Viral Outbreak: The Science of Emerging Disease
Lecture 1 – Dengue Fever: Breaking Epidemic Cycles
Eva Harris, Ph.D.

1. Begin of Lecture 1 (0:15)

[ANNOUNCER:] From the Howard Hughes Medical Institute. The 2010 Holiday Lectures on Science. This year's lectures, "Viral Outbreak: The Science of Emerging Disease", will be given by Dr. Joseph DeRisi, Howard Hughes Medical Institute investigator at the University of California, San Francisco, and by Dr. Eva Harris, Professor of Infectious Diseases at the University of California, Berkeley. The first lecture is titled "Dengue Fever: Breaking Epidemic Cycles." And now to introduce our program, the President of the Howard Hughes Medical Institute, Dr. Robert Tjian.

2. Welcome by HHMI President Dr. Robert Tjian (1:08)

[DR. TJIAN:] Welcome to the Howard Hughes Medical Institute and the 2010 Holiday Lectures on Science. A warm hello to our in-house audience of high school students that have come from throughout the Greater Washington, D.C. area. And also greetings to our webcast audience, especially students at holiday lectures events all across the country from Florida to Washington State, from Wisconsin to North Carolina and so forth. As President of the Howard Hughes Medical Institute, I have the privilege of leading pioneering research as well as educational programs that have national and international impact including these Holiday Lectures tailored particularly to high school biology students. To learn more about HHMI, you can visit our website, www.hhmi.org. The Holiday Lectures are a way for us to enthusiastically celebrate some of the best science that we do in our institute. And I'm particularly pleased to introduce this year's speakers, two extraordinary teachers as well as researchers at the top of their game, who are very excited to tell you about their work. Eva Harris is Professor of Infectious Diseases at the University of California at Berkeley. And Joe DeRisi is a Howard Hughes Medical Institute professor at the University of California at San Francisco. Eva and Joe's themes will be the fascinating and sometimes scary world of viruses and how these viruses can cause disease. I think perhaps most importantly, we hope to also learn how we can fight disease. Now I'm very happy to introduce our first speaker, Eva Harris, to deliver the first lecture. Eva has been a colleague of mine at University of California, Berkeley and I've seen firsthand her passion and enthusiasm for science, particularly for the potential of science to improve the lives of ordinary people. As a young college graduate with a degree in biochemistry, when I first actually met Eva, she had begun to travel to Nicaragua to see if she could make a difference. And, well, let's just say some years later, she is still working at Nicaragua from time to time. And I believe you'll see that she is clearly making a big difference. In her first lecture, Eva will take us to Nicaragua and tell us about the serious impact of a viral disease called dengue fever. It sounds pretty scary. I'll let her explain why this disease has become such a serious epidemic and where in the world it is headed. We now will have a brief video to introduce Eva before her lecture.

3. Profile of Dr. Eva Harris (4:14)

[DR. HARRIS:] Nicaragua is a very beautiful country. It is the second poorest country in the hemisphere after Haiti. And so there are not a lot of resources, but we've been able to come a long way, as they say. We've really put the necessary resources together in such a way that it functions, a lot of effort on quality control. So we've essentially made up I think in quality for the resource constraints.

I think that the key to success, if you wish, has been to really keep it at the grassroots level and to then the primacy of the human relations that is always very important to me and to kind of focus on results. You know, we don't come in at a political level explicitly. You know, we build from the bottom. And

then any government can see the good that that's doing for their country. They can see the impact in the hospitals we work on and health centers that we work with, you know, on the people, you know, so they can actually see that there's something positive there. I think that there's just an overall sense of moving forward in the health and education realm that many people can identify with. This lecture series is an amazing opportunity to be able to connect with and inspire a new generation to be interested in science and to be interested in global science and to be interested in essentially using their intellect and their capacity to change the world. And, you know, they can do that locally. They can do that internationally. But the idea is, you know, to essentially transmit the concept that, you know, with their lives, with their science, with their intellect, they can dream. They can have passion. And they can make a difference.

4. Dr. Harris first visited Nicaragua right after college (6:06)

Thank you. It's really wonderful to be here, a great pleasure and honor. And I'm deeply grateful to Dr. Tjian for inviting me and for the incredible team that we've been working with over all this time, and to HHMI for sponsoring this event, and to you for being here. So what we're going to talk about today is Nicaragua, is dengue fever. And I'm actually going to start with a personal story because, like you, I went to high school and then I went to college. And I graduated from Harvard with a degree in biochemical sciences. And I just always have loved biology; I mean how the molecules work together and how the cells work together in a body. But I've also been always disconcerted and still am by the great inequalities that exist in the world today in terms of wealth, in terms of opportunity, and in terms of access to science education. And so when I finished with college, I went back to New York, which is my hometown. And then from there, I went to Nicaragua because at the time there was a revolution going on. And they were making great strides in the areas of health and education. And as Dr. Tjian said, I wanted to make a difference and I wanted to see what I could. And, of course, I was hopelessly unprepared for what I found. But I had a vision. And that vision was to bring knowledge and tools and resources to the countries where the diseases were endemic and to work with people there to understand what their priorities were in public health and what their priority in infectious diseases were. And one of them was dengue virus.

5. What is dengue fever? (7:26)

And so what is dengue? Well, what you see here are two patients with dengue. There are about 50 million cases of dengue fever which occur every year. And what we see is this is actually a very debilitating and painful disease. In fact, I've had it. But you usually survive; case in point. However, there is a worse form of the disease and this can be actually fatal within 24 to 48 hours. And what we don't understand is why some people have this severe disease. And so there are a lot of issues at the level of the science. But there are a lot of issues at the level of clinical management and public health. And so I try to address all of those. And so what we're going to do now is look a little bit more in detail at dengue. So what you see is that it's essentially a high fever acute onset. And then this muscle and bone pain, it's actually called *la quebradora* or breakbone fever. You might have heard of this. And it has a really extreme retro-orbital pain and it's just very debilitating. But you do have these warning signs, for instance, this abdominal pain and vomiting and especially fluid accumulation. Because what happens in severe dengue is it's essentially an aberrant immune reaction, which leads to leakage of fluid in your circulatory system out. And you end up with shock from low blood volume and then it leads to death very quickly. So the big question is, again, how is it and why is it that certain people can progress this rapidly? So *vamos a Nicaragua!* So we're going to go to Nicaragua and we're going to roll the video and I'm going to tell you a little about the country that we're going to visit.

