 265:739–41. An experimental study that showed that addition of DENV-immune serum enhanced DENV2 infection of peripheral blood leukocytes. The authors proposed the antibody-dependent enhancement hypothesis, which posits that non- or sub-neutralizing antibodies enable DENV to enter and infect mononuclear phagocytic cells to high levels, driving pathogenesis of DHF/DSS. [PubMed: 404559]
6. Halstead SB, O'Rourke EJ. Dengue viruses and mononuclear phagocytes. I. Infection enhancement by non-neutralizing antibody. *J Exp Med*. 1977; 146:201–17. [PubMed: 406347]
7. Halstead SB. Dengue. *Lancet*. 2007; 370:1644–1652. [PubMed: 17993365]
8. Halstead, SB. The Togaviruses. Academic Press, Inc.; 1980. Immunological Parameters of Togavirus Disease Syndromes; p. 107-173.
9. Endy TP, Yoon I-K, Mammen MP. Prospective cohort studies of dengue viral transmission and severity of disease. *Curr Top Microbiol Immunol*. 2010; 338:1–13. [PubMed: 19802574]
10. Endy TP. Human immune responses to dengue virus infection: lessons learned from prospective cohort studies. *Front Immunol*. 2014; 5:183. [PubMed: 24795725]
11. L'Azou M, Moureau A, Sarti E, Nealon J, Zambrano B, Wartel TA, Villar L, Capeding MRZ, Ochiai RL. Symptomatic Dengue in Children in 10 Asian and Latin American Countries. *N Engl J Med*. 2016; 374:1155–1166. [PubMed: 27007959]
12. Gordon A, Kuan G, Mercado JC, Gresh L, Avilés W, Balmaseda A, Harris E. The Nicaraguan pediatric dengue cohort study: incidence of inapparent and symptomatic dengue virus infections, 2004–2010. *PLoS Negl Trop Dis*. 2013; 7:e2462. [PubMed: 24086788]
13. Endy TP, Anderson KB, Nisalak A, Yoon IK, Green S, Rothman AL, Thomas SJ, Jarman RG, Libraty DH, Gibbons RV. Determinants of inapparent and symptomatic dengue infection in a prospective study of primary school children in Kamphaeng Phet, Thailand. *PLoS Negl Trop Dis*. 2011; 5:e975. Analyses of a pediatric cohort study in Thailand showed dramatic variation in the DENV symptomatic:inapparent (S:I) infection ratio over time and between schools. [PubMed: 21390158]

14. Restrepo BN, Piedrahita LD, Agudelo IY, Parra-Henao G, Osorio JE. Frequency and clinical features of dengue infection in a schoolchildren cohort from medellin, Colombia. *J Trop Med*. 2012; 2012:120496. [PubMed: 23304167]
15. Anderson KB, Chunsuttiwat S, Nisalak A, Mammen MP, Libraty DH, Rothman AL, Green S, Vaughn DW, Ennis FA, Endy TP. Burden of symptomatic dengue infection in children at primary school in Thailand: a prospective study. *Lancet*. 2007; 369:1452–1459. [PubMed: 17467515]
16. Runge-Ranzinger S, McCall PJ, Kroeger A, Horstick O. Dengue disease surveillance: An updated systematic literature review. *Trop Med Int Heal*. 2014; 19:1116–1160.
17. Undurraga EA, Edillo FE, Erasmo JN V, Alera MTP, Yoon IK, Largo FM, Shepard DS. Disease burden of dengue in the Philippines: Adjusting for underreporting by comparing active and passive dengue surveillance in Punta Princesa, Cebu City. *Am J Trop Med Hyg*. 2017; 96:887–898. [PubMed: 28093542]
18. Standish K, Kuan G, Avilés W, Balmaseda A, Harris E. High dengue case capture rate in four years of a cohort study in Nicaragua compared to national surveillance data. *PLoS Negl Trop Dis*. 2010; 4:2–6.
19. Wichmann O, Yoon IK, Vong S, Limkittikul K, Gibbons RV, Mammen MP, Ly S, Buchy P, Sirivichayakul C, Buathong R, et al. Dengue in Thailand and Cambodia: An assessment of the degree of underrecognized disease burden based on reported cases. *PLoS Negl Trop Dis*. 2011; 5:1–9.
20. Stanaway JD, Shepard DS, Undurraga EA, Halasa YA, Coffeng LE, Brady OJ, Hay SI, Bedi N, Bensenor IM, Castañeda-Orjuela CA, et al. The global burden of dengue: an analysis from the Global Burden of Disease Study 2013. *Lancet Infect Dis*. 2016; 16:712–723. [PubMed: 26874619]
21. Bhatt S, Gething PW, Brady OJ, Messina JP, Farlow AW, Moyes CL, Drake JM, Brownstein JS, Hoen AG, Sankoh O, et al. The global distribution and burden of dengue. *Nature*. 2013; 496:504–7. [PubMed: 23563266]
22. Sarti E, L’Azou M, Mercado M, Kuri P, Siqueira JB, Solis E, Noriega F, Ochiai RL. A comparative study on active and passive epidemiological surveillance for dengue in five countries of Latin America. *Int J Infect Dis*. 2016; 44:44–49. [PubMed: 26836763]
23. Endy TP, Nisalak A, Chunsuttiwat S, Libraty DH, Green S, Rothman AL, Vaughn DW, Ennis FA. Spatial and temporal circulation of dengue virus serotypes: a prospective study of primary school children in Kamphaeng Phet, Thailand. *Am J Epidemiol*. 2002; 156:52–9. [PubMed: 12076888]
24. Alera MT, Srikiatkachorn A, Velasco JM, Tac-An IA, Lago CB, Clapham HE, Fernandez S, Levy JW, Thaisomboonsuk B, Klungthong C, et al. Incidence of Dengue Virus Infection in Adults and Children in a Prospective Longitudinal Cohort in the Philippines. *PLoS Negl Trop Dis*. 2016; 10:1–14. Analyses of a cohort study of individuals age 6 months to >50 years in the Philippines showed that the incidence of inapparent DENV infections and symptomatic dengue decreased with age, pointing to both protection against infection and disease at older ages in areas with a high force of infection.
25. Graham RR, Juffrie M, Tan R, Hayes CG, Laksono I, Ma’roef C, Erlin, Sutaryo, Porter KR, Halstead SB. A prospective seroepidemiologic study on dengue in children four to nine years of age in Yogyakarta, Indonesia I. Studies in 1995–1996. *Am J Trop Med Hyg*. 1999; 61:412–419. Analyses of a pediatric cohort study in Indonesia showed high infection rates with all four serotypes and that multiple serotype infection sequences caused severe dengue disease. [PubMed: 10497982]
26. Endy TP, Chunsuttiwat S, Nisalak A, Libraty DH, Green S, Rothman AL, Vaughn DW, Ennis FA. 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. [PubMed: 12076887]
27. Morrison AC, Minnick SL, Rocha C, Forshey BM, Stoddard ST, Getis A, Focks DA, Russell KL, Olson JG, Blair PJ, et al. Epidemiology of dengue virus in Iquitos, Peru 1999 to 2005: interepidemic and epidemic patterns of transmission. *PLoS Negl Trop Dis*. 2010; 4:e670. A community-based cohort study of children and adults in Peru showed that neighborhoods with prior high pre-existing anti-DENV seroprevalence were the first infected during the introduction of a new serotype, DENV3, suggesting certain areas were at higher risk of arboviral transmission. [PubMed: 20454609]

