SCIENCE FROM THE BOTTOM UP: MOSQUITO-BORNE DISEASES IN NICARAGUA

Interview with Professor Eva Harris

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Eva Harris *is a Professor of Infectious Diseases and Director of the Center for Global Public Health at UC Berkeley. Her research focuses on mosquito-borne viral diseases including dengue, Zika, and chikungunya in Latin American countries. We chatted with Dr. Harris about the cross-reactive relationships between Zika and dengue antibodies and the potential for certain concentrations of antibodies to enhance disease. But before we even got to our questions, Dr. Harris wanted to share with us what inspired her to start her research program and nonprofit organization in Nicaragua.*

Professor Eva Harris.

EH: Can I just launch right in? I was always interested in science—I did my undergrad at Harvard, and then I came here to UC Berkeley for my PhD in Molecular and Cell Biology. I was at Harvard in the Reagan eighties during the Iran-Contra scandal, and I was very politically active. I wanted to connect politics and science in my career, but at the time there was no way to do this because this was way before global health was a concept. I had decided to go to Berkeley for graduate school, but I postponed that and went to Nicaragua because there was a revolution and I wanted to be part of it. I landed with my pipettes and everyone was unsure what to do with me. I was completely unprepared because I had never been "south of the border." I had traveled very widely but I had never wanted to go to the developing world as a tourist. I wanted to contribute something—but when you're twelve and thirteen you don't have much to contribute. At twenty I still didn't have much to contribute, but I invented as I went along. It eventually became a thirty-year long, multi-million dollar program with hundreds of local workers. It has made a big impact on science in Nicaragua, and it has also become the basis for a large part of our non-profit, the Sustainable Sciences Institute (SSI), which focuses on building scientific capacity in developing countries worldwide. My vision is about doing good science but simultaneously connecting it in a way that makes the world a better place—I'm still an idealist. The vision has never been to have a top-down, vertical, North-South approach but to be more horizontal. Our goal is to directly address problems that are local priorities by applying methodologies in a way that is knowledge-based

and builds from the bottom up. Upon completing my PhD at Berkeley, I wanted to become a bridge between academia and health problems around the world, which eventually became known as global health. But I was doing this fifteen years before the term "global health" was even coined! I received the MacArthur "Genius" Award in 1997, which I used to start the nonprofit SSI while I was building my academic career here at Berkeley, and having a baby. When I came here I was interviewed by a program called Conversations with History. They asked me what I was doing and what it was called, and I wasn't sure what to name it, so I told them International Science… That was my moment to coin "global health," but I didn't! In many ways now, the moniker "global health" has become a way for US universities to have glitzy programs which are less about the welfare of their partners than their own universities. However, that is not what I espouse. I accepted a professorship in Infectious Diseases at Berkeley, which became a platform for me to have the independence I needed to build what I wanted to build. Of course, you pay in academia because you don't really sleep for the next fifty years. I didn't have any experience in dengue, virology, immunology, statistics—none of what I ended up working on! I had my PhD in yeast genetics and I've basically winged it for the rest of my life. I've always thought science as a horse, and I'm just hanging onto the tail, trying to learn as much as I can along the way. After accepting the professorship, I was working on multiple infectious diseases in several different countries. People told me to focus, but I wasn't sure how to because I was interested in everything. Gradually, I forced myself to choose vector-borne diseases and settled on dengue because it was a priority disease in every country in Latin America I worked in. The dengue virus is interesting—it's kind of like a breathing ball (Fig. 1)—and there were many unanswered questions about it and suprisingly little research at the time. We also couldn't use existing animal models because dengue doesn't occur in mice the same way it does in humans. So I established a broad program that spans virology, pathogenesis, and immunology, which my lab studies here at Berkeley, to epidemiology, diagnostics, clinical aspects and control, which we study in close collaboration with my colleagues in Nicaragua. When Zika came along, it expanded everything, since we were able to port laterally from dengue across numerous disciplines. We began about forty new projects in two months! There were so many more questions—pregnancy, microcephaly, which cells does it invade in the placenta, diagnostics, cross-reactivity, etc. But I still continued doing dengue research because that is the focus of my grants. I was able to add supplements to expand all our work into Zika, and we were able to add Zika to all of the studies we had ongoing in Nicaragua, as well as add new studies of pregnant women. So that gives you a little bit of context.