6. Video: Managua: Rapid, Unplanned Urbanization (8:53)

So what you see is the capital and Nicaragua is actually one of the largest countries in Central America, although it's the least densely populated. There are 5 million people and 1.5 million people live in Managua, which is the capital. Now Managua actually was decimated in 1972 by a great earthquake, which leveled it. And actually there are only three buildings that are over two stories high right now and one escalator, which is a big sightseeing event. And you can see that there are a lot of like just kind of open fields in Nicaragua and a very poor water management system, which in many other cities in the tropics you actually don't have access to running water all the time. And, therefore, people have to have their water stored in large barrels, which will store clean water. But as you'll see what Emperatriz is bringing out here are larva. And what these larvae are are larvae for mosquitoes.

7. Dengue virus and vectors (9:45)
And what you're going to see in the rest of this series is that these mosquitoes actually will grow up to become in this case *Aedes aegypti*. And *Aedes aegypti* is a prevalent mosquito throughout the Americas, a little bit in the United States. And what they do is they carry dengue virus. And actually there's another culprit here, which is *Aedes albopictus*, which actually is prevalent and spread throughout all of your backyards too. In fact, you're very lucky today, because you'll be able to actually play around with the adult and larval forms of these, due to the hard work of Laura Kramer and Robin Moudy who are here today to help us with this.

8. Most dengue cases are asymptomatic (10:23)

But the main point is that we have these two mosquitoes, specifically these species that can transmit dengue. And what ends up happening there is that we have, as with many infectious diseases, a pyramid where at the very top is the most severe disease. And then underneath that, we have about 50 million cases of reported disease. And then we have unreported disease. And then at the very bottom of that pyramid are the people who we call asymptomatically infected. So they actually get infected with the virus, but don't actually have clinical symptoms. Now generally that would be a good thing because you'd think you'd be protected in the rest of your life. But, in fact, with dengue and this is the trick we're going to look at later, it actually puts this population at higher risk for severe disease.

9. Rapid global spread of dengue (11:01)

So why are we worried about dengue? Well, it's spreading out of control like a bat out of hell. And the reason is three different ways. We have an increasing number of cases. We have increasing geographic spread. And then we have this issue about co-circulation of the four types or the four flavors of dengue. And that's what causes these problematic immune reactions. So if we look at where dengue is prevalent in the world, there's kind of like a belt around the world, the subtropical and tropical areas. And, in fact, we're lucky enough to have the vectors of dengue in the United States. And this is where the *Aedes albopictus* and a little bit of *Aedes aegypti* are in the United States. And actually there was a big outbreak, or big for the United States, in Key West of confirmed cases, which actually when they looked there were a number of other people that had been infected. I should mention that in the 1700s and 1800s and actually in the 1900s, there was actually a lot of dengue up and down the Eastern seaboard and yellow fever as well. And that actually gradually went away with the increased socioeconomic status and water management. But it's something to keep in mind. And now what we see here in this graph, what you're looking at on the Y-axis is the number of cases in millions that are reported. And over here we have decades. And essentially what you can see is, although there's a cyclic nature, it's going up and up and up and up. And essentially now we're over...we're just, you know, off the charts here.

10. Animation: Dengue Fever Re-emergence in the Americas (12:21)

The other issue and what you're going to see is the geographic expansion. What we can see is from 1960s, you're going to see this spread through the Americas. And what you see is the red here means that there's a large number of cases. And you can actually see it spreading throughout all of the different countries in Latin America. And this is actually not only in the Americas, but also in Southeast Asia and in the Indian subcontinents that we have a huge geographic spread.

11. Multiple types of dengue virus (12:46)
And then the other issue is this co-circulation of the different flavors. So as you can see, we have four different flavors, dengue 1, 2, 3, and 4. They're otherwise known as serotypes. And the problem is that originally, there were only a couple of places where you had all of these four circulating at the same time. But now essentially all of these four viruses are everywhere. And the more that that happens, the more you get the severe disease and epidemics of what we call dengue hemorrhagic fever and shock syndrome.

12. Causes of dengue re-emergence (13:12)

Now it's kind of curious as to why this arose. I told you that, for instance, dengue has been around for a very long time. But what was really curious was the emergence of this severe dengue, of the hemorrhagic fever and shock syndrome that got people really worried because of its fatality rate. And, in fact, the interesting thing is that this actually came about because of World War II in the Southeast Asia because the movement of ships in the Pacific theater moved the mosquito around to different countries. And then the movement of the troops when they were viremic, meaning when they were actually carrying the virus in their own bloodstream moved the different viruses around as well. And so what we're having today in the rest of the world is actually not due to a particular war. But it's due to our lifestyle, which has to do with global trade, global travel, and urbanization. So what you see here, for instance, is a picture of containers of the shipping industry. And here's a barge, which has a bunch of old tires in it. Now in old tires is water. And in the water is larva. And the larvae grow up into mosquitoes. And, in fact, this is how there was a point introduction of a single ship from Southeast Asia that docked in New Orleans. And that's where the whole spread of *Aedes albopictus* into the United States came from. And so you can see that this global trade is a problem for the mosquito. For global travel, this actually looks like my life. But actually what it is is the amount of jet travel in a single day, okay. And so you can imagine that this is real time spread, like the hot zone. You know, it's true. You're actually moving virus all over the place in a very short amount of time. And then we have this issue of urbanization, which is that the greater and greater percentage of the world's population; you can see here on the Y-axis percent of urban population is going up, 1950, 2000, 2030. And the whole world is increasing in urbanization. And many of these cities have poor water planning and waste management. And the bottom line of all of this is that the vectors that transmit dengue are perfectly adapted to your lifestyle and to essentially human habitat.