28. Grange L, Simon-Loriere E, Sakuntabhai A, Gresh L, Paul R, Harris E. Epidemiological risk factors associated with high global frequency of inapparent dengue virus infections. *Front Immunol.* 2014; 5:1–10. [PubMed: 24474949]
29. Halstead SB. Dengue hemorrhagic fever: two infections and antibody dependent enhancement, a brief history and personal memoir. *Rev Cubana Med Trop.* 2002; 54:171–9. [PubMed: 15846943]
30. Thein S, Aung MM, Shwe TN, Aye M, Zaw A, Aye K, Aye KM, Aaskov J. Risk factors in dengue shock syndrome. *Am J Trop Med Hyg.* 1997; 56:566–572. A community- and school-based cohort study in Myanmar confirmed previous observations of high relative risk of DSS during secondary DENV infection as compared to primary DENV infection, and greater risk of DSS associated with secondary DENV2 compared with DENV1, 3, or 4 infections. [PubMed: 9180609]
31. Burke DS, Nisalak A, Johnson DE, Scott McN R. A prospective study of dengue infections in Bangkok. *Am J Trop Med Hyg.* 1988; 38:172–180. [PubMed: 3341519]
32. Sangkawibha N, Rojanasuphot S, Ahandrik S, Viriyapongse S, Jatanasen S, Salitul V, Phanthumachinda B, Halstead SB. Risk factors in dengue shock syndrome: a prospective epidemiologic study in Rayong, Thailand. I. The 1980 outbreak. *Am J Epidemiol.* 1984; 120:653–669. The first large pediatric dengue cohort study, conducted in Thailand, which established that DSS was primarily identified in those experiencing secondary DENV infections and that secondary infection with DENV2 was strong risk factor for DSS. [PubMed: 6496446]
33. Clapham HE, Cummings DAT, Johansson MA. Immune status alters the probability of apparent illness due to dengue virus infection: Evidence from a pooled analysis across multiple cohort and cluster studies. *PLoS Negl Trop Dis.* 2017; 11:1–12.
34. Biswas HH, Ortega O, Gordon A, Standish K, Balmaseda A, Kuan G, Harris E. Early clinical features of dengue virus infection in nicaraguan children: a longitudinal analysis. *PLoS Negl Trop Dis.* 2012; 6:e1562. [PubMed: 22413033]
35. Olkowski S, Forshey BM, Morrison AC, Rocha C, Vilcarromero S, Halsey ES, Kochel TJ, Scott TW, Stoddard ST. Reduced risk of disease during postsecondary dengue virus infections. *J Infect Dis.* 2013; 208:1026–33. [PubMed: 23776195]
36. Liebman KA, Stoddard ST, Reiner RC, Perkins TA, Astete H, Sihuinchu M, Halsey ES, Kochel TJ, Morrison AC, Scott TW. Determinants of heterogeneous blood feeding patterns by *Aedes aegypti* in Iquitos, Peru. *PLoS Negl Trop Dis.* 2014; 8:e2702. [PubMed: 24551262]
37. Harrington LC, Fleisher A, Ruiz-Moreno D, Vermeylen F, Wa CV, Poulson RL, Edman JD, Clark JM, Jones JW, Kitthawee S, et al. Heterogeneous feeding patterns of the dengue vector, *Aedes aegypti*, on individual human hosts in rural Thailand. *PLoS Negl Trop Dis.* 2014; 8:e3048. [PubMed: 25102306]
38. Fischer DB, Halstead SB. Observations related to pathogenesis of dengue hemorrhagic fever. V. Examination of age-specific sequential infection rates using a mathematical model. *Yale J Biol Med.* 1970; 42:329–349. [PubMed: 5419207]
39. Katzelnick LC, Gresh L, Halloran ME, Mercado JC, Kuan G, Gordon A, Balmaseda A, Harris E. Antibody-dependent enhancement of severe dengue disease in humans. *Science.* 2017; 358:929–932. Analysis of a Nicaraguan pediatric cohort study showed an ADE-like relationship between a narrow range of pre-existing anti-DENV binding antibodies and elevated risk of DHF/DSS. In contrast, protection against symptomatic dengue was observed in individuals with high pre-existing anti-DENV binding antibody titers. [PubMed: 29097492]
40. Jarman RG, Holmes EC, Rodpradit P, Klungthong C, Gibbons R V, Nisalak A, Rothman AL, Libraty DH, Ennis FA, Mammen MP, et al. Microevolution of Dengue Viruses Circulating among Primary School Children in Kamphaeng Phet, Thailand. *J Virol.* 2008; 82:5494–5500. [PubMed: 18367520]
41. Rabaa MA, Klungthong C, Yoon IK, Holmes EC, Chinnawirotpisan P, Thaisomboonsuk B, Srikiatkachorn A, Rothman AL, Tannitisupawong D, Aldstadt J, et al. Frequent In-Migration and Highly Focal Transmission of Dengue Viruses among Children in Kamphaeng Phet, Thailand. *PLoS Negl Trop Dis.* 2013; 7:1–14.
42. Bhoomiboonchoo P, Gibbons R V, Huang A, Yoon IK, Buddhari D, Nisalak A, Chansatiporn N, Thipayamongkolgul M, Kalanarooj S, Endy T, et al. The Spatial Dynamics of Dengue Virus in Kamphaeng Phet, Thailand. *PLoS Negl Trop Dis.* 2014; 8:6–11.

