The curious case of the 2010-2011 DENV-2 outbreak in Iquitos, Peru:
Incomplete homologous protection?

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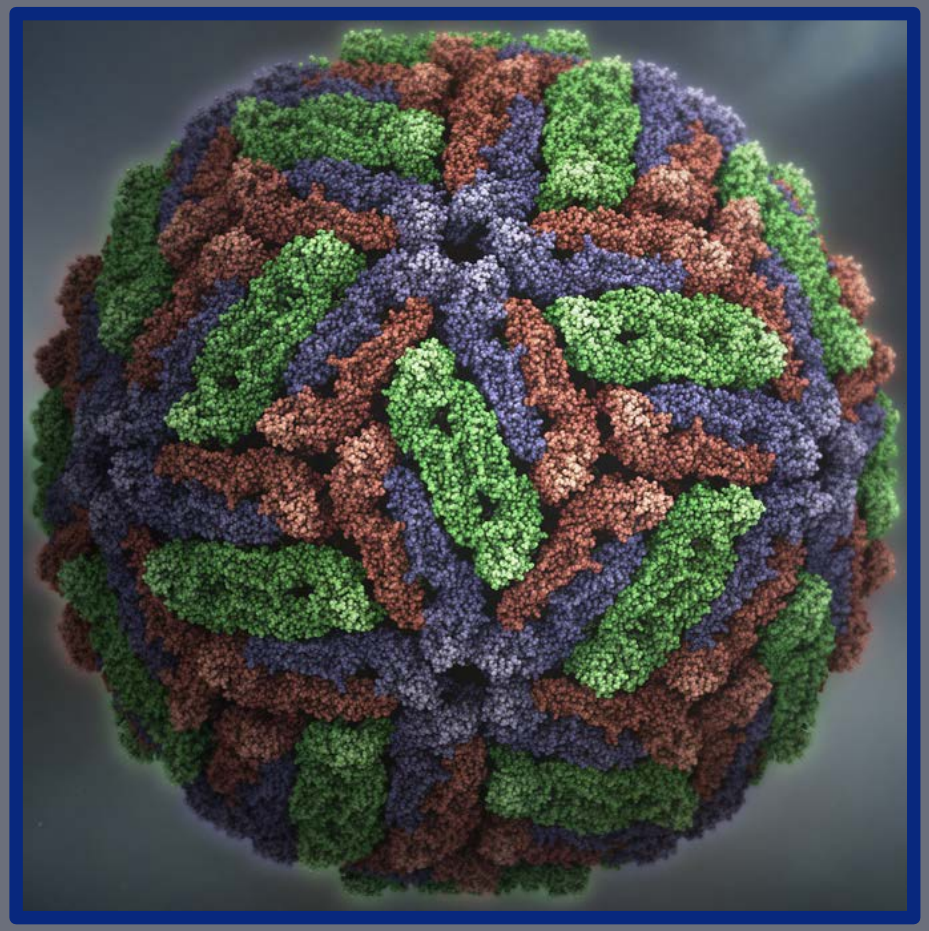
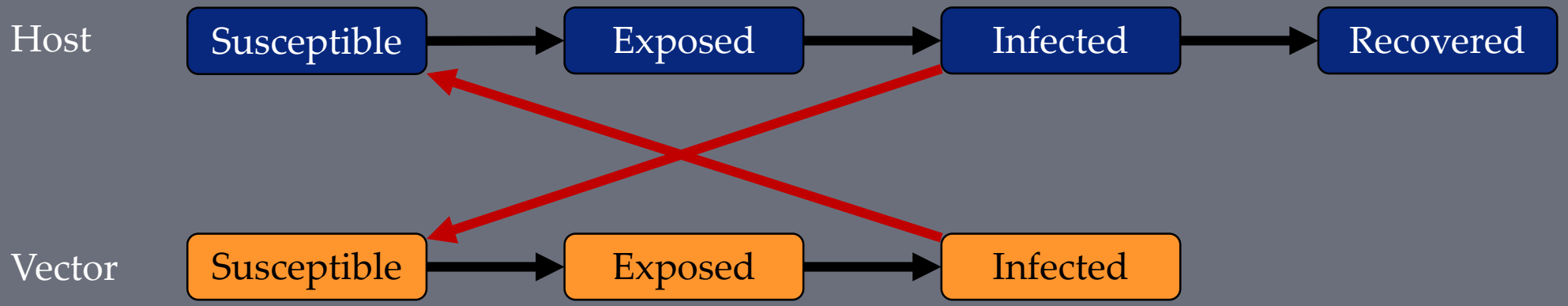


Dengue virus

Dengue virus (DENV) is a mosquito-borne viral infection caused by any of four related, but antigenically distinct virus serotypes (DENV-1, -2, -3, and -4)

DENV transmission



REPORT

Dengue viruses cluster antigenically but not as discrete serotypes

Leah C. Katzelnick[1,2,3,4], Judith M. Fonville[1,2,5], Gregory D. Gromowski[3], Jose Bustos Arriaga[3], Angela Green[4], Sarah L. James[1,2], Louis Lau[4], Magelda Montoya[4], Chunling Wang[4], Laura A. VanBlargan[3], Colin A. Russell[6], Hlaing Myat Thu[7], Theodore C. Pierson[3], Philippe Buchy[8], John G. Aaskov[9,10], Jorge L. Muñoz-Jordán[11], Nikos Vasilakis[12,13,14], Robert V. Gibbons[15], Robert B. Tesh[12,13,14], Albert D.M.E. Osterhaus[5], Ron A.M. Fouchier[5], Anna Durbin[16], Cameron P. Simmons[17,18,19], Edward C. Holmes[20], Eva Harris[4], Stephen S. Whitehead[3], Derek J. Smith[1,2,5,*]

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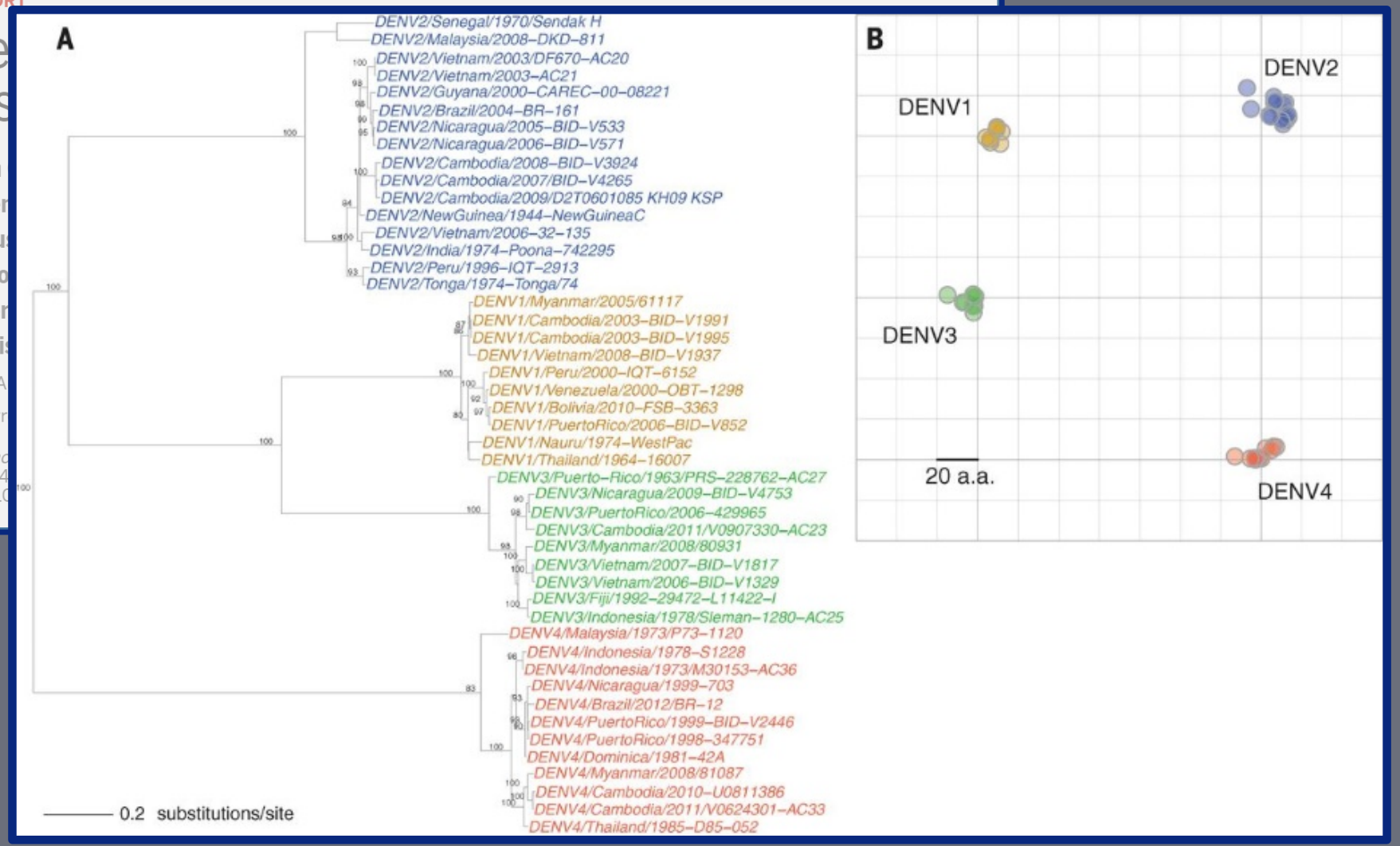
Dengue virus