13. The immune response and severe dengue (15:09)

And so now what I wanted to do is to kind of go into a little bit more, why is it that these four different viruses can come up with this bad reaction. And so imagine that you have a person who is being infected by this red virus, dengue 1. So this person will mount an immune response and will actually either become sick with dengue fever or be asymptomatic, but will clear the virus. And so we're going to have the immune cells, the T-cells and the antibodies. The B-cells are going to remain in the body so that when they have a second infection with that same dengue 1 virus, that red virus, then they actually can be protected by their immune response. And now the problem though is that when you get a green virus, which is the dengue 2 virus, the antibodies and the T-cells only partially can recognize, but they don't neutralize the virus. And because of this funny immune reaction, you actually end up with a much more severe disease in that situation. And what we're going to do tomorrow is look more at the molecular and

immunological detail of that and how we can prevent this problem. But in the meantime, you can see that this is a frigging complicated disease, right? I mean not only do you have the humans, you've got the mosquitoes, you've got the virus. And then on top of that, you can see you've got individual risk factors. We have what we call epidemiological risk factors that have to do with the populations themselves of humans. And then we also have virus risk factors. So we have not only four serotypes, I wish it was that simple; within each serotype, there's genetic diversity. So there are genotypes within the serotypes. And then there's clades within the genotypes. And there are strains within the clades. And these can actually have different potential for disease severity as well. So only... you get the severe disease only kind of when this all comes together as the perfect storm. So you can imagine that to understand this disease severity, you have to understand a lot of different factors.

14. A broad-based public health research approach (16:52)

So when I started in my lab working on this 15 years ago, I decided to take a really broad approach that would move from the molecular virology, where we understand how the virus actually replicates, to an animal model of the disease so that we can understand how the disease and the immune reaction can happen. And we were very concerned about having diagnostics out there to be able to identify the disease properly. And then to study with the epidemiology is really the study of human populations. And so what we tried to do here is to have a series of studies, which I'm going to tell you about in a moment, in the communities and in the hospitals and then actually how we can do community-based interventions to stop this whole story. And then the last piece, I'll tell you tomorrow about another effort, which has been to really just build scientific capacity throughout the world so that every country can actually deal with their own problems by themselves. So what I'm going to tell you about now briefly is our cohort study and to kind of give you a sense of what this kind of epidemiological research looks like in real life. So this is just a picture. It's called an epidemic curve. And what you're seeing here again are cases on the Y-axis. And then along the X-axis, what we see are months. However, each bar in this histogram is a single day. And what we're looking at is an epidemic in Rio de Janeiro in Brazil. And so every day you're getting about 3,000 cases reported in a single city, okay. And I just told you that there are way more cases than are even reported. So this is a really intense epidemic when it happens. And now you can say do you see do you see anything curious about this curve? So there's this dip here. And you say, well, that's kind of interesting. I wonder what happened to the mosquito. Or do you think the virus was actually evolving in a certain way? I don't know. But you know what it was? *Carnaval*, that's right. You know, February, it's *Carnaval* in Brazil. And so people were sick and they're going to their doctors. And their doctors are like out there *samba-ing* and like there's no one to report the dengue to. Or maybe they were just like, well, you know, just go dance it off, whatever. So, you know, this is why we say you really need to know your own population so that you can interpret this in a proper way.

15. Pediatric cohort study to track dengue epidemics (18:48)

But this is our own data. This is Nicaragua. And this is a cohort study that we've had going on since 2004 and we're following 4,000 children over time. And what you can see is that from about August to January of every year, we have a peak of dengue incidence. And what we want to do is we want to understand really the natural history of the transmission of this. And what we do is we call it a cohort study because you have a group of children and you follow them forward in time, over time. And this allows a very different kind of analysis than what we call a cross-sectional study, which is only one point in time. And you'll see why this is so important. So what we are interested in is to use this to understand the spectrum of disease in children, to characterize the natural dengue transmission, to be able to investigate the immunological and the viral determinants of both dengue and severe dengue; and then also to be able to develop a site where we can actually test new drugs and vaccines so that we can prevent dengue in the future. So what do we actually do? Well, you mean we actually go to children's

households. We collect and test blood samples. We provide medical care. And we establish what's called good clinical and laboratory practice, which is a very kind of high level of standard so that I can participate in international studies and trials.

16. Cohort study detects symptomatic and asymptomatic infections

So the way the design of this study is such that we go forward in time like I mentioned. And then every year we take what's called a healthy blood sample. So why do we do that? Well, we collect antibodies in immune cells, which are in the blood, and we gather data. And then what we do is we measure every year the number of dengue antibodies. Now this also has a service for the population because we test for hemoglobin. And hemoglobin will tell you whether your child is anemic. And if they're anemic, then they get iron supplementation. So this is another really important part of our work. And I think that anyone who works internationally or just in communities needs to keep this in mind that what you do is you have a scientific reason for doing it. But you also must give back to the communities and to the countries in which you work. And so everything we do has a scientific objective and then essentially a giving-back objective. And so this is useful for the families themselves. It's useful for our science because what we do is we look and we measure the antibodies in year one and year two. And if we have an increase by fourfold or more in antibodies, that means that that child was exposed to dengue virus, mounted an immune response, and we can read out that immune response by an increase in antibodies. So we know that that patient or that child had an infection between year one and year two. Okay, now what we do is we actually can follow if they come to the health center every time they have any kind of a medical anything, you know. So we have thousands of consults. But everything that looks potentially like dengue or a fever, we actually work it up and we look for dengue antibodies and for virus in that sample. So what this allows us to do is to identify all of the children that had a symptomatic case of dengue, okay. So now when we compare that, what we can see is that we can calculate the total number of infections. And then we can calculate the kids that actually got sick. And then we know by subtracting that the kids that were actually infected and never got sick. And then we can say now what is it about those kids? What do they have in their antibodies or their immune cells that were able to protect them from getting sick? Or what is it that these children had that allowed them to get sick or even to have severe dengue? So for the scientific objective, we can then look at the immune factors and the viral factors that differentiated these children who either were infected over the same time period who either got sick or didn't get sick. And that's really important for understanding how to make a good vaccine or how to treat people. Now what it also serves though is, as I've said, something useful for the country. So what you're seeing here is a graph where we see on what we call the X-axis all these different health centers. So there are 21 health centers in the whole city of Managua with these 1.5 million residents. And what we're looking at in the different colors is the number of cases that are reported to the health system, okay, in different colors. And so now what I'm going to show you is our cohort study. Wow. So do you think that there's actually a lot more dengue right in the health center where we're working? No, that's right. No. In fact, I can show you because this is the health center, only the kids that weren't in the cohort. This is because we're actually testing for dengue and looking for dengue. And what we see is that there's actually 20-fold more dengue than anybody is actually aware of. And this is really important because when you want to actually make a case, an economic case to make a drug or a vaccine, you want to be able to say, look; it costs this much. And if you're off by like 20 or 50-fold, you know that's a problem. And as a ministry of health, you also need to know how much disease you have in your country. And this is not just in Nicaragua. It's actually now there are studies coming out in all countries that have about this same effect. So this is really important for the ministries to know. The other important thing and this is my last piece of data is that, in fact, lot of childhood dengue actually doesn't look like the dengue that we have as our case definition. And I mentioned that part of this study was to look at what does pediatric, what does childhood dengue look like? And you can see that these are laboratory confirmed cases. So we look for the virus. We look for antibodies. We can say you had dengue. Now did it look like what we think dengue looks like? Well, if you're 11 or 12, yeah, maybe it