43. Liebman, Ka, Stoddard, ST., Morrison, AC., Rocha, C., Minnick, S., Sihuinchu, M., Russell, KL., Olson, JG., Blair, PJ., Watts, DM., et al. Spatial Dimensions of Dengue Virus Transmission across Interepidemic and Epidemic Periods in Iquitos, Peru (1999–2003). *PLoS Negl Trop Dis*. 2012; 6:e1472. [PubMed: 22363822]
44. Reiner RC, Stoddard ST, Forshey BM, King AA, Ellis AM, Lloyd AL, Long KC, Rocha C, Vilcarromero S, Astete H, et al. Time-varying, serotype-specific force of infection of dengue virus. *Proc Natl Acad Sci U S A*. 2014; 111:E2694–702. [PubMed: 24847073]
45. Imai N, Dorigatti I, Cauchemez S, Ferguson NM. Estimating Dengue Transmission Intensity from Sero-Prevalence Surveys in Multiple Countries. *PLoS Negl Trop Dis*. 2015; 9:1–19.
46. Ferguson NM, Donnelly Ca, Anderson RM. Transmission dynamics and epidemiology of dengue: insights from age-stratified sero-prevalence surveys. *Philos Trans R Soc Lond B Biol Sci*. 1999; 354:757–768. [PubMed: 10365401]
47. Simmons CP, McPherson K, Van Vinh Chau N, Hoai Tam DT, Young P, Mackenzie J, Wills B. Recent advances in dengue pathogenesis and clinical management. *Vaccine*. 2015; 33:7061–7068. [PubMed: 26458808]
48. Balmaseda A, Hammond SN, Pérez L, Tellez Y, Saborío SI, Mercado JC, Cuadra R, Rocha J, Pérez MA, Silva S, et al. Serotype-specific differences in clinical manifestations of dengue. *Am J Trop Med Hyg*. 2006; 74:449–456. [PubMed: 16525106]
49. Montoya M, Gresh L, Mercado JC, Williams KL, Vargas MJ, Gutierrez G, Kuan G, Gordon A, Balmaseda A, Harris E. Symptomatic versus inapparent outcome in repeat dengue virus infections is influenced by the time interval between infections and study year. *PLoS Negl Trop Dis*. 2013; 7:e2357. [PubMed: 23951377]
50. Katzelnick LC, Montoya M, Gresh L, Balmaseda A, Harris E. Neutralizing antibody titers against dengue virus correlate with protection from symptomatic infection in a longitudinal cohort. *Proc Natl Acad Sci U S A*. 2016; 113:728–33. A subset analysis of a Nicaraguan pediatric cohort study showed that level of pre-existing neutralizing antibody titer was associated with the probability of having a symptomatic as opposed to inapparent DENV infection, with interacting factors including the annual S:I ratio and time between infections. [PubMed: 26729879]
51. Endy TP, Nisalak A, Chunsuttiwat S, Vaughn DW, Green S, Ennis FA, Rothman AL, Libraty DH. Relationship of preexisting dengue virus (DV) neutralizing antibody levels to viremia and severity of disease in a prospective cohort study of DV infection in Thailand. *J Infect Dis*. 2004; 189:990–1000. A nested case-control analysis of non-severe and severe dengue cases from a pediatric cohort study in Thailand showed that the level of pre-infection neutralizing antibodies to the secondary infecting viral isolate was associated with reduced severity of secondary DENV3 but not DENV2 infection. [PubMed: 14999601]
52. Laoprasopwattana K, Libraty DH, Endy TP, Nisalak A, Chunsuttiwat S, Ennis FA, Rothman AL, Green S. Antibody-dependent cellular cytotoxicity mediated by plasma obtained before secondary dengue virus infections: potential involvement in early control of viral replication. *J Infect Dis*. 2007; 195:1108–16. [PubMed: 17357046]
53. Sirivichayakul C, Sabchareon A, Limkittikul K, Yoksan S. Plaque reduction neutralization antibody test does not accurately predict protection against dengue infection in Ratchaburi cohort, Thailand. *Viol J*. 2014; 11:48. [PubMed: 24620925]
54. Buddhari D, Aldstadt J, Endy TP, Srikiatkachorn A, Thaisomboonsuk B, Klungthong C, Nisalak A, Khuntirat B, Jarman RG, Fernandez S, et al. Dengue virus neutralizing antibody levels associated with protection from infection in Thai cluster studies. *PLoS Negl Trop Dis*. 2014; 8:e3230. [PubMed: 25329173]
55. Corbett KS, Katzelnick L, Tissera H, Amerasinghe A, de Silva AD, de Silva AM. Preexisting neutralizing antibody responses distinguish clinically inapparent and apparent dengue virus infections in a Sri Lankan pediatric cohort. *J Infect Dis*. 2015; 211:590–9. [PubMed: 25336728]
56. Moodie Z, Juraska M, Huang Y, Zhuang Y, Fong Y, Carpp LN, Self SG, Chambonneau L, Small R, Jackson N, et al. Neutralizing Antibody Correlates Analysis of Tetravalent Dengue Vaccine Efficacy Trials in Asia and Latin America. *J Infect Dis*. 2017; doi: 10.1093/infdis/jix609
57. Kliks SC, Nisalak A, Brandt WE, Wahl L, Burke DS. Antibody-dependent enhancement of dengue virus growth in human monocytes as a risk factor for dengue hemorrhagic fever. *Am J Trop Med Hyg*. 1989; 40:444–51. A case-control analysis of children in a pediatric cohort study in Thailand

showed that undiluted pre-infection serum samples from those who subsequently acquired severe dengue enhanced DENV2 infection in freshly isolated primary human monocytes significantly more than serum from those who subsequently developed inapparent DENV infections. [PubMed: 2712199]