Dengue virus

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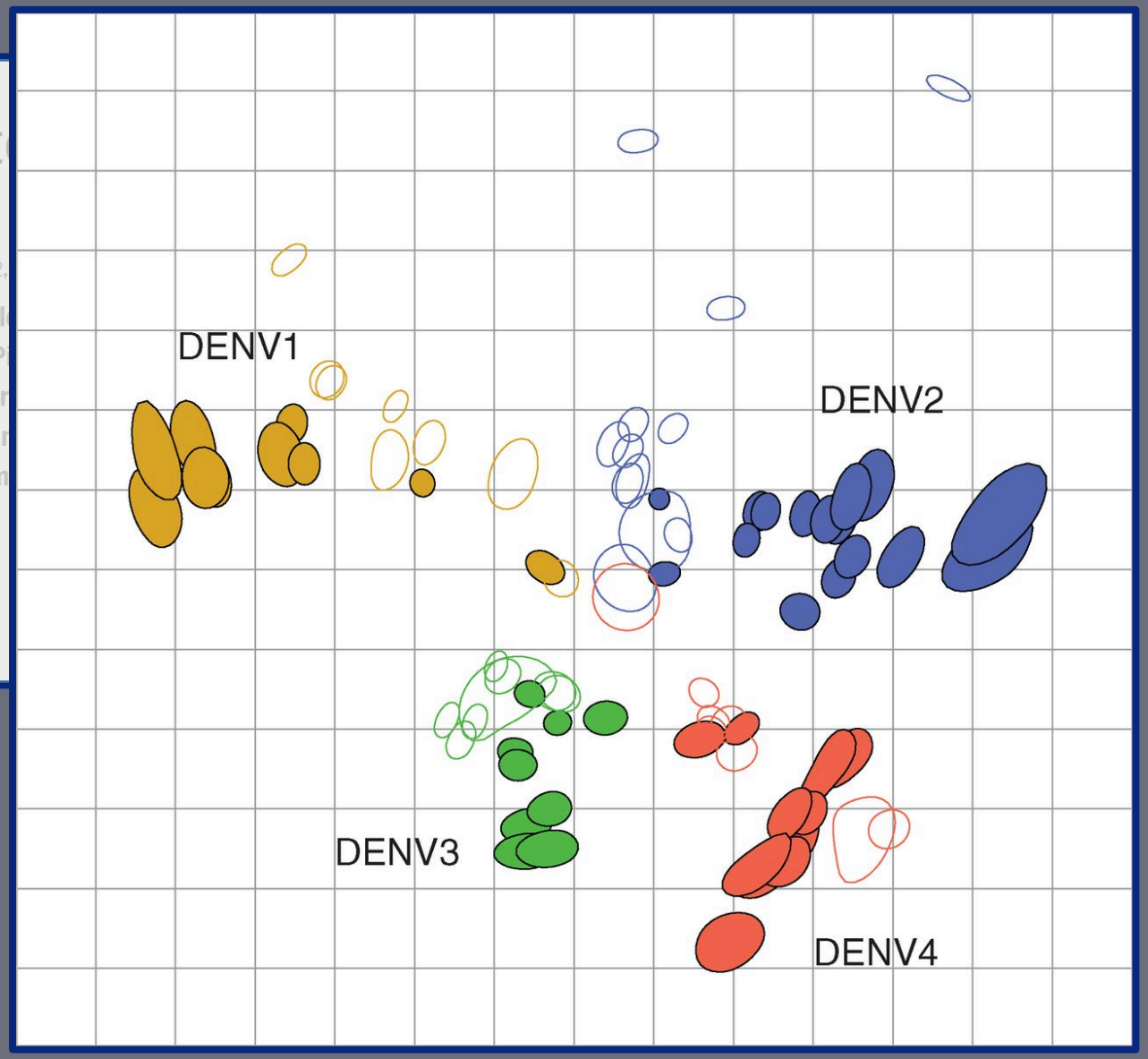

Dengue virus

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discret

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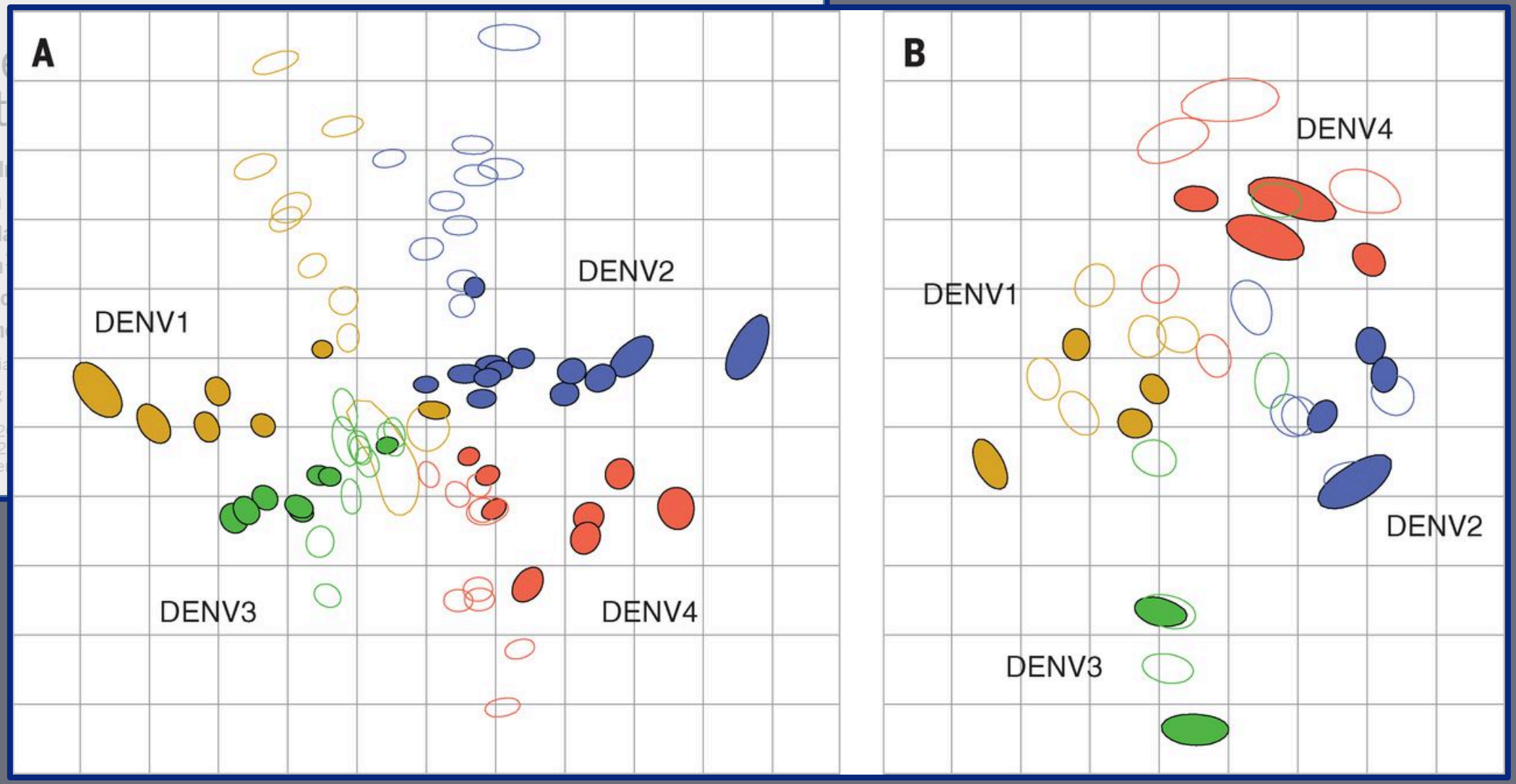

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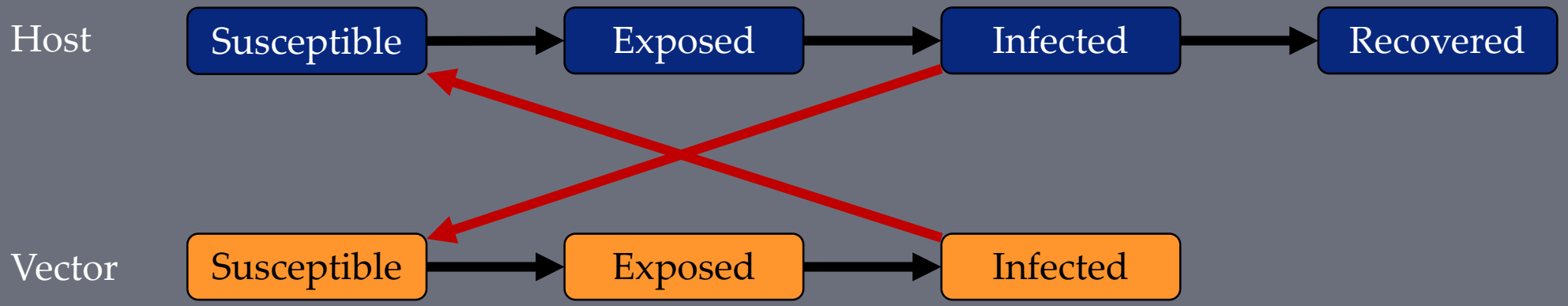