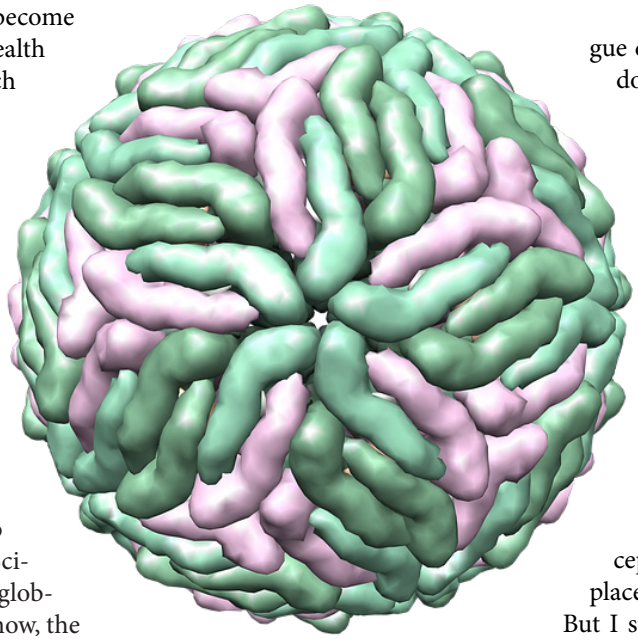

Figure 1: *Dengue virus 3D structure.*[1]

> ***"Our goal is to address local priorities by applying methodologies in a way that is knowledge-based and builds from the bottom up."***

BSJ: Let's talk about your research on Zika first. What does it mean to be seropositive?

EH: Seropositive just means that you have been exposed to the pathogen of interest and therefore your body has developed antibodies to that pathogen, which we can measure. In this case, it is tricky because dengue and Zika viruses are very closely related antigenically, and there is a lot of antibody cross-reactivity. All the standard methods we had for serologically detecting dengue virus infection were now criss-crossed with Zika virus infection. However, using fifteen years of samples from patients with dengue and Zika, we immediately developed a sensitive and Zika-specific assay. This was the Zika NS1 BOB ELISA, a blockade of binding (BOB) ELISA that is based on a viral protein that is secreted from infected cells called non-structural protein 1 (NS1). A lot of people were using NS1 as an antigen, but both dengue and Zika antibodies can recognize Zika NS1. We worked with a small company that had developed human monoclonal antibodies to Zika virus. A monoclonal antibody is produced by a single B cell (a white blood cell that secretes antibodies) that is fused to make an immortal cell. The resulting hybridoma only secretes antibodies of that particular clonal lineage, that in this case recognize a specific site on Zika virus NS1. We label that antibody with an enzyme (which can be detected colorimetrically) and then compete it against the antibodies in patients' sera. So, if you have had Zika, you will also have antibodies against that one Zika-specific site that can displace the labeled monoclonal antibody, reducing the color measured in the assay. But if you have dengue, you won't have an antibody to that

Zika-specific site, so you maintain the labelled antibody and the color. In this way, we can distinguish individuals who have been exposed and have developed antibodies to Zika from those who have developed antibodies to dengue.

BSJ: Your study sample was divided into three groups: pediatric, adult, and family. Across those three groups what factors had the largest influence on Zika seroprevalence (level of Zika pathogens exhibited within a population)?