58. Laoprasopwattana K, Libraty DH, Endy TP, Nisalak A, Chunsuttiwat S, Vaughn DW, Reed G, Ennis FA, Rothman AL, Green S. Dengue Virus (DV) enhancing antibody activity in preillness plasma does not predict subsequent disease severity or viremia in secondary DV infection. *J Infect Dis.* 2005; 192:510–9. [PubMed: 15995967]
59. Anderson KB, Gibbons RV, Thomas SJ, Rothman AL, Nisalak A, Berkelman RL, Libraty DH, Endy TP. Preexisting Japanese encephalitis virus neutralizing antibodies and increased symptomatic dengue illness in a school-based cohort in Thailand. *PLoS Negl Trop Dis.* 2011; 5:e1311. [PubMed: 21991398]
60. Clapham HE, Quyen TH, Kien DTH, Dorigatti I, Simmons CP, Ferguson NM. Modelling Virus and Antibody Dynamics during Dengue Virus Infection Suggests a Role for Antibody in Virus Clearance. *PLoS Comput Biol.* 2016; 12:1–15.
61. Lai CY, Williams KL, Wu YC, Knight S, Balmaseda A, Harris E, Wang WK. Analysis of Cross-Reactive Antibodies Recognizing the Fusion Loop of Envelope Protein and Correlation with Neutralizing Antibody Titers in Nicaraguan Dengue Cases. *PLoS Negl Trop Dis.* 2013; 7:1–11.
62. Puschnik A, Lau L, Cromwell EA, Balmaseda A, Zompi S, Harris E. Correlation between dengue-specific neutralizing antibodies and serum avidity in primary and secondary dengue virus 3 natural infections in humans. *PLoS Negl Trop Dis.* 2013; 7:e2274. [PubMed: 23785536]
63. Andrade DV, Katzelnick LC, Widman DG, Balmaseda A, de Silva AM, Baric RS, Harris E. Analysis of Individuals from a Dengue-Endemic Region Helps Define the Footprint and Repertoire of Antibodies Targeting Dengue Virus 3 Type-Specific Epitopes. *MBio.* 2017; 8:e01205–17. [PubMed: 28928210]
64. Ackerman ME, Barouch DH, Alter G. Systems serology for evaluation of HIV vaccine trials. *Immunol Rev.* 2017; 275:262–270. [PubMed: 28133810]
65. van Panhuis WG, Gibbons RV, Endy TP, Rothman AL, Srikiatkachorn A, Nisalak A, Burke DS, Cummings DAT. Inferring the serotype associated with dengue virus infections on the basis of pre- and postinfection neutralizing antibody titers. *J Infect Dis.* 2010; 202:1002–10. [PubMed: 20738205]
66. Waggoner JJ, Balmaseda A, Gresh L, Sahoo MK, Montoya M, Wang C, Abeynayake J, Kuan G, Pinsky BA, Harris E. Homotypic Dengue Virus Reinfections in Nicaraguan Children. *J Infect Dis.* 2016; 214:986–93. [PubMed: 26984144]
67. Forshey BM, Reiner RC, Olkowski S, Morrison AC, Espinoza A, Long KC, Vilcarromero S, Casanova W, Wearing HJ, Halsey ES, et al. Incomplete Protection against Dengue Virus Type 2 Re-infection in Peru. *PLoS Negl Trop Dis.* 2016; 10:e0004398. [PubMed: 26848841]
68. Clapham HE, Rodriguez-Barraquer I, Azman AS, Althouse BM, Salje H, Gibbons RV, Rothman AL, Jarman RG, Nisalak A, Thaisomboonsuk B, et al. Dengue virus (DENV) neutralizing antibody kinetics in children after symptomatic primary and postprimary DENV infection. *J Infect Dis.* 2016; 213:1428–1435. [PubMed: 26704615]
69. Cauchemez S, Ferguson NM. Methods to infer transmission risk factors in complex outbreak data. *J R Soc Interface.* 2012; 9:456–469. [PubMed: 21831890]
70. Helb DA, Tetteh KKA, Felgner PL, Skinner J, Hubbard A, Arinaitwe E, Mayanja-Kizza H, Ssewanyana I, Kanya MR, Beeson JG, et al. Novel serologic biomarkers provide accurate estimates of recent *Plasmodium falciparum* exposure for individuals and communities. *Proc Natl Acad Sci.* 2015; 112:E4438–E4447. [PubMed: 26216993]
71. Katzelnick LC, Fonville JM, Gromowski GD, Bustos Arriaga J, Green A, James SL, Lau L, Montoya M, Wang C, VanBlargan LA, et al. Dengue viruses cluster antigenically but not as discrete serotypes. *Science.* 2015; 349:1338–43. [PubMed: 26383952]
72. Kochel TJ, Watts DM, Halstead SB, Hayes CG, Espinoza A, Felices V, Caceda R, Bautista CT, Montoya Y, Douglas S, et al. Effect of dengue-1 antibodies on American dengue-2 viral infection and dengue haemorrhagic fever. *Lancet.* 2002; 360:310–2. [PubMed: 12147378]