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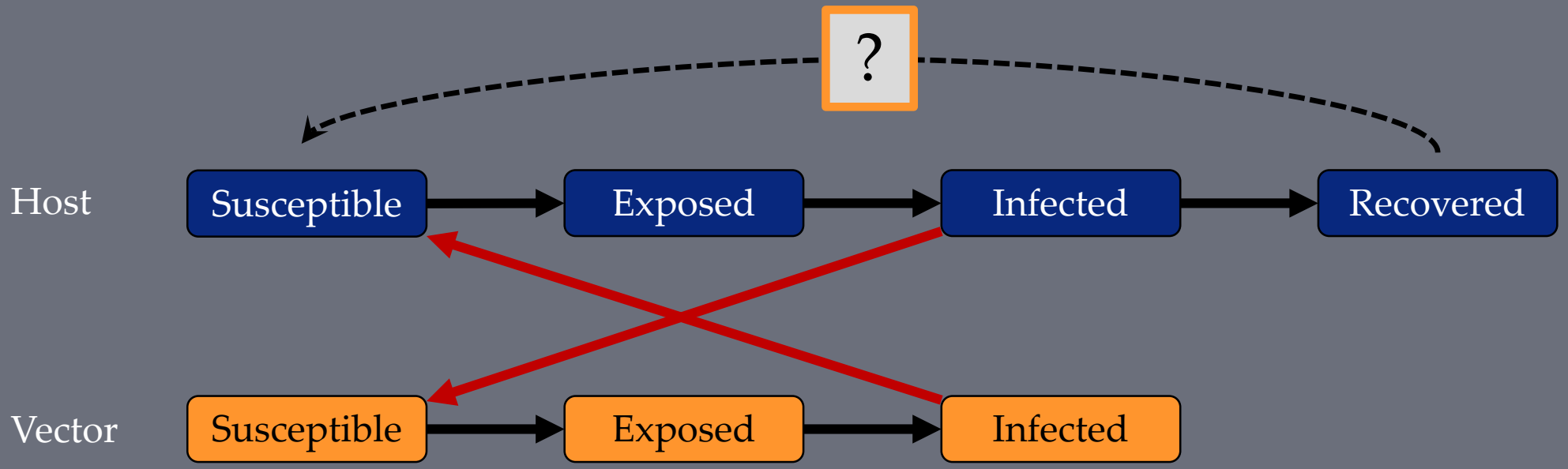
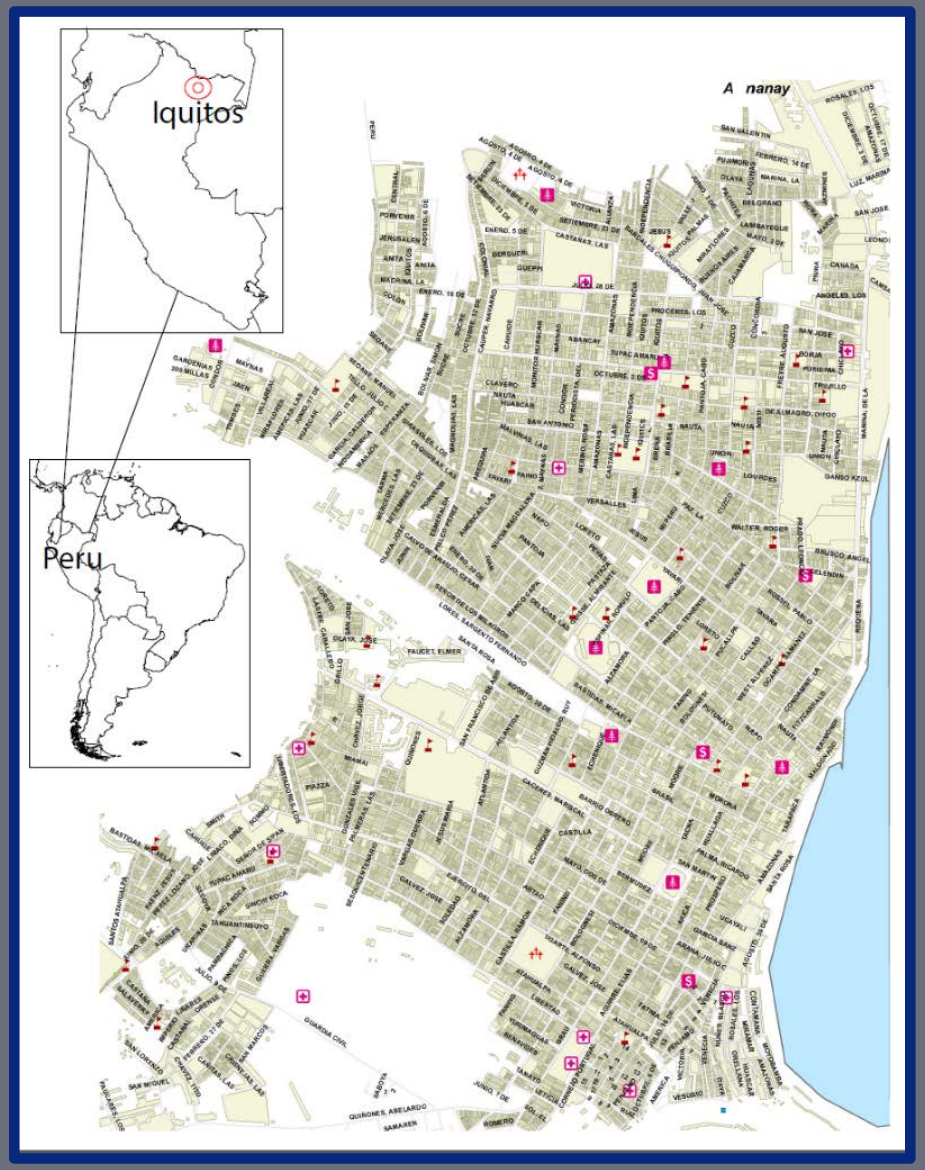




Iquitos, Peru

- Iquitos has a population of 400,000. The city, located at the beginning of the Amazon river, is relatively isolated from other large cities.
- DENV-1 was introduced in 1991, DENV-2 in 1995, DENV-3 in 2001 and DENV-4 in 2008-2009. A massive AA-DENV-2 outbreak occurred in 2010/2011.
- The Scott lab at UC Davis began extensive studies of dengue in Iquitos in 1999 and thus were able to collect data during two `virgin-soil' invasions

PRNT in Iquitos

The primary tool for identifying serostatus in cohorts has been PRNT 70 (samples that reduced the number of plaques by 70%)

- Cut-off dilutions were set at 1:60 for DENV-1, DENV-2, and DENV-3, and 1:40 for DENV-4 (all after the addition of virus).
- For routine seroprevalence studies conducted in prior to 2005, positivity (e.g., 70% reduction at 1:60 dilution) was based on samples tested at 1:60 dilution (after the addition of virus).
- For routine seroprevalence studies conducted after 2005, positivity (e.g., 70% reduction at 1:60 dilution) was based on titers were estimated by probit regression, using a dilution series of 1:40, 1:80, 1:160, and 1:640, after the addition of virus.

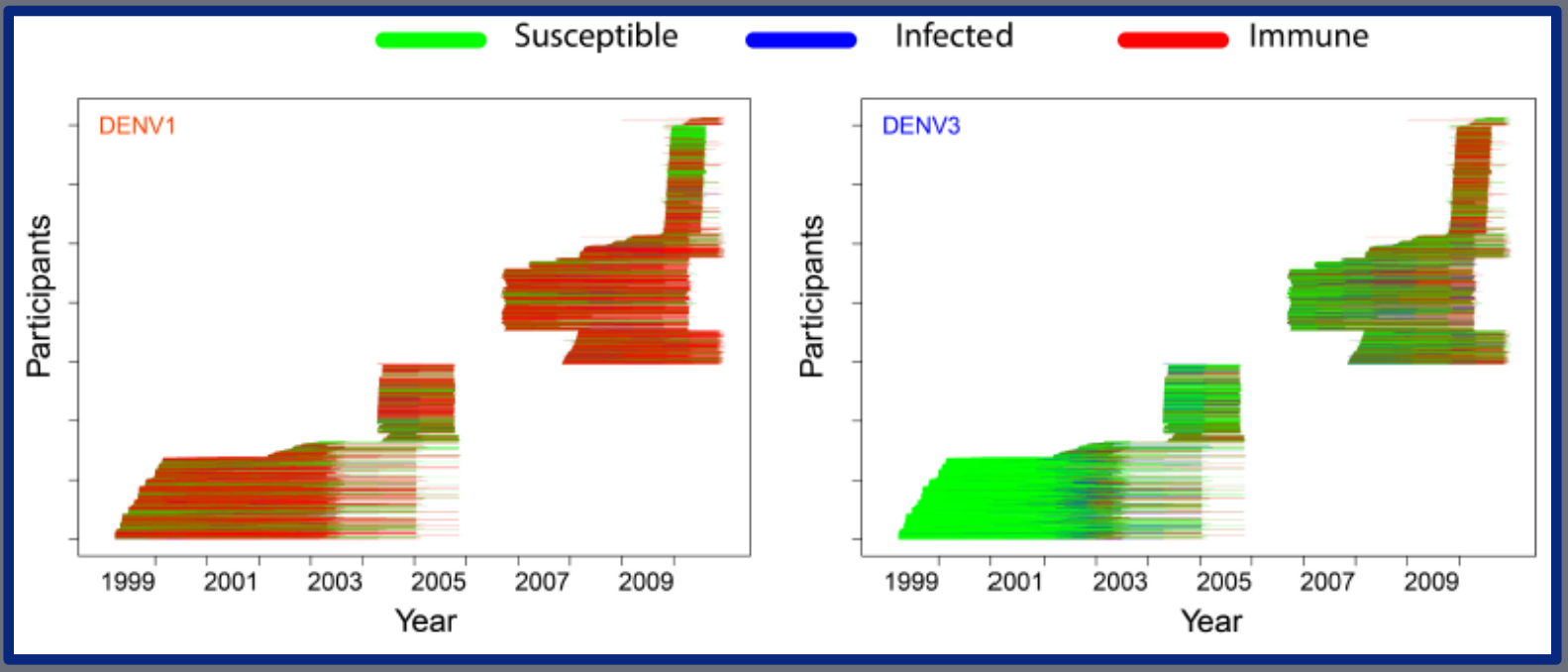
PRNT in Iquitos

- For quantifying end point titers, samples were diluted four-fold from 1:40 to 1:10240 and tested in duplicate; final titers were estimated by probit regression.