EH: You would think that when a new pathogen is introduce into a naïve population, everyone would be equally exposed. But that was not the case, and we observed differences in Zika seroprevalence risk across both age and sex. We saw that females were slightly more exposed. In our original pediatric cohort of 3,700 children we observed an interesting linear rise in seroprevalence over age, but we wanted to examine not only how kids ages two to fourteen are impacted, but how the adults are infected as well. So we expanded our study to our household cohort, and we observed that Zika seroprevalence risk was more flat across ages in adults compared to the children's cohort. We then compared seroprevalence across the whole household study—kids and adults (Fig. 2). As before, we observed Zika seroprevalence risk increased with age and then flattened out. We were wondering why this was, and we noticed that kids who were obese or overweight had slightly higher seroprevalence. Because of that, we started looking at body mass index (BMI), as well as what turned out to be the best correlate: body surface area (BSA). When you examine variables one by one in a univariate analysis, both age and BSA are significant. Another significant variable is school session. Children in Nicaragua go to school either in the morning or the afternoon. The kids that went in the afternoon were also at a higher risk of seroprevalence. However, in a multivariate model, BSA was revealed as the greatest significant risk factor for getting Zika infections. Then we realized, not only do childeren get bigger as they get older, but the afternoon session was when the older kids went to school. Everything was collinear with body size. Additionally, mosquitoes are attracted to carbon dioxide. If you're bigger, you breathe out more carbon dioxide. Women sometimes breathe more rapidly than men, and when you are overweight or obese, you can also breathe more rapidly. We also added seroprevalence to our spatial analysis, because every child in our cohort study has a GPS point for their house. If you notice, in Fig. 3, the purple is clustered at the western end of our study site, around the cemetery. We then went to the cemetery and measured all the mosquito breeding sites there. *Aedes aegypti*, the biggest mosquito carrier of Zika and dengue viruses, breeds in clean water around people's homes. In other community-based projects, we explain to people why they should clean up standing water around their homes—but no one is doing that in a cemetery. In fact, the cheapest tombstone one can buy in the cemetery is a cross with two little holders for flowers on either side. These flower-holders fill with rainwater—even if you are not bringing water for the flowers, these holders are collecting water anyway.

"In a multivariate model, body surface area was revealed as the sole significant risk factor for getting Zika infections."

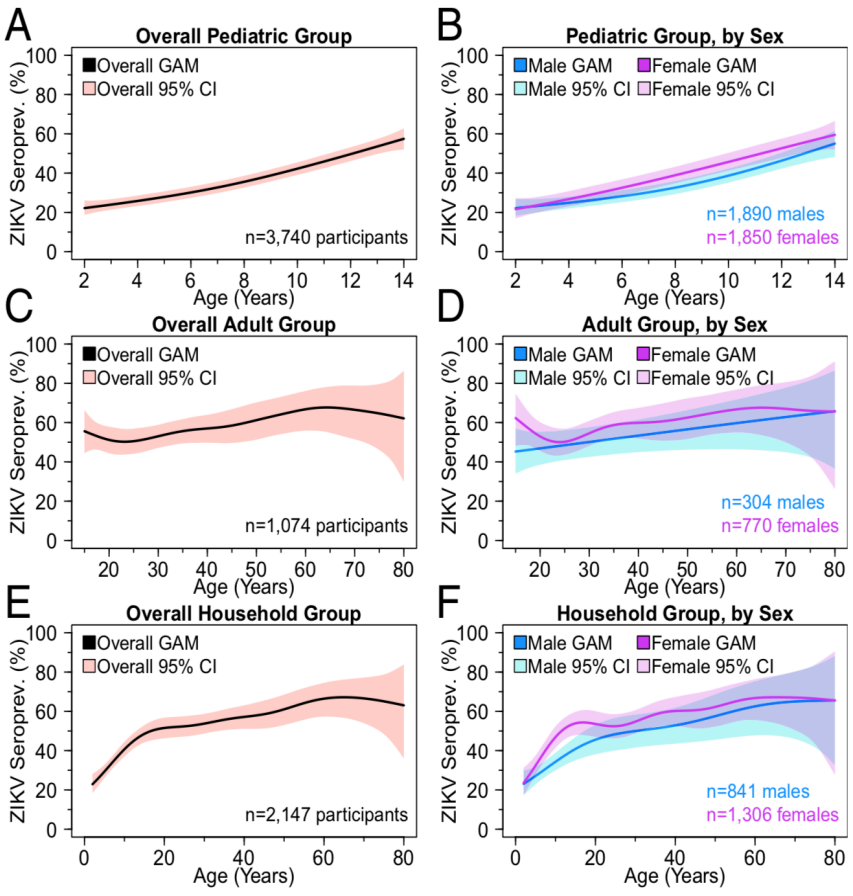

Figure 2: *Zika seroprevalence in pediatric, adult, and household cohorts.*[1]

Additionally, some crypts are broken and water can seep in, which makes wonderful mosquito breeding grounds.