73. Watts DM, Porter KR, Putvatana P, Vasquez B, Calampa C, Hayes CG, Halstead SB. Failure of secondary infection with American genotype dengue 2 to cause dengue haemorrhagic fever. *Lancet*. 1999; 354:1431–1434. A student cohort study in Peru showed high incidence of secondary infection with American genotype DENV2 in those with pre-existing DENV1 immunity, but unexpectedly no cases of DHF/DSS, suggesting that prior DENV1 immunity differentially protected against severe dengue in infections with the DENV2 American genotype as opposed to the Asian genotype. [PubMed: 10543670]
74. OhAinle M, Balmaseda A, Macalalad AR, Tellez Y, Zody MC, Saborío S, Nuñez A, Lennon NJ, Birren BW, Gordon A, et al. Dynamics of dengue disease severity determined by the interplay between viral genetics and serotype-specific immunity. *Sci Transl Med*. 2011; 3:114ra128. An analysis of pediatric cohort and hospital dengue studies in Nicaragua demonstrated specific interaction between primary infections with either DENV1 or DENV3 and subsequent risk of severe dengue during infection with one clade of DENV2 as compared to another.
75. Messer WB, Yount B, Hacker KE, Donaldson EF, Huynh JP, de Silva AM, Baric RS. Development and characterization of a reverse genetic system for studying dengue virus serotype 3 strain variation and neutralization. *PLoS Negl Trop Dis*. 2012; 6:e1486. [PubMed: 22389731]
76. Wahala WMPB, Donaldson EF, de Alwis R, Accavitti-Loper MA, Baric RS, de Silva AM. Natural strain variation and antibody neutralization of dengue serotype 3 viruses. *PLoS Pathog*. 2010; 6:e1000821. [PubMed: 20333252]
77. Magaret C, M J, Shao J, Carpp L, Fiore-Gartland A, Benkeser D, Girerd-Chambaz Y, Langevin E, Frago C, Guy B, et al. Viral genetic diversity and protective efficacy of a CYD-TDV tetravalent dengue vaccine in a phase 3 trial in Asia. American Society for Tropical Medicine and Hygiene Annual Meeting. 2017 Abstract #622.
78. Henein S, Swanstrom J, Byers AM, Moser JM, Shaik SF, Bonaparte M, Jackson N, Guy B, Baric R, de Silva AM. Dissecting antibodies induced by a chimeric yellow fever-dengue, live-attenuated, tetravalent dengue vaccine (CYD-TDV) in naïve and dengue exposed individuals. *J Infect Dis*. 2016; 7292:351–358.
79. Patel B, Longo P, Miley MJ, Montoya M, Harris E, de Silva AM. Dissecting the human serum antibody response to secondary dengue virus infections. *PLoS Negl Trop Dis*. 2017; 11:e0005554. [PubMed: 28505154]
80. Messer WB, Yount BL, Royal SR, de Alwis R, Widman DG, Smith SA, Crowe JE, Pfaff JM, Kahle KM, Doranz BJ, et al. Functional transplant of a dengue virus serotype 3 (DENV3)-specific human monoclonal antibody epitope into DENV1. *J Virol*. 2016; 90:5090–7. [PubMed: 26962223]
81. Gallichotte EN, Menachery VD, Yount BL, Widman DG, Dinnon KH, Hartman S, de Silva AM, Baric RS. Epitope Addition and Ablation via Manipulation of a Dengue Virus Serotype 1 Infectious Clone. *mSphere*. 2017; 2:1–11.
82. Gallichotte EN, Widman DG, Yount BL, Wahala WM, Durbin A, Whitehead S, Sariol CA, Crowe JE, de Silva AM, Baric RS. A new quaternary structure epitope on dengue virus serotype 2 is the target of durable type-specific neutralizing antibodies. *MBio*. 2015; 6:e01461–15. [PubMed: 26463165]
83. Dejnirattisai W, Wongwiwat W, Supasa S, Zhang X, Dai X, Rouvinsky A, Jumnainsong A, Edwards C, Quyen NTH, Duangchinda T, et al. A new class of highly potent, broadly neutralizing antibodies isolated from viremic patients infected with dengue virus. *Nat Immunol*. 2015; 16:785.
84. Rouvinski A, Guardado-Calvo P, Barba-Spaeth G, Duquerroy S, Vaney M-C, Kikuti CM, Navarro Sanchez ME, Dejnirattisai W, Wongwiwat W, Haouz A, et al. Recognition determinants of broadly neutralizing human antibodies against dengue viruses. *Nature*. 2015; 520:109–13. [PubMed: 25581790]
85. Garcia-Bates TM, Cordeiro MT, Nascimento EJM, Smith AP, Soares de Melo KM, McBurney SP, Evans JD, Marques ETA, Barratt-Boyes SM. Association between Magnitude of the Virus-Specific Plasmablast Response and Disease Severity in Dengue Patients. *J Immunol*. 2013; 190:80–87. [PubMed: 23203929]
86. Zompi S, Montoya M, Pohl MO, Balmaseda A, Harris E. Dominant cross-reactive B cell response during secondary acute dengue virus infection in humans. *PLoS Negl Trop Dis*. 2012; 6:e1568. [PubMed: 22448292]

87. Mathew A, West K, Kalayanarooj S, Gibbons RV, Srikiatkachorn A, Green S, Libraty D, Jaiswal S, Rothman AL. B-cell responses during primary and secondary dengue virus infections in humans. *J Infect Dis.* 2011; 204:1514–22. [PubMed: 21930609]
88. Parameswaran P, Liu Y, Roskin KM, Jackson KKL, Dixit VP, Lee J-Y, Artiles KL, Zompi S, Vargas MJ, Simen BB, et al. Convergent antibody signatures in human dengue. *Cell Host Microbe.* 2013; 13:691–700. [PubMed: 23768493]
89. Hadjilaou A, Green AM, Coloma J, Harris E. Single-Cell Analysis of B Cell/Antibody Cross-Reactivity Using a Novel Multicolor FluoroSpot Assay. *J Immunol.* 2015; 195:3490–3496. [PubMed: 26320246]
90. Andrade, P., Coloma, J., Harris, E. ELISPOT-based “Multi-Color FluoroSpot” to study type-specific and cross-reactive responses in memory B cells after dengue and Zika virus infections. In: Kalyuzhny, AE., editor. *Handbook of Elispot: Methods and Protocols.* 2018.
91. Mangada MM, Endy TP, Nisalak A, Chunsuttiwat S, Vaughn DW, Libraty DH, Green S, Ennis Fa, Rothman AL. Dengue-specific T cell responses in peripheral blood mononuclear cells obtained prior to secondary dengue virus infections in Thai schoolchildren. *J Infect Dis.* 2002; 185:1697–1703. [PubMed: 12085313]
92. Mongkolsapaya J, Dejnirattisai W, Xu X, Vasanawathana S, Tangthawornchaikul N, Chairunsri A, Sawasdivorn S, Duangchinda T, Dong T, Rowland-Jones S, et al. Original antigenic sin and apoptosis in the pathogenesis of dengue hemorrhagic fever. *Nat Med.* 2003; 9:921–927. [PubMed: 12808447]
93. Hatch S, Endy TP, Thomas S, Mathew A, Potts J, Pazoles P, Libraty DH, Gibbons R, Rothman AL. Intracellular cytokine production by dengue virus-specific T cells correlates with subclinical secondary infection. *J Infect Dis.* 2011; 203:1282–1291. [PubMed: 21335561]
94. Weiskopf D, Angelo MA, de Azeredo EL, Sidney J, Greenbaum JA, Fernando AN, Broadwater A, Kolla RV, De Silva AD, de Silva AM, et al. Comprehensive analysis of dengue virus-specific responses supports an HLA-linked protective role for CD8+ T cells. *Proc Natl Acad Sci.* 2013; 110:E2046–E2053. [PubMed: 23580623]
95. Rothman AL. Immunity to dengue virus: a tale of original antigenic sin and tropical cytokine storms. *Nat Rev Immunol.* 2011; 11:532–543. [PubMed: 21760609]
96. Simon-Lorière E, Duong V, Tawfik A, Ung S, Ly S, Casadémont I, Prot M, Courtejoie N, Bleakley K, Buchy P, et al. Increased adaptive immune responses and proper feedback regulation protect against clinical dengue. *Sci Transl Med.* 2017; 9:eaa15088. [PubMed: 28855396]
97. Villar L, Dayan GH, Arredondo-García JL, Rivera DM, Cunha R, Deseda C, Reynales H, Costa MS, Morales-Ramírez JO, Carrasquilla G, et al. Efficacy of a tetravalent dengue vaccine in children in Latin America. *N Engl J Med.* 2015; 372:113–123. [PubMed: 25365753]
98. Capeding MR, Tran NH, Hadinegoro SRS, Ismail HIHM, Chotpitayasunondh T, Chua MN, Luong CQ, Rusmil K, Wirawan DN, Nallusamy R, et al. Clinical efficacy and safety of a novel tetravalent dengue vaccine in healthy children in Asia: a phase 3, randomised, observer-masked, placebo-controlled trial. *Lancet.* 2014; 6736:1–8.
99. Hadinegoro SR, Arredondo-García JL, Capeding MR, Deseda C, Chotpitayasunondh T, Dietze R, Muhammad Ismail HIH, Reynales H, Limkittikul K, Rivera-Medina DM, et al. Efficacy and Long-Term Safety of a Dengue Vaccine in Regions of Endemic Disease. *N Engl J Med.* 2015; 373:1195–206. [PubMed: 26214039]
100. Anderson KB, Gibbons RV, Cummings DAT, Nisalak A, Green S, Libraty DH, Jarman RG, Srikiatkachorn A, Mammen MP, Darunee B, et al. A shorter time interval between first and second dengue infections is associated with protection from clinical illness in a school-based cohort in Thailand. *J Infect Dis.* 2014; 209:360–8. Analysis of a pediatric cohort study in Thailand showed that the average time from primary DENV infection to inapparent secondary DENV infection was significantly less than the time to symptomatic or severe secondary dengue, suggesting that heterotypic antibodies wane from protective to potentially harmful levels. [PubMed: 23964110]
101. Reich NG, Shrestha S, King AA, Rohani P, Lessler J, Kalayanarooj S, Yoon I-K, Gibbons RV, Burke DS, Cummings DAT. Interactions between serotypes of dengue highlight epidemiological impact of cross-immunity. *J R Soc Interface.* 2013; 10:20130414. [PubMed: 23825116]