- For seroprevalence studies, test viruses were DENV-1 16007 (DHF case from Thailand, 1964), DENV-2 16681 (Asian I genotype; DHF case from Thailand, 1964), DENV-3 IQD1728 (DF case from Peru, 2002), and DENV-4 1036 (DF case from Indonesia, 1976).

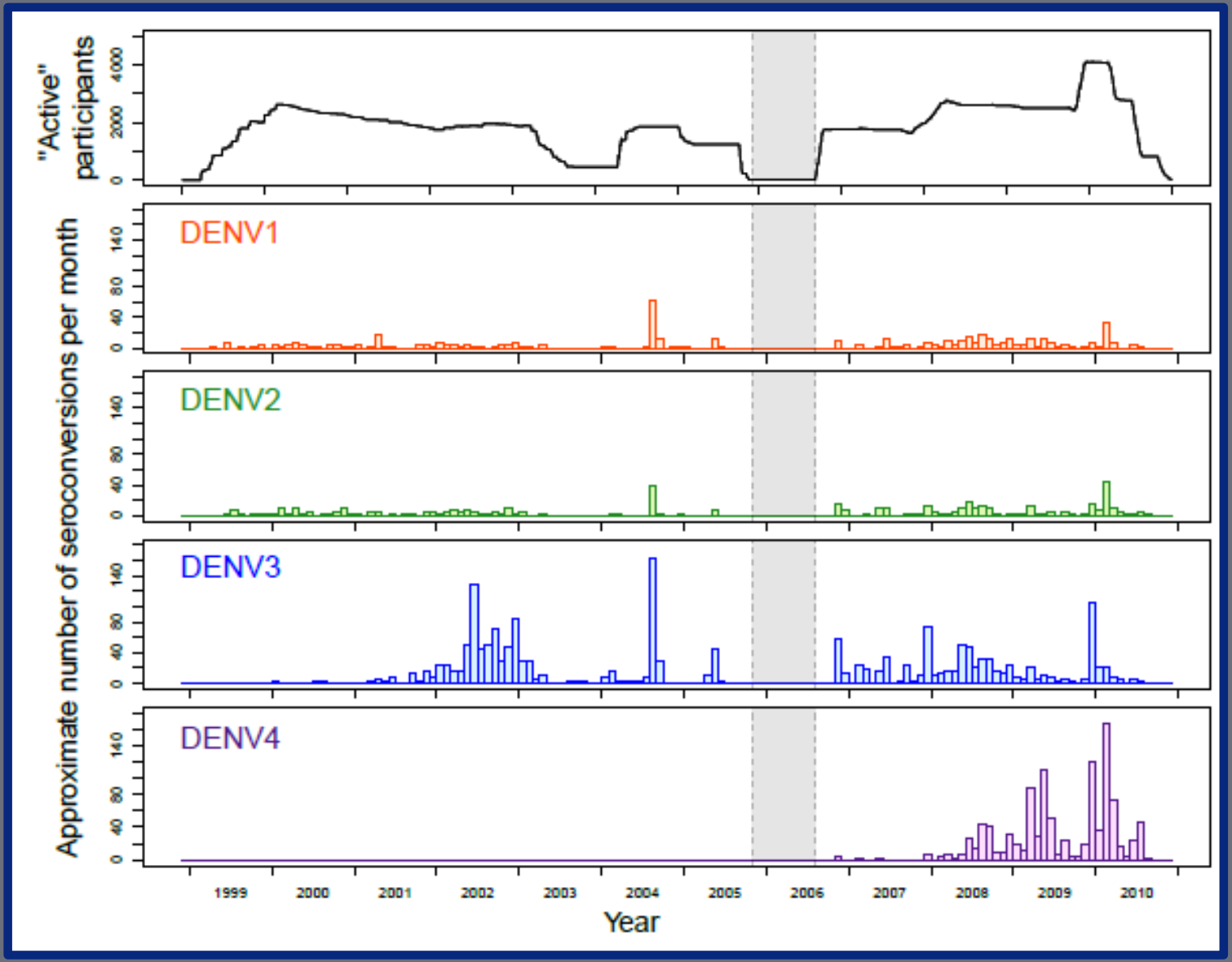
- DENV-2 16681 (Asian genotype) was selected for the seroprevalence assays because previous experiments in our laboratory showed that this strain minimized a cross-reactive response from DENV-1 infection.

Longitudinal Cohorts In Iquitos



- Since 1999, longitudinal cohorts of ~3,000-4,000 have been maintained. Each individual's serostatus to all four serotypes is evaluated every 6-9 months they are participants.

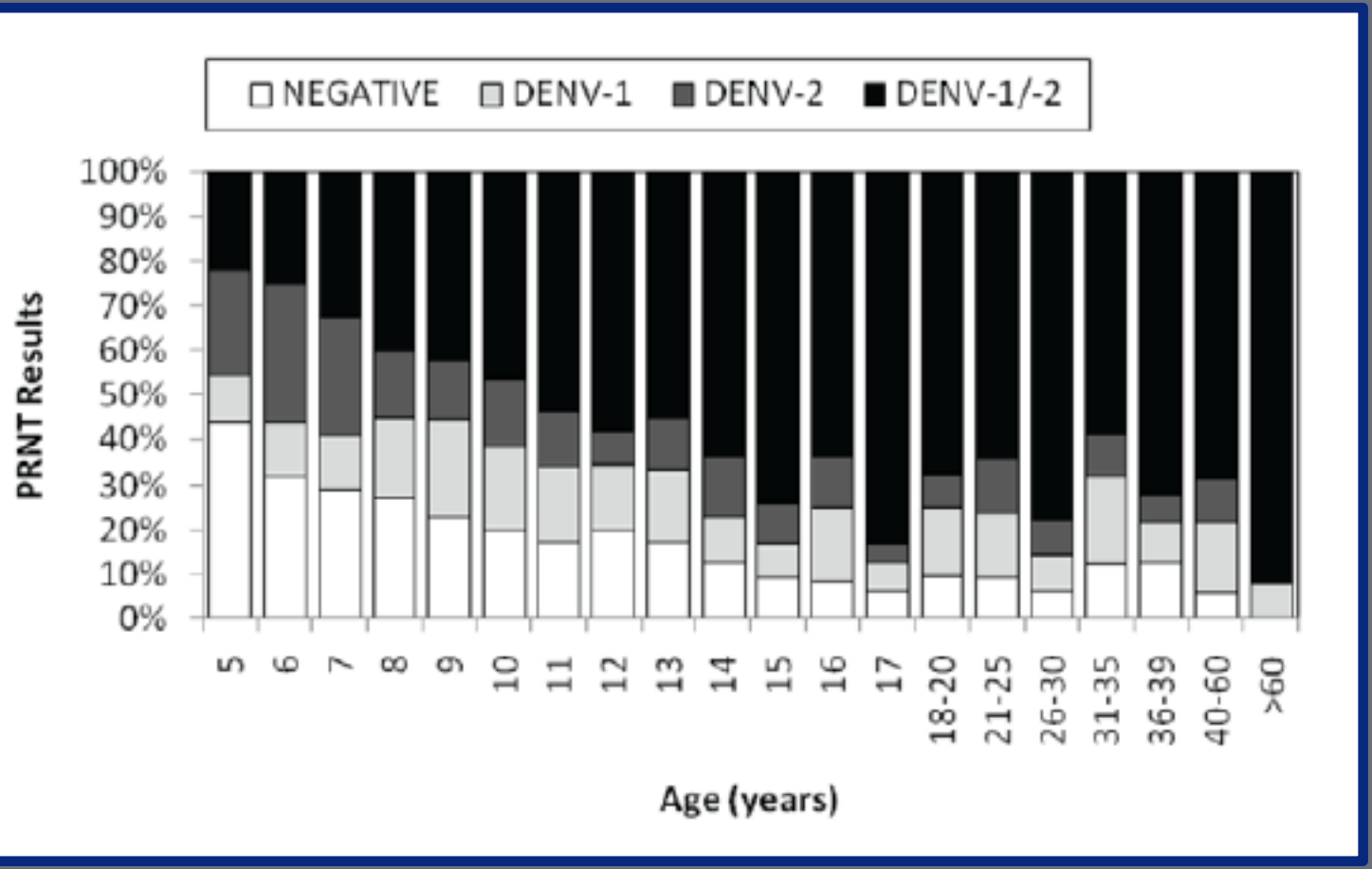
Longitudinal cohort



Longitudinal Cohorts In Iquitos

- Since 1999, longitudinal cohorts of ~3,000-4,000 have been maintained. Each individual's serostatus to all four serotypes is evaluated every 6-9 months they are participants.
- Around 16,000 participant's serotype-specific serostatus have been measured several (2-14) times (~50,000 blood samples)

Population-level infection patterns



Morrison et al, PLoS NTD 2010

Back in 1999, at the initiation of the first longitudinal cohort in Iquitos, initial seroprevalence surveys indicated the vast majority of individuals had already been exposed to both DENV-1 and DENV-2.

As would be expected, there was a large dependence on age.