does. But, in fact, when you're really young, maybe you're not saying I have *myalgias* and *arthralgias*. And I have, you know...so you actually are reporting just a fever. And, in fact, more children have a fever that doesn't look like dengue and they're never even being recorded. So now on top of this 20-fold more dengue than we even thought, now we're fully missing 25% or a quarter of all of the childhood dengue that's out there, okay. So this is actually useful for clinicians and the Ministry of Health et cetera. So what I wanted to show you is how we can actually use this kind of study to be able to direct health care benefits, to deliver important epidemiological and clinical data to the country, and then also to be able to set the ground for the immunological and virological research that goes on in collaboration between my lab at Berkeley and our colleagues in Nicaragua.

17. Q&A: Would disease severity be worse if infected by all four serotypes? (25:05)

So that was what I wanted to start with. And I think we're going to stop here for a couple questions. And I'm going to just open the floor to see if A, you understood and B, you have any curiosities. And I'll be able to take a couple questions. Yes.

[STUDENT:] I was wondering since there are four types of serotypes of dengue if a person had three types of dengue if they got the fourth one, would the reaction be worse or would they be used to it since they had three different strains?

[DR. HARRIS:] Can I hire you? No. That's a brilliant question because it's exactly what we're trying to figure out. And it's exactly what this cohort can tell us. It's been thought that it's the second infection that's the worst. And because essentially you have a certain set of antibodies and they can...we'll talk about this tomorrow, but they can essentially create this over-exuberant immune response. But the thought is that once you get to third, and fourth infections, there's this kind of enough cross reactivity that you are protected. But that's kind of dogma and we don't know. And the coolest thing is that now we're seven years into this cohort and we just got funded for another couple of years. And what we can actually do is prove whether you have less dengue when you have more infections because that has big implications for vaccine development. Like what if you actually don't need a full response to all four viruses in a vaccine because you have to give all four viruses together? So what if you could really only get three and that would still be protective, for instance, you know? So it's a really good question.

18. Q&A: Would a live or a dead vaccine be better? (26:27)

Mm-hmm, yes.

[STUDENT:] If you were going to do a vaccine, would you do like a live vaccine? Or would you do more of a dead one?

[DR. HARRIS:] Yeah. So the leading candidates right now, in fact, there's a phase IIB trial and one going into phase III, which are live attenuated vaccines. And so the one that's going...yeah, anyway, so these allow...what that means is that you have a vaccine which is alive, but doesn't make you sick. And that actually allows you to have a very good antibody response and a T-cell response. And so that was of the leading candidates. But the problem is that since you have to put all four together and they're live, they actually interfere sometimes with each other. And so meaning like the immune response can get muted to one or another. So there are other sets of kind of what we call second generation vaccines, which are subunit vaccines in a prime boost strategy. So there are kind of second generation vaccines that are exploring other approaches.

[STUDENT:] ...it would be like an every three month type thing?

[DR. HARRIS:] No, you would actually -- well, unfortunately, you often need to boost, which is another issue with these. So right now, you need kind of two boosts to get the best reaction. But the idea is that there are these live attenuated and then others, which are subunit or killed vaccines, which are being tried as well.

19. Q&A: Who funds your research programs? (27:36)

[STUDENT:] My question is...

[DR. HARRIS:] Yeah.

[STUDENT:] ...regarding funding. From - - a town in Nicaragua and you go all over the world. And I'm wondering who funds you? Who gives you the money? Is it from a grant or from an institution?

[DR. HARRIS:] Okay. So see these dark circles under my eyes? I write lots of grant proposals, anything. I mean this work in Nicaragua for me is the most important. And so I mean from the National Institutes of Health I get a lot of funding from them. There were pass-through organizations through the Gates Foundation, which for a while was supporting this, and WHO, anything, you know, foundations. We have a foundation in Switzerland. You know, if HHMI is interested...no, just kidding. And so, you know, I mean anything, I'll do anything to continue this work and I keep working on this. But it's, you know, as with anything, you've got to work hard to find the money.

20. Q&A: Is global warming helping spread dengue to the United States? (28:20)

Yes, in the gray sweater.

[STUDENT:] Is global warming the reason why this virus has come to the United States?

[DR. HARRIS:] You know, a very good point. Actually there is spread of the vector of the mosquito, yes, because of global warming. But actually the reason that we don't have disease as much in the United States and the reason that it got wiped out here for a while is actually because of socioeconomic status and because of the way of life. So for instance, the big change was screened windows and air conditioning and not being outside while the bugs were biting. So, in fact, you can have virus. Like the *albopictus* is all over Europe. *Albopictus* is in the United States. And it has the potential to cause disease, but not as much because people are simply not in contact as much as they are like when you get out where there's like no window. It's just always open, you know. And so there's no idea for screens or anything. But that's a very good...what good questions we get here, excellent.