BSJ: Could you define neutralizing antibodies and explain the concept of cross-neutralization?

EH: For measuring neutralizing antibodies, there is a different kind of assay consisting of cells, antibodies, and a virus. If the antibody binds to the virus in a way that blocks it from infecting the cell, then the infection is neutralized. One can measure infection either by a plaque assay or by flow cytometry using a labelled antibody that will essentially color a cell upon infection. Then one can perform a dilution series of the serum or monoclonal antibody in question and add that dilution series to the virus. If the antibody neutralizes infection, then at high concentration no plaques, or no colored cells, are obtained. As the serum or antibody is diluted, more and more plaques are obtained. We can measure the presence of neutralizing antibodies using an NT50 value, or neutralizing titer 50—the concentration of serum or monoclonal antibody that reduces the amount of plaques or colored cells by 50%. That value can be used to compare antibody neutralizing potency. Dengue is caused by four different virus serotypes, and antibodies can cross-react with these serotypes and some can cross-neutralize different serotypes. Other antibodies are type-specific—these are powerful and will protect you from future disease upon infection with the same serotype. Anti-Zika virus antibodies are not only cross-reactive with dengue viruses, they can be cross-neutralizing to some extent as well. So one question was: even though there is cross-neutralization, could we still use these methods to distinguish dengue from Zika? If done properly, we can.

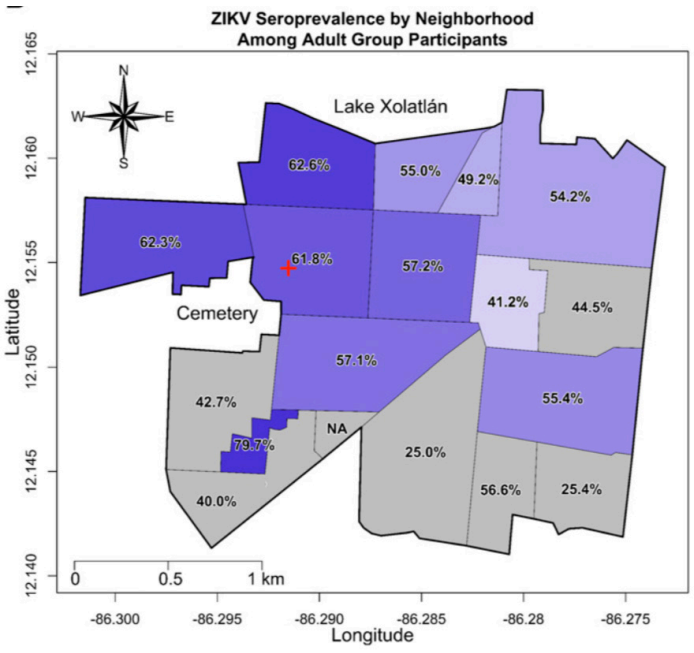

Figure 3: *Spatial distribution of Zika seroprevalence.*[1]