102. Sanofi Pasteur. New analysis of long-term Dengvaxia® data found differences in vaccine performance based on prior dengue infection - Company will ask regulators to update product label to reflect new information.
103. Halstead SB. Dengvaxia sensitizes seronegatives to vaccine enhanced disease regardless of age. *Vaccine*. 2017; 35:6355–6358. [PubMed: 29029938]
104. Ng S, Lopez R, Kuan G, Gresh L, Balmaseda A, Harris E, Gordon A. The Timeline of Influenza Virus Shedding in Children and Adults in a Household Transmission Study of Influenza in Managua, Nicaragua. *Pediatr Infect Dis J*. 2016; 35:583–586. [PubMed: 26910589]
105. Kuan G, Ramirez S, Gresh L, Ojeda S, Melendez M, Sanchez N, Collado D, Garcia N, Mercado JC, Gordon A, et al. Seroprevalence of Anti-Chikungunya Virus Antibodies in Children and Adults in Managua, Nicaragua, After the First Chikungunya Epidemic, 2014–2015. *PLoS Negl Trop Dis*. 2016; 10:e0004773. [PubMed: 27322692]
106. Waggoner JJ, Gresh L, Vargas MJ, Ballesteros G, Tellez Y, Soda KJ, Sahoo MK, Nuñez A, Balmaseda A, Harris E, et al. Viremia and Clinical Presentation in Nicaraguan Patients Infected With Zika Virus, Chikungunya Virus, and Dengue Virus. *Clin Infect Dis*. 2016; 63:1584–1590. [PubMed: 27578819]
107. Balmaseda A, Stettler K, Medialdea-Carrera R, Collado D, Jin X, Zambrana JV, Jaconi S, Cameroni E, Saborio S, Rovida F, et al. Antibody-based assay discriminates Zika virus infection from other flaviviruses. *Proc Natl Acad Sci U S A*. 2017; 114:8384–8389. [PubMed: 28716913]
108. Balmaseda A, Zambrana JV, Collado D, Garcia N, Saborío S, Elizondo D, Mercado JC, Gonzalez K, Cerpas C, Nuñez A, et al. Comparison of four serological methods and two RT-PCR assays for diagnosis and surveillance of Zika. *J Clin Microbiol*. 2018; doi: 10.1128/JCM.01785-17
109. Mishra N, Caciula A, Price A, Thakkar R, Ng J, Chauhan L, Jain K, Espinosa D, Montoya Cruz M, Balmaseda A, et al. Diagnosis of Zika virus infection by peptide array and ELISA. *MBio*. In press.
110. Premkumar L, Collins M, Graham S, Liou G-JA, Lopez CA, Jadi R, Balmaseda A, Brackbill JA, Dietze R, Camacho E, et al. Development of envelope protein antigens to serologically differentiate Zika from dengue virus infection. *J Clin Microbiol*. 2017; doi: 10.1128/JCM.01504-17
111. Chotiwan N, Brewster CD, Magalhaes T, Weger-Lucarelli J, Duggal NK, Rückert C, Nguyen C, Luna SMG, Fauver JR, Andre B, et al. Rapid and specific detection of Asian-and African-lineage Zika viruses. 2017; 538:1–14.
112. Tsai WY, Youn HH, Brites C, Tsai JJ, Tyson J, Pedroso C, Drexler JF, Stone M, Simmons G, Busch MP, et al. Distinguishing Secondary Dengue Virus Infection from Zika Virus Infection with Previous Dengue by a Combination of 3 Simple Serological Tests. *Clin Infect Dis*. 2017; 65:1829–1836. [PubMed: 29020159]
113. Kuan G, Gordon A, Aviles W, Ortega O, Hammond SN, Elizondo D, Nunez A, Coloma J, Balmaseda A, Harris E. The Nicaraguan Pediatric Dengue Cohort Study: study design, methods, use of information technology, and extension to other infectious diseases. *Am J Epidemiol*. 2009; 170:120–129. [PubMed: 19435864]
114. Khuntirat B, Love CS, Buddhari D, Heil GL, Gibbons RV, Rothman AL, Srikiatkachorn A, Gray GC, Yoon IK. Absence of neutralizing antibodies against influenza A/H5N1 virus among children in Kamphaeng Phet, Thailand. *J Clin Virol*. 2015; 69:78–80. [PubMed: 26209384]
115. Toledo J, George L, Martinez E, Lazaro A, Han WW, Coelho GE, Runge Ranzinger S, Horstick O. Relevance of Non-communicable Comorbidities for the Development of the Severe Forms of Dengue: A Systematic Literature Review. *PLoS Negl Trop Dis*. 2016; 10:1–18.
116. Alsan MM, Westerhaus M, Herce M, Nakashima K, Farmer PE. Poverty, Global Health, and Infectious Disease: Lessons from Haiti and Rwanda. *Infect Dis Clin North Am*. 2011; 25:611–622. [PubMed: 21896362]
117. Ezzati M, Lopez AD, Rodgers A, Hoorn S Vander, Murray CJL, Risk C. Selected major risk factors and global and regional burden of disease. 2002; 360:1347–1360.
118. Coloma J, Harris E. Sustainable Transfer of Biotechnology to Developing Countries. *Ann N Y Acad Sci*. 2008; 1136:358–368. [PubMed: 17954678]