21. Spread of mosquito vector, spread of dengue (29:18)

So now what do we do about this, right? So what I'm going to tell you about is this community-based intervention that we've been working on in Nicaragua to try and address this issue of in the here and now. I mean we're working on vaccines, right? But that's not now. So how can we actually prevent this disease in the moment? So, well, I told you about this problem, right, global trade and urbanization. And you can see like a lot of the trash for instance is plastics and the tires and all of that can actually be breeding grounds for mosquitoes. So when you look here, this is a picture similar to what you saw before. What you see is Latin America. And what we're looking at now is the distribution of this mosquito. *Aedes aegypti* in the seventies and then in the late nineties. And it's actually worse now. Now look at this. This looks real similar, right? This is where the disease is. So this shows you that where you have mosquitoes with the right conditions, you have disease. And it also shows you that if you can get rid of the mosquitoes, you can get rid of the disease. So even though having the mosquito because it's a

vector-borne disease, the mosquito is transmitting the virus and making things more complicated; it also actually gives you an entry, another entry point for control of that disease, right? Because now you don't only have the human and the virus, if you can get rid of that central point, which is the mosquito, then you can also control the disease.

22. Large-scale mosquito control programs (30:40)

So actually this is interesting because the reason that this looks so nice and clean is because there was a huge campaign in the Americas in the sixties to get rid of *Aedes aegypti* because it also spreads yellow fever. And there was a lot of fear about yellow fever. And so, big campaign, a lot of insecticides, DDT, the Army involved, the health...but what happened is that it was very effective. But then once they stopped paying into this and actually supporting this and it was very expensive, boom, the mosquito came right back. And on its tail came the dengue. So another thing at the kind of more micro-level...that was like the whole continent, right now let's just look at Fortaleza, Brazil. This is the work of Pontes et al which was published in 2000. And what you can see here is this is the effects of a government-run program where they're coming and sending out teams that go into people's homes and they get rid of the water where, you remember how you saw that larvae growing around there. So they get rid of where the mosquitoes are growing. And then you can see that when they have what we call 100% coverage, that means whatever percent coverage, that the teams are going into those homes and getting rid of where the mosquitoes breed. And what you see is as a result is that if you then measure how many houses have mosquitoes growing in them, then you can see that there's an inverse relation. So if you get rid of where the mosquitoes breed, you tend not to have mosquitoes and vice versa. And now let's look at dengue. So you can see that pretty much, you know, whenever you get..., if you do not get rid of where the mosquitoes breed, there are mosquitoes and then there's dengue, okay, and then conversely the opposite. Now this actually shows you that you can be effective, right? If you get rid of mosquitoes, you don't have dengue. However, when you depend entirely on an external force, this is a problem. Because if you look here, what happened here? Well, there was a cholera epidemic. So all the public health force went, whoop, cholera. And then what'd they do? Well, they didn't check their houses and then da, da, da, da, and then they got a big dengue epidemic, okay. So you need to actually transfer the responsibility, you know, from this kind of top-down situation into the communities. But first what we need to understand is the mosquito lifecycle. And this is something, like I said, some of you all have...those of you who are here are going to have a really fun time playing around with this.

23. Video: The Mosquito Life Cycle (32:43)

But if we roll this video, what I'm going to show you is how the mosquito goes. So this is the mosquito at the end of the day. But actually it lays eggs and these eggs grow. And they can actually be desiccated and then they come back. And with the presence of water, they actually can then emerge and become the larvae that you saw. So this is called the larva. And this is a siphon here and it's breathing the air. And then what it will do is it will grow in clean water. Especially the *Aedes aegypti* grows in clean water. And you can see and actually it's cool because different species actually dance differently. But then they become pupae. And the pupae you can see will then split open in the back of the exoskeleton and they will actually emerge into mosquitoes. And the mosquitoes will then land and will probe. And what you can see is now what happens to the virus as it enters the mosquito. So it's going to enter and it's going to go into the midgut. And then it's going to start replicating. And it's actually going to go out through the midgut and then disseminate to the other body organs. But the most important is going to the salivary glands because then the salivary glands will then allow this mosquito to then deliver the virus back into the next person that it bites, okay. So that's how it works.

24. Community-based mosquito education strategies (33:54)

Now what we're going to go on is there are a lot of posters. This is a poster from Brazil and, you know, they're right on. You know, they say "stop the mosquito." And then you can stop dengue. However, when we actually went out into all of the barrios, what we call the neighborhoods in Nicaragua, and we started talking to people, we're saying what is it that you need to know? Like what do you need to know so that you can actually do some kind of intervention around dengue? They're like, it's not the mosquito, right? It's the lifecycle because what matters, you know, all of those interventions are about the water. Like no one is running around swatting mosquitoes. They're actually getting rid of the water where the mosquitoes breed. And yet no one had ever told people that the mosquitoes breed in water. And so like why would you spend all your time running around, you know, with the water and all this work when you know...and it was like completely illogical. But yet, it had never occurred to any public health campaign that that was illogical, right? And so finally, we're like, oh; now we get it. And, ding, that big click was...now, all the kids, this is Nicole explaining, the eggs to the pupae to the larvae. And it takes about a week, a little bit over a week to get the pupae. So you don't actually need to be scrubbing around and getting rid of the water every day. You just do it once a week. And as long as you can *cortar el ciclo*, as long as can cut the cycle every week, you'll never get pupae or mosquitoes, right? So that's totally logical. And once you deliver it in that way and it wasn't even our idea, right, it was the community saying, oh; now we understand. To get rid of the disease, we need to get rid of the mosquito. To get rid of the mosquito, we just cut the lifecycle. And that became the whole issue that they were working around.

25. Video: Mosquito Vector Education Program in Nicaragua (35:30)

And so what you see in this video is we worked with a number of different barrios and the different residents and they came up with just phenomenal ways of expressing this. This is the lifecycle. And this is they're telling you all. And this is how you, you know, scrub the barrels and this is what you do. So there's like all these cool different ways that they had to transmit the information about, you know, how this thing is spread, what are the interventions that you can do. They made these stencils and they put them up all over Managua to explain the lifecycle and how you can can *cortar el ciclo*. They made posters. And then they went, you know, resident to resident. This is what we call a *brigadista* and she is also a total *reggatonera*, but she's out there like explaining the lifecycle and getting down with the people and showing; if you just take your clean water in your very own household, you look and there are these frigging little things in it. It's so gross, you know. People get really upset. Like, you know, it's very motivating to see that their own water is full of this stuff, you know. And so then they get really motivated to actually do something about it. And this is Magdalena, who's been working with us for a while and she's, you know, explaining in every household how this thing works.