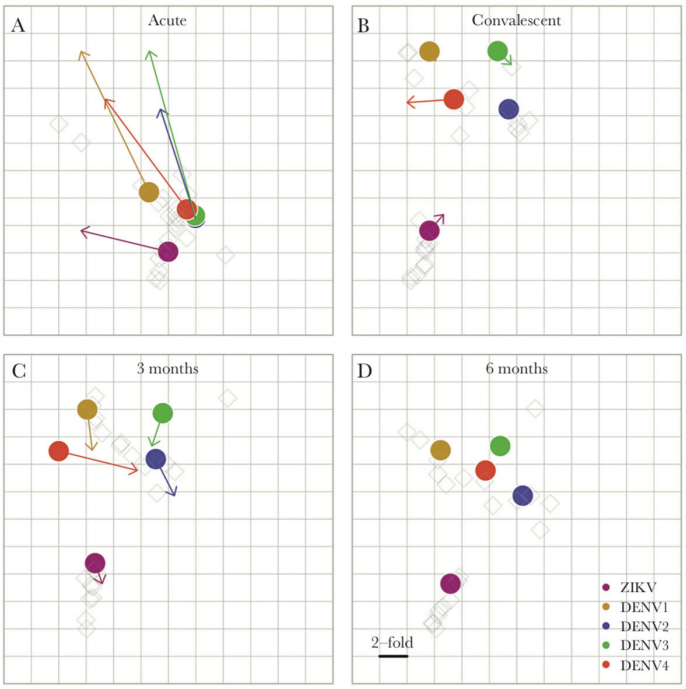

Figure 4: *Antigenic map of dengue 1-4 and Zika viruses.*[2]

BSJ: Does cross-neutralization of Zika occur in dengue-immune individuals? What does that tell us about Zika's presence in the dengue serocomplex?

EH: The short answer is yes; you can have cross-reactivity and cross-neutralization. The question is: what is the magnitude? If you've had a dengue virus (DENV) infection in the past, you will have higher neutralization to dengue virus in the future. Cross-neutralization of Zika virus (ZIKV) also occurs in dengue-immune individuals, but on a much smaller scale than it does for other dengue serotypes. Then we asked the opposite question: if you've had Zika, do you cross-neutralize DENV? You do, but again, at a much lower level than ZIKV. We then made an antigenic cartography map (Fig. 4) where we plot distance as a function of NAb titers. We essentially plot each virus as a ball in three-dimensional space. When you collapse that into two dimensions, you can see where those balls are in relation to each other, and what we found is that early after infection, ZIKV was in the same region as dengue—that's why people thought they were very similar. But as time went on, ZIKV really pulled away from the dengue viruses on this antigenic map and therefore we believe Zika virus is in a distinct serocomplex from dengue viruses. I direct a big grant that brings together academic groups from around the country to investigate adaptive immunity to dengue and Zika. Dr. Aravinda de Silva's group at the University of North Carolina, Chapel Hill, has developed a method for pulling out certain subsets of antibodies, for instance, antibodies that recognize DENV 2 (one of four DENV

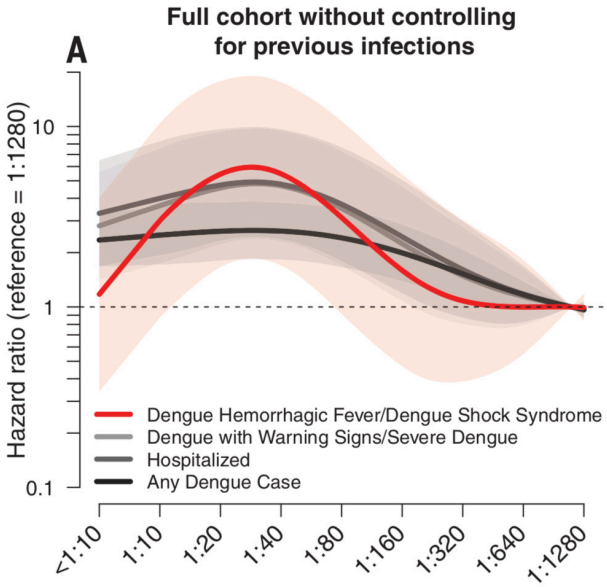

Figure 5: *Dengue antibody titers illustrate a peak enhancement between 1:21 and 1:80 NAb ratios.*[3]

serotypes). This allows us to study a polyclonal mix of antibodies but remove all the cross-reactive antibodies and be left with just the type-specific antibodies. Using this method, we found that in both travelers and endemic populations, even though there are a ton of dengue and Zika virus cross-reactive antibodies, they're really not contributing to the Zika neutralizing antibody titer. What's really contributing are Zika type-specific antibodies. In other words, dengue antibodies are not necessarily cross-neutralizing to ZIKV even if they are cross-reactive.

BSJ: Given all the research with the cross-neutralization between dengue and Zika, what would you say to claims about the potential of single possible vaccine for both Zika and dengue?