119. Gibbons RV, Nisalak A, Yoon IK, Tannitisupawong D, Rungsimunpaiboon K, Vaughn DW, Endy TP, Innis BL, Burke DS, Mammen MP, et al. A model international partnership for community-based research on vaccine-preventable diseases: The Kamphaeng Phet-AFRIMS Virology Research Unit (KAVRU). *Vaccine*. 2013; 31:4487–4500. [PubMed: 23933334]
120. Halstead SB, Scanlon JE, Umpaivit P, Udomsakdi S. Dengue and chikungunya virus infection in man in Thailand, 1962–1964. IV. Epidemiologic studies in the Bangkok metropolitan area. *Am J Trop Med Hyg*. 1969; 18:997–1021. The first dengue cohort study, which provided key insights into dengue, including high incidence of infection, high levels of hospitalized dengue in 6–12-month old infants and children ages 3–4, and isolation of DENV primarily in *Aedes aegypti* mosquitoes. [PubMed: 4390977]
121. Russell PK, Yuill TM, Nisalak A, Udomsakdi S, Gould DJ, Winter PE. An insular outbreak of dengue hemorrhagic fever. II. Virologic and serologic studies. *Am J Trop Med Hyg*. 1968; 17:600–608. [PubMed: 4970512]
122. Porter KR, Beckett CG, Kosasih H, Tan RI, Alisjahbana B, Rudiman PIF, Widjaja S, Listiyaningsih E, Ma'Roef CN, McArdle J, et al. Epidemiology Of Dengue And Dengue Hemorrhagic Fever In A Cohort Of Adults Living In Bandung, West Java, Indonesia. *Am J Trop Med Hyg*. 2005; 72:60–66. [PubMed: 15728868]
123. Comach G, Blair PJ, Sierra G, Guzman D, Soler M, Quintana MC de, Bracho-Labadie M, Camacho D, Russell KL, Olson JG, et al. Dengue Virus Infections in a Cohort of Schoolchildren from Maracay, Venezuela: A 2-Year Prospective Study. *Vector-Borne Zoonotic Dis*. 2009; 9:87–92. [PubMed: 18788903]
124. Balmaseda A, Hammond SN, Tellez Y, Imhoff L, Rodriguez Y, Saborío SI, Mercado JC, Perez L, Videá E, Almanza E, et al. High seroprevalence of antibodies against dengue virus in a prospective study of schoolchildren in Managua, Nicaragua. *Trop Med Int Heal*. 2006; 11:935–942.
125. Mammen MP, Pimgate C, Koenraadt CJM, Rothman AL, Aldstadt J, Nisalak A, Jarman RG, Jones JW, Srikiatkachorn A, Ypil-Butac CA, et al. Spatial and temporal clustering of dengue virus transmission in Thai villages. *PLoS Med*. 2008; 5:e205. [PubMed: 18986209]
126. Yoon I-K, Getis A, Aldstadt J, Rothman AL, Tannitisupawong D, Koenraadt CJM, Fansiri T, Jones JW, Morrison AC, Jarman RG, et al. Fine scale spatiotemporal clustering of dengue virus transmission in children and *Aedes aegypti* in rural Thai villages. *PLoS Negl Trop Dis*. 2012; 6:e1730. [PubMed: 22816001]
127. Yoon I-K, Rothman AL, Tannitisupawong D, Srikiatkachorn A, Jarman RG, Aldstadt J, Nisalak A, Mammen MP, Thammapalo S, Green S, et al. Underrecognized mildly symptomatic viremic dengue virus infections in rural Thai schools and villages. *J Infect Dis*. 2012; 206:389–98. [PubMed: 22615312]
128. Yoon IK, Srikiatkachorn A, Hermann L, Buddhari D, Scott TW, Jarman RG, Aldstadt J, Nisalak A, Thammapalo S, Bhoomiboonchoo P, et al. Characteristics of mild dengue virus infection in Thai children. *Am J Trop Med Hyg*. 2013; 89:1081–1087. [PubMed: 24127167]
129. Tien NTK, Luxemburger C, Toan NT, Pollissard-Gadroy L, Huong VTQ, Van Be P, Rang NN, Wartel TA, Lang J. A prospective cohort study of dengue infection in schoolchildren in Long Xuyen, Viet Nam. *Trans R Soc Trop Med Hyg*. 2010; 104:592–600. [PubMed: 20630553]
130. Balmaseda A, Standish K, Mercado JC, Matute JC, Tellez Y, Saborío S, Hammond SN, Nuñez A, Avilés W, Henn MR, et al. Trends in patterns of dengue transmission over 4 years in a pediatric cohort study in Nicaragua. *J Infect Dis*. 2010; 201:5–14. [PubMed: 19929380]
131. Sirivichayakul C, Limkittikul K, Chanthavanich P, Jiwariyavej V, Chokejindachai W, Pengsaa K, Suvannadabba S, Dulyachai W, Letson GW, Sabchareon A. Dengue infection in children in Ratchaburi, Thailand: a cohort study. II. Clinical manifestations. *PLoS Negl Trop Dis*. 2012; 6:e1520. [PubMed: 22389735]
132. Sabchareon A, Sirivichayakul C, Limkittikul K, Chanthavanich P, Suvannadabba S, Jiwariyavej V, Dulyachai W, Pengsaa K, Margolis HS, Letson GW. Dengue infection in children in Ratchaburi, Thailand: A cohort study. I. Epidemiology of symptomatic acute dengue infection in children, 2006–2009. *PLoS Negl Trop Dis*. 2012; 6:2006–2009.