26. Top-down and bottom-up approaches to mosquito control (36:34)

And so what I want to contrast for you now is this kind of top-down approach, which we saw which was, you know, this kind of pan-American approach or the Brazilian Ministry of Health coming in. And, you know, what happens there is it's often effective, but it's large in verticals. It's expensive and it ignores the communities and the differences that we have. And it has often a short-term impact like we saw with both of those cases. So the alternative is to do what we call a bottom-up approach. And this is actually where you work with local leadership and local people. And this really involves an intensive community involvement with responsibility. It's adaptive and it can actually be sustainable. So if we just take that concept of the top down and the bottom up and then we translate that into mosquito control, let's look at what that means. So we have the traditional institutional mosquito control, which actually relies a lot on chemicals, which are actually not good for your health it turns out. The larvicides are put into drinking water and they're potentially harmful. It's resource intensive and it's actually unsustainable. And we contrasted with a community-based approach, which is actually very importantly evidence-based like I showed you. You show people their own evidence, their own larvae in their own water. And

they actually then get motivated to do something about it. It's peer-led and it's what we call green because it actually doesn't depend on insecticides or chemicals. And actually I'm going to show you some data that actually shows that it's equally effective if not more than the traditional approach. So this sounds fabulous. But what's the difference? I mean the problem is this motivational gap, right? I mean, man, you come back and you're tired. I mean then you get out and it's like 100 and some degrees every day. And you don't want to be like scrubbing the barrels and like emptying them. I mean why are you going to do this? I mean you might know it. But to get the energy to do it, you've got to be like motivated. And I mean it's the same thing, right, with STDs, HIV. You know, you know you're supposed to use a condom. But like does everybody always use a condom? I mean, you know, there's knowledge and then there's practice. And so the big question here is how do you actually bridge that bridge between the knowledge and the gut practice?

27. A community-based mosquito control program (38:34)

And so what we came up with, we meaning the communities that we work with and the nonprofit that we work with called CIET and ourselves, was this idea which is called SEPA, which is kind of a mouthful, Socializing Evidence for Participatory Action. But in Spanish, the word *saber, sepa* is actually "to know." So it's kind of a little trick on words there. And so the idea was to motivate the community participation in mosquito control to use as an evidence-based approach, which was actually going to use as evidence people's behavior and knowledge and their capacity to control dengue, the mosquito indices in their own homes. And another thing that we added, which was the level of antibodies to dengue virus in their children's saliva, which allowed us to show them whether their child had gotten infected in a certain point of time. And so that was like really motivating to know that your own kid had gotten infected with dengue or not. And so this kind of evidence was what really was the click for motivating. And then the idea was that they would then come up with the interventions and that it was actually...then they would do it year after year and they would refine it. And then as they refined it, they actually learned this whole methodology of translating evidence into action and into policy.

28. Measuring the effectiveness of community-based programs (39:42)

And so what I'm going to show you now is kind of how this worked. This is the greater Managua area. And we chose representative sites so we call them barrios. So they're really just neighborhoods. And we took 120 households in every one of these 30 neighborhoods. And we chose 10 of them that where we did what's called the intervention where we did the action. And then 20 of them where we did not do the action and then those serve as our controls or what we call our reference neighborhoods. And you can see that they have very different socioeconomic levels. Some have the dirt roads. Some are nicely paved. And this is actually based on a lot of data. These are 4,000 households that represent 23,000 people. And then every year, we do a cycle where we measure all of the larvae and the pupae in 26,000 water containers. So this is actually based on some heavy numbers. We take these saliva samples from over 3,000 children and then we combine all that data together. And the way it actually works is that this went on for four years. And now we just got funded to do what's called a random controlled trial, which can actually prove that this does or does not work. But this was our pilot study to see whether there was actually something in this approach. And so we do these measurement cycles every year. We do the household visits in October and February. What I'm going to show you now is the data from the water containers, what we call entomological surveys. Entomology is the study of insects. So these are looking for the insects in these people's homes. And then we also went twice to collect the saliva from the children. And remember how I showed you in our cohort study, we compared two points in time and we looked for an increase in the number of antibodies against dengue. So we do that here too. We look and see whether there was an increase in the number of antibodies against dengue. And then that shows us that that child was infected during that time period. Okay, so that's where the evidence comes from.

29. Reduction of mosquito vectors by community-based programs (41:31)

So what you can see is now we're going to look at this question of the children's infection rate with dengue. So this doesn't mean that they necessarily were sick. It means that they actually experienced an infection. And again remember, if they've had an infection, that puts them at risk for the more severe disease. So this is actually important. And so what you see is in the year 2004 when we started, we're looking at the relative percent infection in our reference in our intervention sites. And, of course, there's no difference, right, because we didn't do any interventions. So everything is the same. Now however in 2007, and we actually saw this change happen right away, we actually get a 60% reduction in the children that were infected with dengue in the barrios that did the activities versus the barrios or the neighborhoods that did not, okay. So that's, you know, a pretty profound effect. You can actually reduce the number of children infected with dengue if you do this kind of activity. So another thing that was important as evidence...so that was the infection rate in the kid's saliva. Now let's talk about those entomological data or essentially the data about how much larvae and pupae are growing in these homes, okay. So what's interesting is if you look at the intervention in this case, which is the SEPA movement, we can see now on our Y-axis is the percent of, in this case, barrels that have larvae or pupae in them, okay. So if you do this program, you actually end up dropping down by about a third the number of barrels that have larvae in them. And now if you look at the houses, right, so barrels are where they have the clean water, but the house is really what we care about because that's where people live. And you see exactly the same reduction. About a third of the houses essentially have less larvae growing in them. So now we compare that to the traditional approach, which is to go in and put larvicide inside the homes or in the drinking water, which again could be a health risk. And you see that if you put the larvicide in the barrel, you get a reduction in the number of larvae. That makes sense. But now look at this problem. If you actually look at the houses, there's no effect. So that's like - - wow. Then you think about what's the mosquito going to do? I don't want to go here. I want to go over here and lay eggs in this water, right. So, you know, your barrel doesn't have the larvae. But another receptacle in the house is producing all of the mosquitoes. So it's really important, right? What we care about is whether the house is positive for mosquitoes, not necessarily if just one container is positive. And so this shows you that this is actually a more effective approach because it's taking the whole house as the entity for activity, not just a particular receptacle. So this way shows that this actually works.