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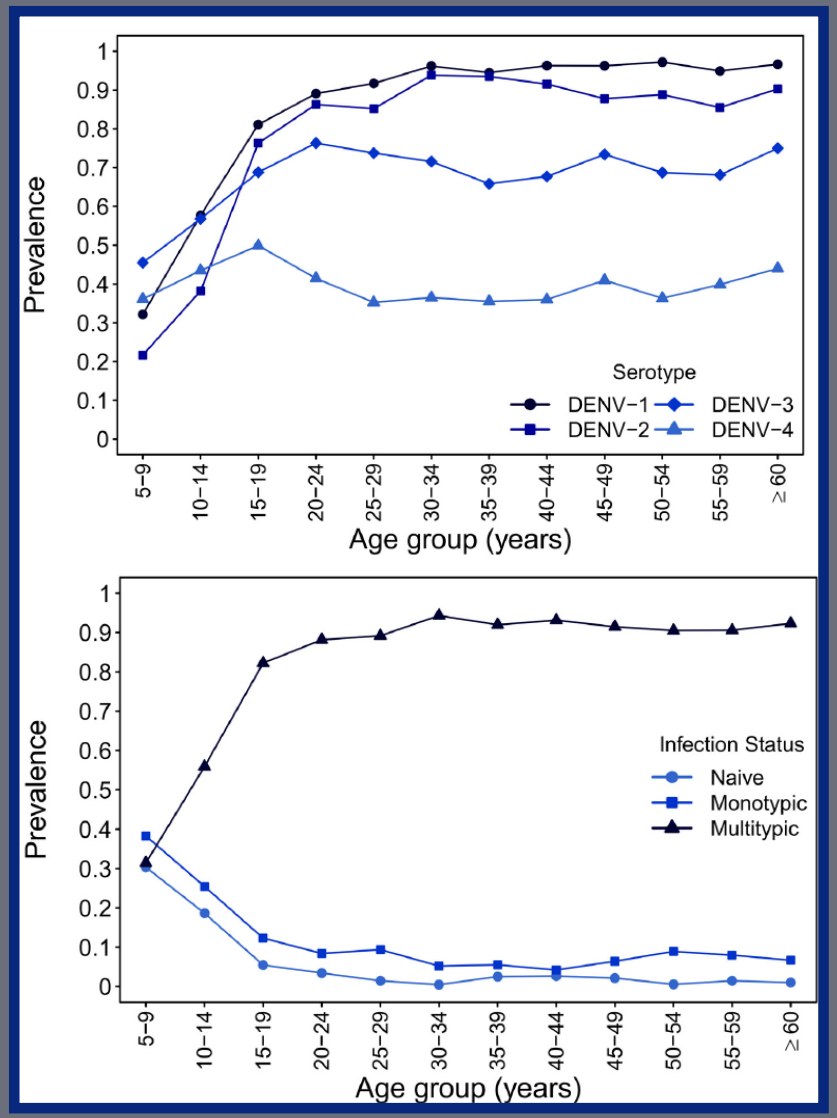

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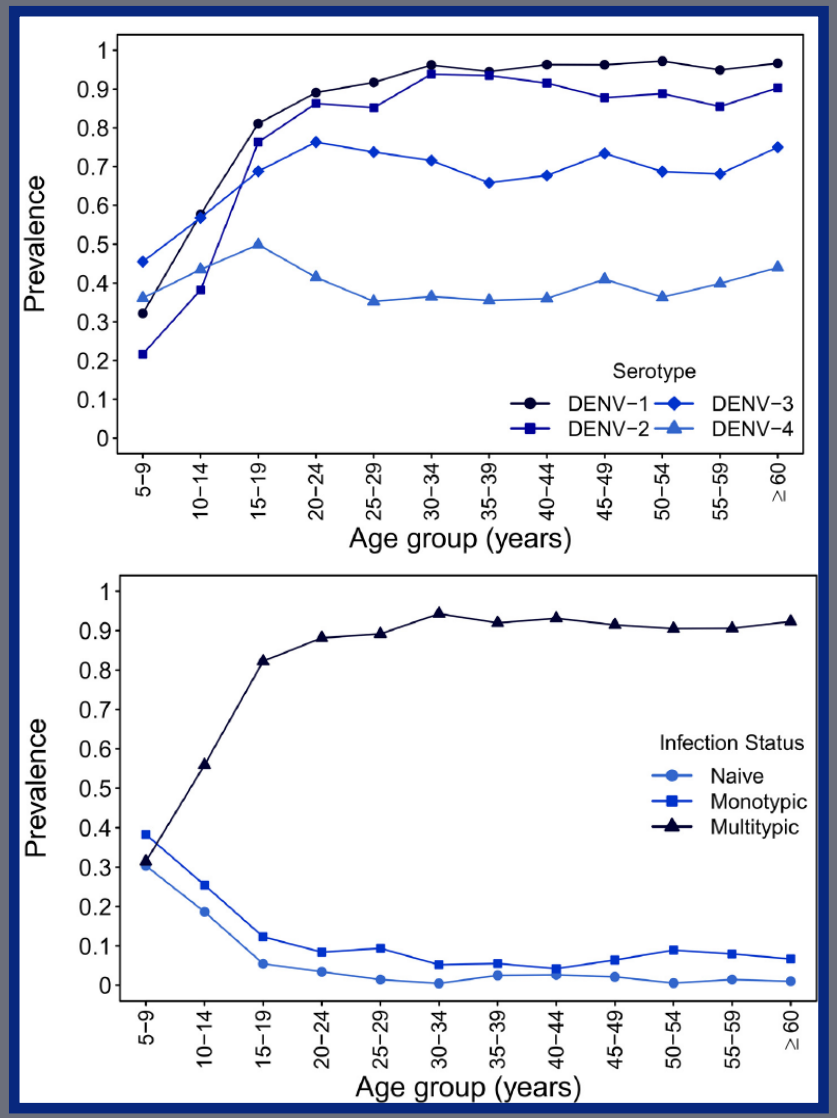
This pattern within the PRNT data was maintained through later cohorts (data here collected March-June 2010)

Population-level infection patterns



For DENV-2, using the age distribution of the population, we can get an estimate of the age-distribution of susceptibles.

Assuming the probability of disease is independent of age*, we can estimate the age distribution of disease.

If p_i is the percent of age class i that is still susceptible and a_i is the percent of the population that is of age class i, then if q_i is the probability the next case is of age class i, we have

$$q_i = \frac{p_i a_i}{\sum_j p_j a_j}$$

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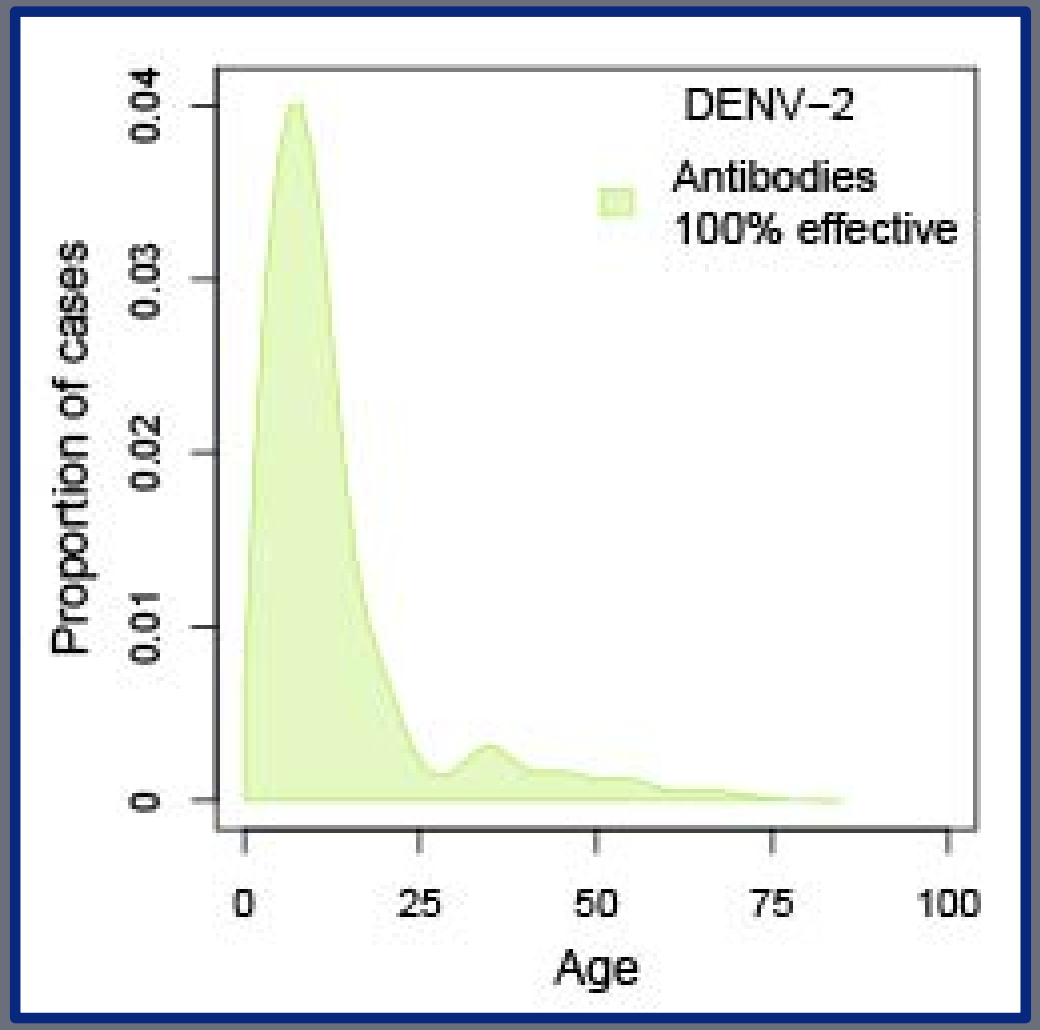