133. Yoksan S, Tubthong K, Kanitwithayanun W, Jirakanjanakit N. Laboratory assays and field dengue vaccine evaluation at Ratchaburi province, Thailand: a preliminary result. *J Clin Virol.* 2009; 46:S13–S15. [PubMed: 19800560]
134. Kosasih H, Alisjahbana B, Nurhayati, de Mast Q, Rudiman IF, Widjaja S, Antonjaya U, Novriani H, Susanto NH, Jusuf H, et al. The Epidemiology, Virology and Clinical Findings of Dengue Virus Infections in a Cohort of Indonesian Adults in Western Java. *PLoS Negl Trop Dis.* 2016; 10:1–18.
135. Vong S, Khieu V, Glass O, Ly S, Duong V, Huy R, Ngan C, Wichmann O, William Letson G, Margolis HS, et al. Dengue incidence in urban and rural cambodia: Results from population-based active fever surveillance, 2006–2008. *PLoS Negl Trop Dis.* 2010; 4:2006–2008.
136. Argüello DF, Tomashek KM, Quiñones L, Beltran M, Acosta L, Santiago LM, Biggerstaff BJ, Garcia-Rivera EJ, Sun W, Pollissard-Gadroy L, et al. Incidence of dengue virus infection in school-aged children in Puerto Rico: A prospective seroepidemiologic study. *Am J Trop Med Hyg.* 2015; 92:486–491. [PubMed: 25646256]
137. Stoddard ST, Forshey BM, Morrison AC, Paz-Soldan Va, Vazquez-Prokopec GM, Astete H, Reiner RC, Vilcarromero S, Elder JP, Halsey ES, et al. House-to-house human movement drives dengue virus transmission. *Proc Natl Acad Sci U S A.* 2013; 110:994–9. A pediatric and adult community cohort study in Peru that incorporated contact tracing of febrile patients demonstrated that house-to-house movement of humans based on social networks drove heterogeneity in DENV transmission. [PubMed: 23277539]
138. Tam CC, Tissera H, de Silva AM, De Silva AD, Margolis HS, Amarasinge A. Estimates of dengue force of infection in children in Colombo, Sri Lanka. *PLoS Negl Trop Dis.* 2013; 7:e2259. [PubMed: 23755315]
139. Tissera H, Amarasinghe A, De Silva AD, Kariyawasam P, Corbett KS, Katzelnick L, Tam C, William Letson G, Margolis HS, De Silva AM. Burden of dengue infection and disease in a pediatric cohort in urban Sri Lanka. *Am J Trop Med Hyg.* 2014; 91:132–137. [PubMed: 24865684]
140. Capeding MR, Chua MN, Hadinegoro SR, Hussain IIHM, Nallusamy R, Pitisuttithum P, Rusmil K, Thisyakorn U, Thomas SJ, Huu Tran N, et al. Dengue and Other Common Causes of Acute Febrile Illness in Asia: An Active Surveillance Study in Children. *PLoS Negl Trop Dis.* 2013; 7:e2331. [PubMed: 23936565]
141. Dayan G, Arredondo JL, Carrasquilla G, Deseda CC, Dietze R, Luz K, Costa MSN, Cunha RV, Rey LC, Morales J, et al. Prospective cohort study with active surveillance for fever in four dengue endemic countries in Latin America. *Am J Trop Med Hyg.* 2015; 93:18–23. [PubMed: 26013373]
142. Dhar-Chowdhury P, Paul KK, Haque CE, Hossain S, Lindsay LR, Dibernardo A, Brooks WA, Drebot MA. Dengue seroprevalence, seroconversion and risk factors in Dhaka, Bangladesh. *PLoS Negl Trop Dis.* 2017; 11:1–24.

Highlights

- Cohort studies measure disease rates, transmission parameters, and risk factors
- Cohorts identify correlates of protection and risk and inform vaccine trial design
- They provide critical data in outbreaks and enable development of diagnosis assays
- Cohorts build research capacity and inform local and international policy-making

Table 1

Ten ways cohort studies promote scientific research and infectious disease control

1	Estimate basic infection and disease incidence, transmission parameters, and risk factors
2	Identify correlates of protection and disease risk
3	Enable scientific studies of well-characterized samples with advanced scientific techniques
4	Provide longitudinal samples to study kinetics of antibody and biomarker levels
5	Inform vaccine trial design and evaluation
6	Serve as sentinels during outbreaks to inform local and international policy decision-making
7	Collect high-quality samples for diagnostic assay development
8	Enable holistic studies of multiple diseases and environmental and socioeconomic factors
9	Increase understanding of individual and intrinsic differences that drive immunity to pathogens
10	Foster infectious disease infrastructure, research, and control in disease-affected countries in close collaboration with Ministries of Health

Table 2

Community-, school-, or household-based longitudinal prospective cohort studies of dengue (in chronological order)

Location	Years
Bangkok, Thailand [120]	1962–1964
Koh Samui Island, Thailand [121]	1966–1967
Rayong, Thailand [32]	1980–1981
Bangkok, Thailand [31,32]	1980–1981
Yangon, Myanmar [30]	1984–1988
Iquitos, Peru [73]	1993–1996
Yogyakarta, Indonesia [25]	1995–1996
Kamphaeng Phet I, Thailand [15,23,26]	1998–2002
Iquitos, Peru (2 studies during this period) [27]	1999–2005
West Java, Indonesia [122]	2000–2004
Maracay, Venezuela [123]	2001–2002
Managua, Nicaragua [124]	2001–2003
Kamphaeng Phet II, Thailand [125–128]	2004–2006
Long Xuyen, Vietnam [129]	2004–2007
Managua, Nicaragua [12,113,130]	2004–present
Ratchaburi, Thailand [131–133]	2005–2009
Iquitos, Peru [35,44]	2006–2010
West Java, Indonesia [134]	2006–2009
Kampong Cham, Cambodia, [135]	2006–2008
Patillas, Puerto Rico [136]	2007–2008
Iquitos, Peru [67,137]	2007-present
Colombo, Sri Lanka [55,138,139]	2008–2010
Preparatory studies for CYD-TDV Phase III vaccine trials: Indonesia, Malaysia, Philippines, Thailand Vietnam [140]	2010–2011
Preparatory studies for CYD-TDV Phase III vaccine trials: Brazil, Colombia, Puerto Rico, Mexico [141]	2010–2011
Medellin, Colombia [14]	2010–2011
CYD-TDV Phase III vaccine trial placebo controls [11]	2011–2014
Dhaka, Bangladesh [142]	2012
Cebu City, Philippines [17,24]	2012-present