30. Building sustainable local capacity for vector control (44:10)

And now what we're going to show you is how this works. And we're going to go a little bit back because what we do is we then feedback this information from the serology, from the surveys, and entomology. Again, we analyze the data together with the communities. And then again, they come up with the interventions. And then they do this every year over and over again. And as they do this, you can see that we actually first work with...we first started this working with a nonprofit called CIET. And we sent a facilitator who got integrated into the community and identified who it was in the community who actually cared about health in their community. And these were called the *brigadistas*. But really soon, we actually withdrew the facilitator and it only became a delegate from the barrios that came to CIET. And so essentially after one year, we transferred the whole authority for making the decisions and how they're going to do the entomology searches and everything. And all the decisions were made by the people who lived in the barrio right away. And that's how this worked because you think nothing is going to work unless you have real ownership and buy-in from the people doing it. And to do that, they need to own it. They need to make their own decisions. And so that was the whole point here. And they did it together with, you know, whatever kind of organizations happened in that community. Sometimes it was churches. Sometimes it was schools. Sometimes it was dance organizations. Sometimes it was, you know, whatever was working. And every barrio is different. But it didn't matter, you know. Everyone came up with their own solution once they understood what the problem was. But the cool thing is that when you get the whole neighborhood involved, there are all kinds of good stuff that

happens because this is really about social mobilization. It's about getting people involved. And so what happened? Well, you know, a lot of these are really poor communities. They have a violence problem, and a lot of gangs. And so what ended up happening is that in those barrios that the gang members actually became *brigadistas*. And they actually started working going to people's houses. "This is the larvae, ma'am," you know. And so they actually became a real social, positive aspect. In Nicaragua there's a lot of very different religious and political views. And often people are like super polarized along those lines. But actually in this since they were all working together with this common goal, they forgot about those differences and they worked together. It was really nice. And over time actually the *brigadistas*, even though they were not necessarily literate, they became known to the whole communities including the Ministries of Health as the person to go to for a health problem. And so then they became invited to sit on the boards of the Ministry of Health and all this. And then really importantly since they were managing their own money, I mean it was like $50 a month, you know, to buy soda after they went and helped the residents figure out whether they had larvae in their homes. But they learned how to do financial management. And then these same groups then made cooperatives, sewing cooperatives, others, you know, so that they actually became their own economic systems as well.

31. Video: Health Brigades in Managua's Neighborhoods (46:44)

And so what I'm going to show you now is what these *brigadas* looked like, if you can roll this video. So you can see that they're going to be very different types. A lot of children got involved. It really depended on what the neighborhood was like. So we have the children involved. This is Juan Emilio Menocal which is one of the *barrios* with a very active group. This is Maria. She's one of the *brigadistas*. And her mom used to say that she could only do this with the sinks because if she was going to do this with barrels, she might fall in and drown. So, you know, she was only the one who could do the sinks. This is a woman. She's looking for the eggs because the eggs sit there. Now what do you do? Remember how the tires have water? What do you do? They fill them with earth, duh. I mean no one had thought of this. It's brilliant. You make stairways out of old tires. You fill them with dirt and then there's no water inside them. They made elasticized covers for the barrels. And then they were able to sell them. And then they formed a sewing cooperative. It was totally cool. And then we have these fabulous parties every year called *encuentros*. And you can see that they come up with all kinds of ways to actually get rid of the mosquito. Again, I have nothing to do with any of this. Okay.

32. Video: Mosquito Reggaeton, a Dengue Rap Song (47:42)

And so now in the next one, what I want to end this little series with or this lecture is, so this is Israel Rodriguez. And so I spend a lot of time in Nicaragua and these guys get to know me. And it turns out that they find out that I grew up in New York and like hip-hop era and I love dancing. And so every year, they would come up with a new *reggaeton*, which is like Latino hip-hop, on dengue for me, okay. So this is my favorite and I'm going to show you because it's really cool. We added subtitles. So how many of you speak Spanish or understand Spanish? *Que bien, bueno*. What you're going to see nonetheless is in English. We have subtitles that are going to tell you and it actually tells you what to do to get rid of the mosquito. It's really cool. So hit it, Israel.

[ISRAEL:] Nicaragua!
We have to cut the life cycle of the "little nails!"
Because in 8 days they convert to pupae, And in 11 they evolve into mosquitoes!
If you're not careful, they'll give you dengue!
The plague of mosquitoes must be eliminated.
And together we can do it!
Talking with people is how we can solve it.

And eliminating -- YO! -- those "little nails" and pupae.
Good morning ma'am! I've come to show you
How you can get rid of dengue.
Brushing the barrel down, down, down.
To one side, to one side, and to the other.
Looking for clean water to grow.
Because they want to convert into "little nails" and pupae.
And you have to check in 8 days.
If it turns positive you'll laugh.
I think this is something that we have to do.
We have to break the lifecycle!
And together we can do it!
Throwing away the junk!
This is a dream that is coming true.
Thanks to SEPA *brigadistas* that are making it happen!
Prix Boy!
Eva Productions.

[DR. HARRIS:] So I didn't ask for that, but...I just gave them a little money to make a CD and, you know, this is what happened. So anyway, you can see that it really is infectious, you know. People get the idea and then they have all different kinds of ways that like I certainly didn't dream of.

33. Q&A: Does dengue fever only spread through mosquito vectors? (49:46)

And so with that, I'm going to end this particular piece of the lecture and see if this has incited any questions in your minds. Yes.

[STUDENT:] I was wondering, can dengue fever only spread through mosquito vectors?

[DR. HARRIS:] Yes, actually. Right now, that's the only way. And you actually will get kind of what's called focal transmission in a household. But it's not because it's going from person to person, but rather because the mosquitoes that are infected will get infected in that house and will spread, you know, around those houses in that area, for instance. But for right now and as far as we know, it's really mosquito to human. Good question.

34. Q&A: What protects children who are infected but remain asymptomatic? (50:23)

Yes.

[STUDENT:] You mentioned that there were some cases where the children were asymptomatic to the cases.

[DR. HARRIS:] Mm-hmm.

[STUDENT:] So have you made any discoveries of what those children have in common that makes them asymptomatic to them?