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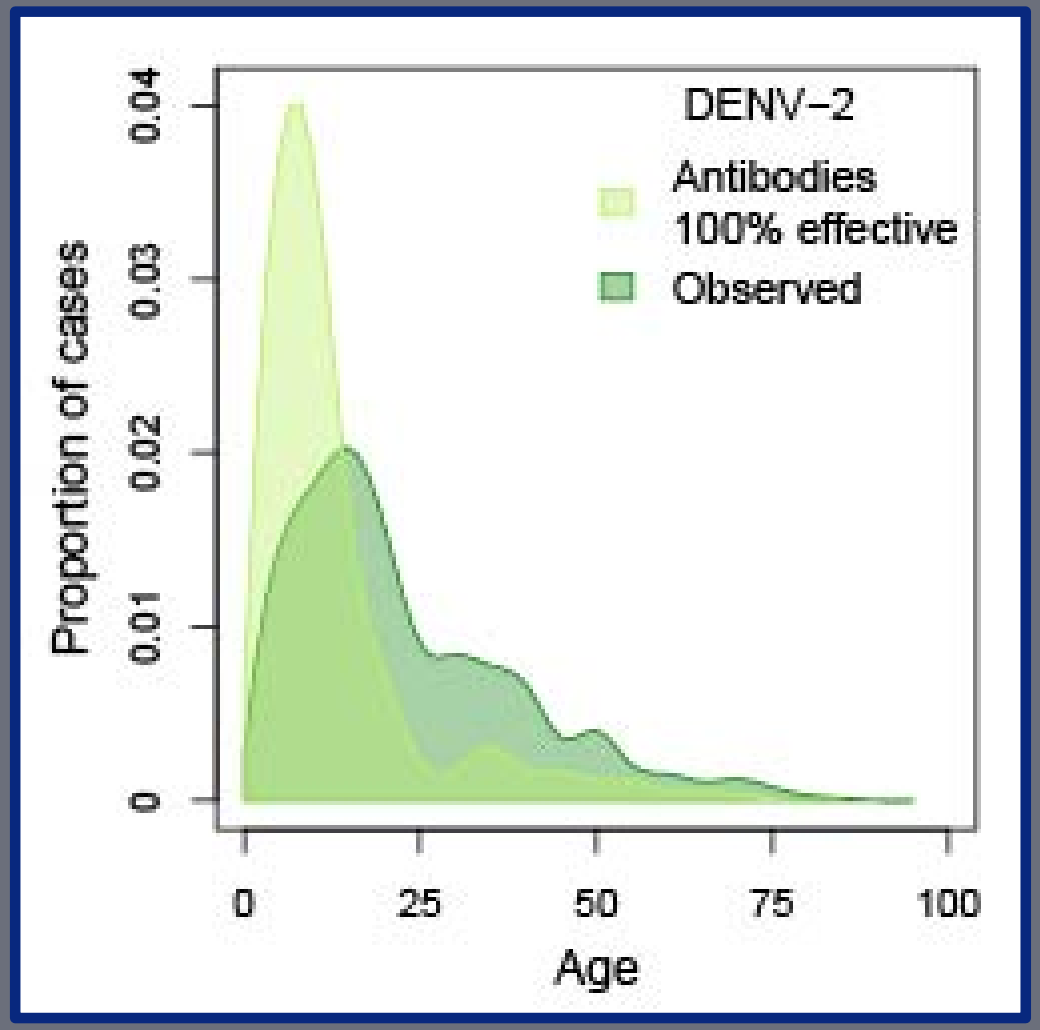

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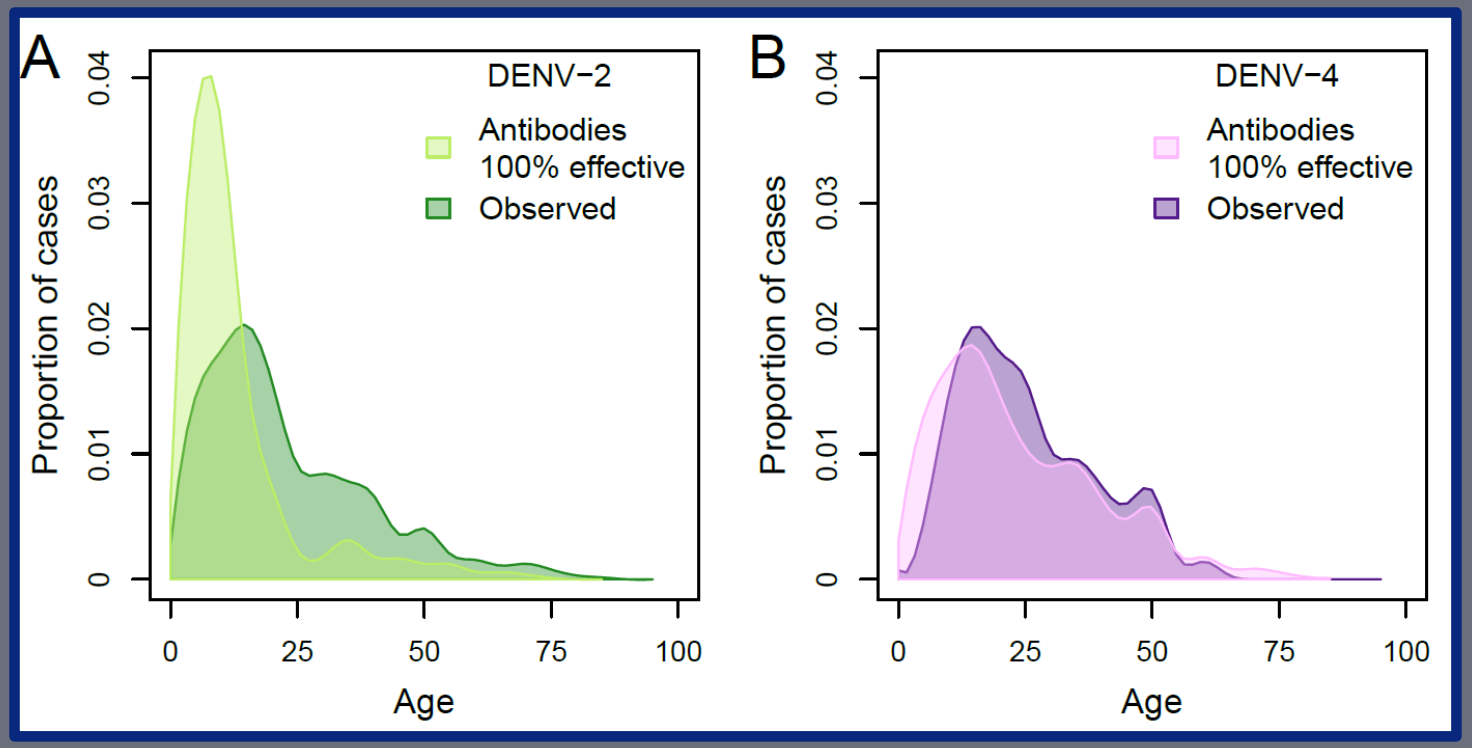
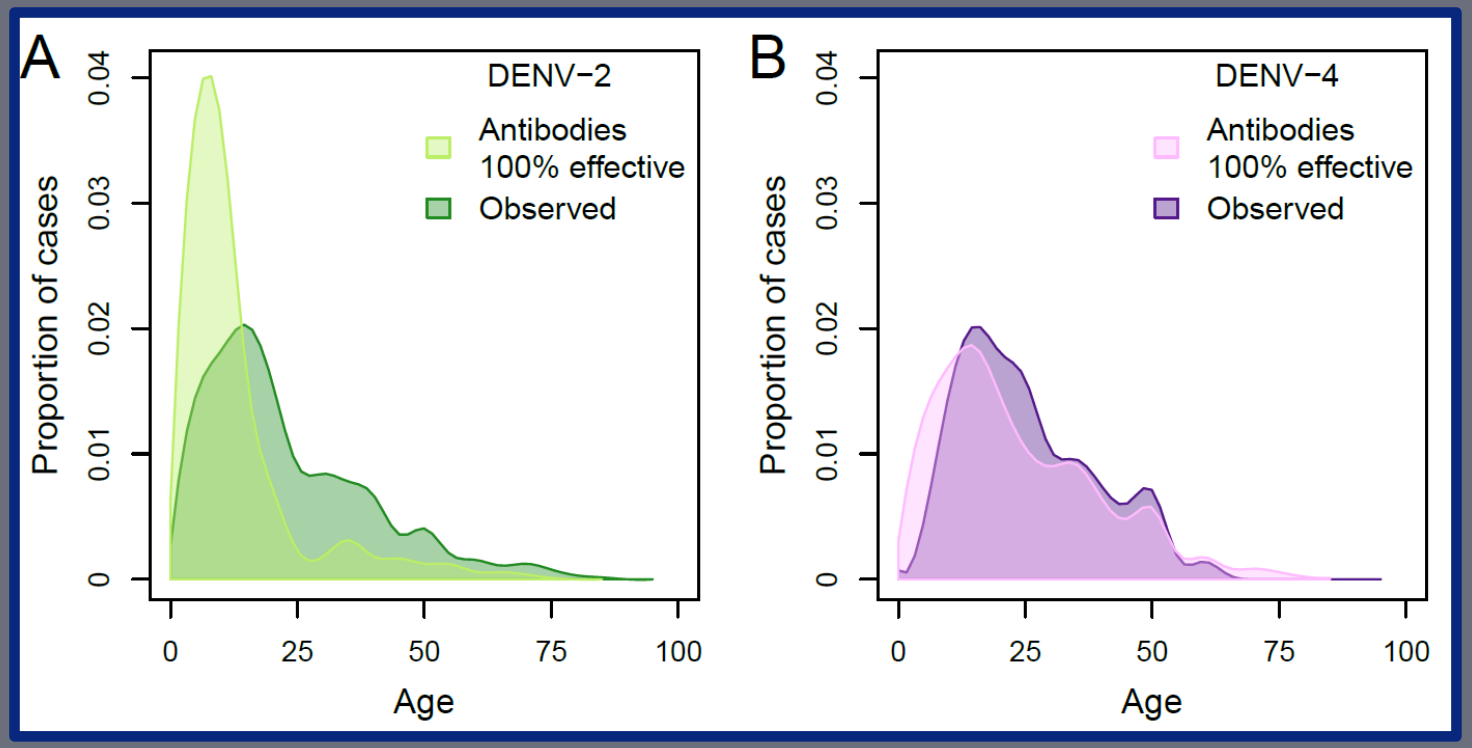


At the same time as this DENV-2 outbreak, there was a continuation of the DENV-4 invasion.