EH: Initially, before we did that last set of experiments, we thought that the presence of cross-reactive antibodies against both dengue and Zika was a positive sign for a single vaccine. But the fact that the most potent Zika neutralizing antibodies are Zika-specific means that you couldn't have a dengue vaccine that would work against Zika. What people are working on, since the dengue vaccine has four different serotypes, is adding Zika as a fifth virus. A dengue vaccine isn't going to work against Zika, but both viruses could potentially be included in one vaccine.

BSJ: We've spent a lot of time talking about neutralizing antibodies, but at some concentrations antibodies can enhance disease. Can you explain the concept of antibody-dependent enhancement (ADE)?

EH: Antibody-dependent enhancement is a concept that's been around for a really long time, and it has to do with the fact that there are multiple ways for a virus to enter certain target immune cells. One is through what we call a cognate receptor, which is essentially a receptor that recognizes the virus and brings it into the cell. But you can also have antibodies to that virus that recognize the virus but don't actually neutralize it, as we discussed above. This causes an immune complex where that virus is still alive even though it's bound to an antibody. The constant region (Fc) of that antibody interacts with Fc receptors on the target cell surface, which bring the antibody and the live virus into the cell. So there are two routes into an Fc receptor-bearing cell: one through the cognate receptor, and another through the Fc receptor. In general, the Fc receptor route is only supposed to bring in dead or neutralized viruses, but if you have an antibody that has not neutralized your virus, it gives your virus a "stealth" way of entering the cell. This way, the virus does not trigger the innate immune response within that cell. Having this extra route ends up increasing the infection of that immune cell, which then activates T cells, which secrete cytokines. Then a cytokine "storm" is created that leads to pathogenesis. In the paper you are referring to, we didn't actually show ADE by this mechanism, but we did show antibody-enhanced disease in human populations, meaning that there is an increased risk for severe disease in people with a specific concentration of pre-existing antibody than those with more or less of that antibody.

BSJ: In this study you showed that subjects with antibody ratios between 1:21 and 1:80 were at a significantly larger risk for dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS)—the most severe cases of dengue symptoms. How does this ratio relate to the concepts of antibody-dependent enhancement and antibody-enhanced disease?

EH: At this point, we have evidence to show that certain concentrations of antibodies cause antibody-enhanced disease, which is a non-mechanistic immune correlate. Theoretically, we could be seeing ADE, which is a mechanistic correlate. The idea is if you have no antibodies, the virus is only getting into the cell via the cognate receptor. If you have many, many antibodies, even if they're not great, they fully coat the virus so it can't get into the cell. But if you have antibodies that are not great, and you don't have enough of them to fully coat or neutralize the virus, they actually help the disease by forming an immune complex and allowing the virus to enter the cell via the Fc receptor. That's why we observed a greater risk of DHF/DSS at that particular range of antibody concentration. There has been a huge controversy in the field over how to measure enhancing antibodies. It's generally

"The dengue vaccine that's been licensed actually causes ADE in dengue-naïve people."

done in vitro by taking a cell line with only Fc receptors—no cognate receptors. You treat these cells with DENV and get no infection until you add antibodies. Then with a dilution series, you obtain a curve similar to the one in Fig. 5 because there's no other way into the cell unless a certain amount of antibodies enable the virus to enter the cell via the Fc receptor route. The question is: what is that level of antibodies in a human? We avoided a lot of this controversy because we weren't testing any assay in vitro, we were just observing the natural antibody titers of children with disease.

BSJ: What implications does your research in ADE have on dengue vaccination?

EH: Big—because the dengue vaccine that has been licensed actually can cause ADE in some dengue-naïve children. A lot of us saw this coming and warned that ADE could be an outcome. But the company went ahead, and children were vaccinated in the Philippines, and it turns out that there are reports of more severe disease in dengue-naive vaccinated children, which are currently being investigated. The company has changed its label to only recommend vaccination in dengue-immune individuals. The fact that we showed ADE can occur in humans concomitantly with the company's change in its label was a big deal. Now that vaccine can no longer be used in dengue-naïve children, so this study had a big impact.

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