[DR. HARRIS:] That's exactly where we're going. We don't have the answer yet, but we finally have set up a whole series of techniques. And, in fact, Joe and I are thinking of collaborating in this area that can look at what we call the repertoire of antibodies and of B-cells, for instance. And so what we're trying to understand is is there a certain type of antibody that is more effective or less effective? And

actually we have some inroads into that because it looks like there is a certain...as you'll see, there's a virus and an antibody will interact with a particular part of that virus. And there are certain what we call epitopes that can be more neutralizing or more enhancing. And so it's actually we're finally getting an array of technologies that can actually use these whole serum samples. A lot of times you look at a single antibody, like a monoclonal antibody and a single virus. But what we want to look at is the whole gamish, you know. And so that's what's been like challenging. But now we finally have a bunch of techniques that we can actually apply to the serum to ask exactly that question. Good.

35. Q&A: Would putting screens in windows solve the problem? (51:32)

Right behind you.

[STUDENT:] From an earlier question about global warming, if they did put screens on the windows and if the barrels were inside the houses, would there be more cases of dengue? Or would that kind of...

[DR. HARRIS:] Yeah. See, it's hard. I mean there are a lot of things you could think of if you had the resources to do them, you know. And so for instance, in industrialized countries that is an option, or just making sure that you have 24 hours a day of water. But in many countries, there's huge poverty and a lot of big issues. And so you push on that side as well to do poverty alleviation programs. And that will have an effect. But in the meantime, you know, no one is thinking of putting screens in every window in Managua. So the idea is, you know, to clean up in the meantime, you know, where they're breeding outside. Good question.

36. Q&A: Do dengue symptoms differ between infants and adults? (52:21)

Okay, ah, okay, yes.

[STUDENT:] Do the symptoms or the risk change as you get older?

[DR. HARRIS:] Very good, yes, actually. You tend to have...the children tend to shock more easily and it has to do with the fragility of their capillaries. So that you like -- what I didn't show is that you have a big peak in infants, for instance. It has to do also with maternal transfer of antibodies that can make enhanced disease. But in children, you also have more fragile capillaries and you can go into shock easier, which is why a lot of evidence has been on pediatric dengue, on children's dengue. When we did studies across all ages in Nicaragua and this has been shown in others, adults tend to bleed more. So you get more internal bleeding, especially in the GI tract and just bleeding out more in adults for instance. That's a very good question.

37. Q&A: How is Dengue coming to the US? We don’t use water barrels. (53:05)

Okay, yes.

[STUDENT:] Why is dengue spreading back in the United States if we have clean water systems and clean hospitals?

[DR. HARRIS:] Yeah, exactly. So that is one of the questions that a lot of people are starting to look at now. And I mean essentially there's just a lot more dengue circulating in the world, you know. So then you're going to have repeated introductions. So before you only had, you know, one person every 20 years who might have been in Asia and come home. But, you know, as you see that the number of cases is going up, so you have a lot of travelers that are coming back. And then more and more times that you

start reintroducing, one of that is going to escape, you know. There are some really interesting studies I can talk to you about later, okay? I've got to go to somewhere else in the room.

38. Q&A: Is dengue a rural problem? (53:43)

Yes, go for it, whoever.

[STUDENT:] So is dengue as big a problem in rural areas? And if so, how does the SEPA objective work with that?

[DR. HARRIS:] Yes, a very good point. It's been always thought of being a big urban problem. But a lot of it has to do with how the water is stored. For instance, in Cambodia, there's an interesting study that's just coming out how it's in rural Cambodia there are a lot of problems also. And they have these kinds of ceramic places where they store water, which are really great breeding sites for the mosquitoes. And so as long as you have a certain level of density of population, you can get the transmission. It just gets worse when you're in an urban situation because there's just that many more people and that much more transmission that can happen focally. Good point.

39. Q&A: How do you control larvae if you can't afford to dump water? (54:28)

Okay, yes.

[STUDENT:] You said that they dump out of the water. But what if they need that clean water? Like what if they don't have access to more clean water and they can't dump it out?

[DR. HARRIS:] Right, very good point. So a lot of times at least -- so many countries, no. Many cities will have intermittent water. And so like where I live in Managua, you have like 11 hours per day of water, you know. So we just time it so that you're scrubbing out and then you're refilling it during that time, you know. But that's a good point. And if you're kind of messed up, I won't use any other words. If you don't have any water at all and then what you do is, that you're very careful about how you seal the barrel. So what's called hermetically sealed means you put something where like nothing can get in. But it's really important because we actually did a study and we found that if you had a badly placed lid, you actually had more larvae than if you had, you know -- so that's why I really like that elasticized material because you can think of trying to like get some like metal thing that's like perfectly - - , you know. Whereas, if you just think, oh; that's cool. Elasticized material does the same thing. So those are the two kinds of options that you would have. One more question.

40. Q&A: Are elderly people at greater risk from dengue? (55:34)

Okay, yes.

[STUDENT:] As people get older, they get more fragile. So would they be more prone to dengue than little kids?

[DR. HARRIS:] Yeah. But there's actually an interesting thing about whom mosquitoes bite more in terms of there's an age relation there. But what happens is that as you get older, at least when you have a lot of dengue is that you're already immune so that you really see the worst dengue in children. Although in new places where dengue enters, then you start seeing adults. And, in fact, you do see it in elderly. And like in Puerto Rico when it first started coming in, you would see dengue and severe dengue in elderly people. But what generally happens is you get so much that, you know, by the time you're even...like in our situation, by the time kids are 10, 97% of them have been exposed to dengue. So you

can imagine that just by the time you're older, you're just like either dead or immune, you know. Okay, thank you.

41. Closing remarks by HHMI President Dr. Robert Tjian (56:36)

[DR. TJIAN:] Wow. I think that's the first speaker we've had who danced. Eva, that was a fantastic lecture to start off the series. I think it's not often that we get to see the convergence of science, which you'll see in a subsequent lecture by Eva, and social issues, which I think we can all relate to. So thank you. And really as always, I thank the audience. I mean there were so many more questions that you would like to have fielded. But please search Eva out and ask her these questions that you didn't have a chance to do so. And I have to tell you, you know, I'm kind of a mosquito magnet. So this is really freaking me out. So in the next lecture, Joe DeRisi will take us deeper into the realm of emerging viruses and show us how new technologies are revolutionizing our ability to hunt for new viruses and to quickly identify them.