Using the same approach, we do not see a significant mismatch between expected and observed cases for DENV-4.

Population-level infection patterns



Simply, we can modulate the percent we estimate susceptible in the population and recalculate the expected age distribution of cases from

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to

$$q_i = \frac{(\gamma * p_i + 1 - \gamma) a_i}{\sum_j (\gamma * p_j + 1 - \gamma) a_j}$$

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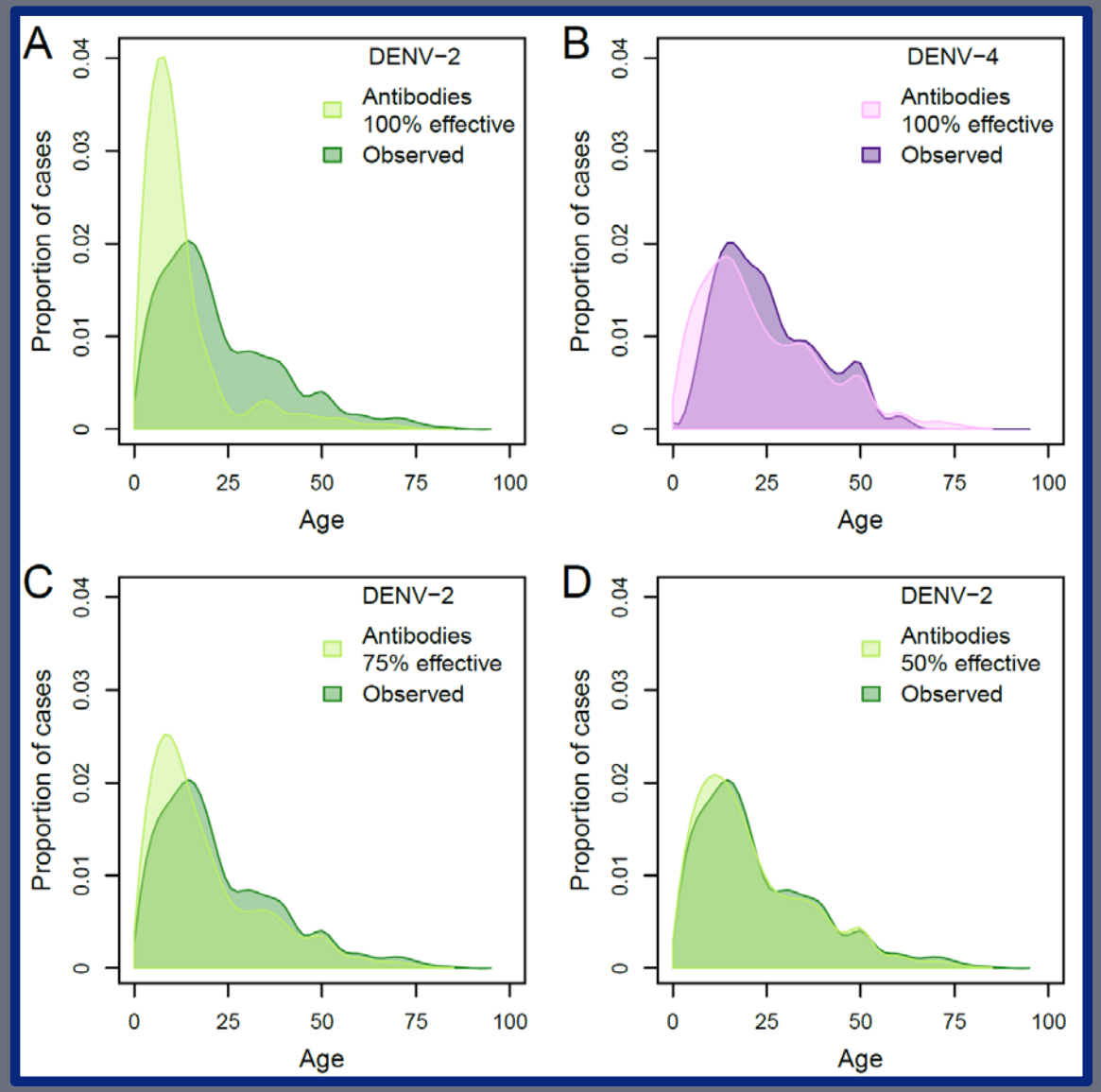
Population-level infection patterns



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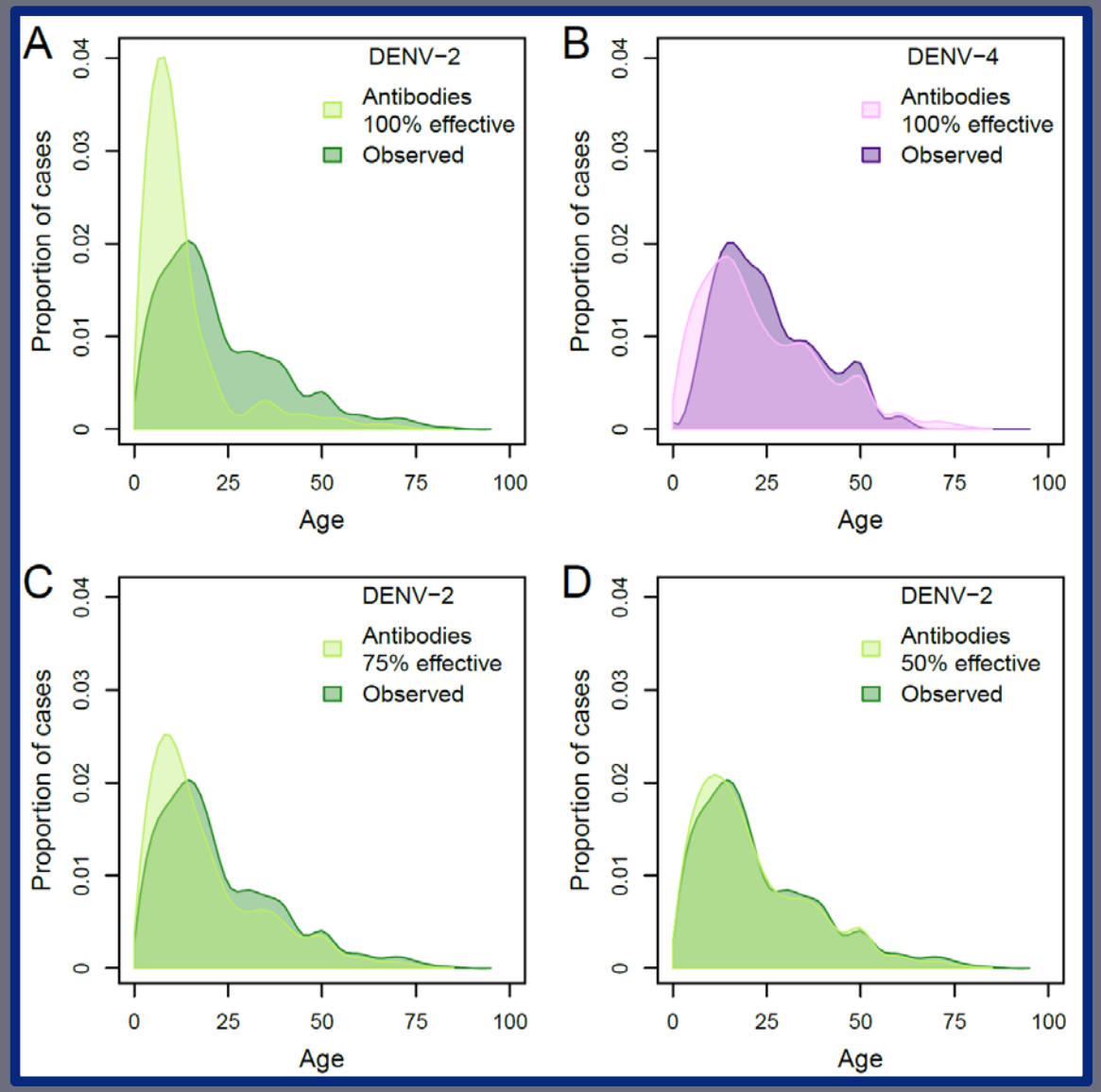

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We estimate homologous protection of 35.1% (95% CI: 0%-65.2%)

Individual-level infection patterns

The results from the population-level analysis indicate that some individuals with pre-existing DENV-2 neutralizing antibodies prior to the 2010 epidemic were infected by AA-DENV-2 and presented with clinically apparent disease.

To address this possibility, we examined the serological histories of individuals who had symptomatic or clinically inapparent AA-DENV-2 infections and who had provided two or more samples (median 4 samples; range, 2–7 samples) in a longitudinal cohort study.

Table 1. Pre-epidemic DENV-2 neutralizing antibody prevalence among symptomatic and inapparent DENV-2 infections.

	Symptomatic	Inapparent	All
Median age in 2010 (IQR[1])	17 (13–42)	27 (16–45)	19 (14–43)
DENV-2 antibody prevalence[2]	43% (26/60)	76% (13/17)	51% (39/77)
< = 15yrs (born 1995 or later)	17% (4/24)	25% (1/4)	18% (5/28)
> 15yrs (born prior to 1995)	61% (22/36)	92% (12/13)	69% (34/49)
Geometric mean titer[3]	267	418	310

[1] IQR indicates interquartile range (i.e., 25th and 75th percentiles).
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Using this data we could identify inapparent infections. Again, subsetting this group by only looking at those individuals that were both infected with AA DENV-2 and had provided 2 or more samples, we found a strong age dependence.

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Overall, 76% had DENV-2 neutralizing antibodies prior to infection, a prevalence similar to the study population at-large (73%) but higher than the symptomatic infections (i.e., 43%; age-adjusted odds ratio 4.2, 95% confidence interval 1.1–17.7).

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Among these AA-DENV-2-infected individuals (symptomatic and inapparent), the proportion with pre-existing DENV-2 neutralizing antibodies was consistent for years leading up to the 2010–2011 epidemic, as was the magnitude of antibody titers.

Table 2. Pre-epidemic DENV-2 neutralizing antibody profiles of participants with subsequent confirmed DENV-2 infection in 2010–2011. Seroprevalence is based on a cutoff titer of 60. Geometric mean titers (GMT) are based on samples with detectable titers.

	Years prior to infection			
	3 to 2 yrs	2 to 1 yrs	1 to 0.5 yrs	≤ 0.5 yrs
N[1]	54	64	60	34
Seroprevalence (95% CI)	59% (45%-72%)	59% (46%-72%)	58% (45%-71%)	59% (41%-75%)
Geometric mean titer[2]	320	337	263	370

[1] If more than one sample was available for a participant within a particular time interval, only one sample was used in the calculations.

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Three individuals that were monotypic for DENV-2 at the start of the study experienced virologically-confirmed acute DENV-2 infections during the 2010–2011 epidemic; two were inapparent and one was clinically apparent. These data suggest that AA-DENV-2 did infect individuals with pre-existing high titer DENV-2-neutralizing antibodies and are consistent with the notion that DENV-2 antibodies provided partial protection against disease.

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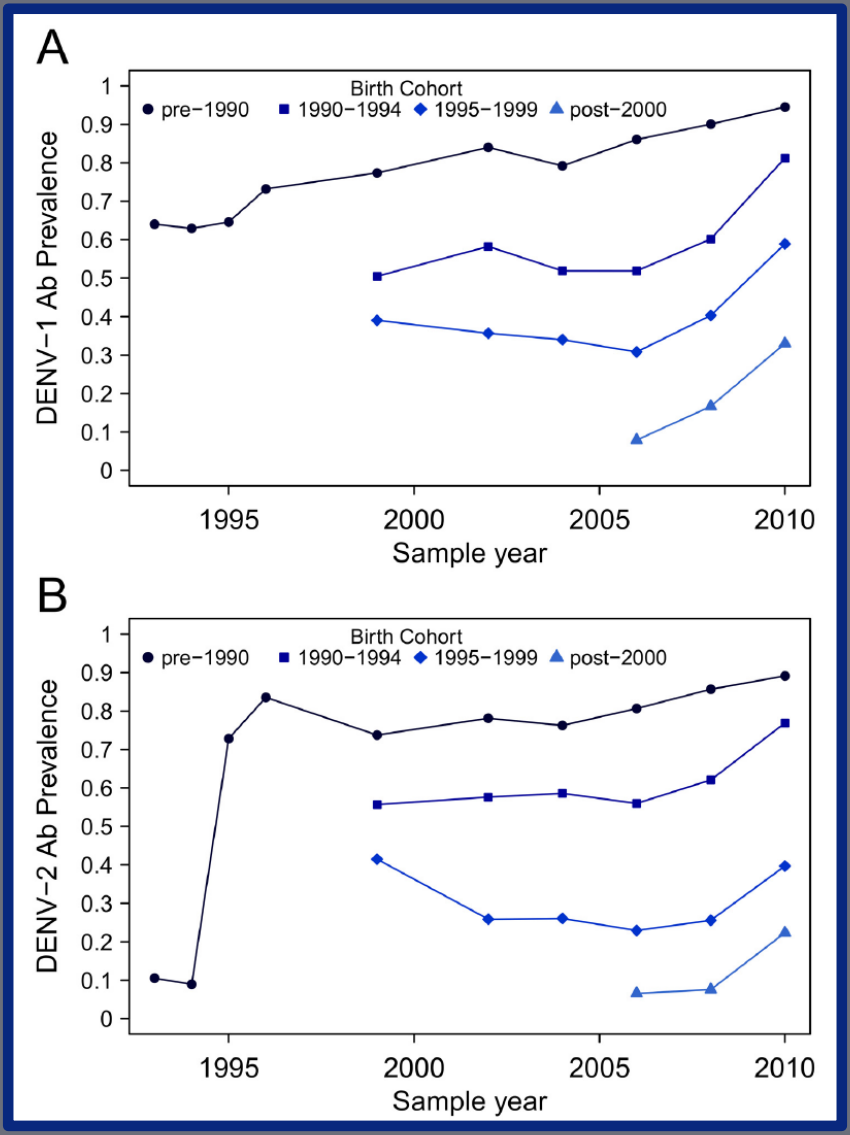

To address cross-reaction's role in these patterns, we utilized multiple cross-sectional samples from cohort studies conducted in Iquitos since the early 1990s

If cross-reaction was driving the patterns, we would expect DENV-2 antibody prevalence in adults to increase incrementally over the years (many of which were dominated by heterologous transmission)

Yet, among individuals born prior to 1995, the observed pattern was consistent with DENV-2 neutralizing antibodies generated largely during the 1995 outbreak.

Individual-level infection patterns

Our results suggest the possibility that Am-DENV-2 infection failed to generate antibodies that robustly neutralize AA-DENV-2.

To address this, we utilized pre-epidemic serum collected between 2006 and 2010 from individuals who were later detected with AA-DENV-2 infections (n = 21) and compared end point titers against two strains of Am-DENV-2 and two strains of AA-DENV-2. Robust neutralizing titers were observed against both genotypes, although pre-epidemic titers were two-fold higher against Am-DENV-2 compared with AA-DENV-2.

In contrast, post-epidemic serum (2011–2012) collected from previously DENV-2-naïve individuals who were infected during the 2010–2011 epidemic (n = 14) did not have higher titers using Am-DENV-2 compared with AA-DENV-2.

Table 3. Neutralization of Am-DENV-2 and AA-DENV-2 test viruses using human serum from longitudinal cohort studies. Geometric mean titers (GMTs) for the individual test viruses and the average of the two GMTs are shown.

	American genotype DENV-2 test viruses			American/Asian genotype DENV-2 test viruses		
Sera, collection years	IQT2124	IQT2913	Average	NFI1159	NFI1166	Average
Pre-epidemic, 2006–2010 (n = 24)	200	526	363	139	203	171
Post-epidemic, 2011–2012 (n = 14)	327	1150	739	871	927	899

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Concerns

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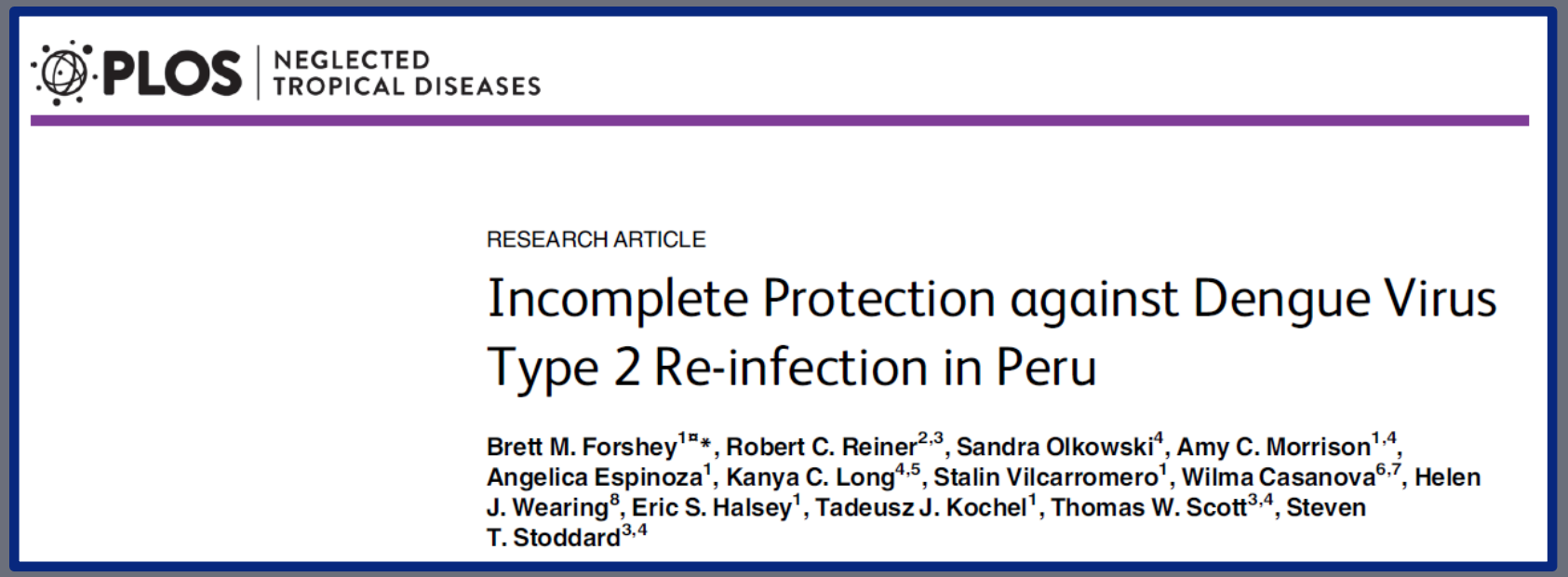
Observations

- PRNT70 at 1:60 dilution is relatively conservative. When we went up to PRNT90, we had only a modest reduction in seroprevalence (67% versus 73%)
- Among individuals who had pre-existing DENV-2 neutralizing antibodies and were infected in 2010/2011, more than half had titers >300 (using the 70% threshold).
- Completely independent of PRNT, the average age of cases was considerably higher than expected.
  - Most people >20 yrs old have been exposed to 2 serotypes, which should make them **less** likely to show up to clinic

Questions?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RESEARCH ARTICLE
Incomplete Protection against Dengue Virus Type 2 Re-infection in Peru
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