Viral Outbreak: The Science of Emerging Disease
Discussion – Biology of the Mosquito Vector
Laura D. Kramer, Ph.D.; Robin M. Moudy, Ph.D.; and Eva Harris, Ph.D.

1. Life cycle and ecology of different mosquitoes (00:05)

[DR. KRAMER:]We're going to start with the mosquito life cycle. You saw this and I just quickly want to go through that mosquitoes go through metamorphosis. They are holometabolous which means that they have a complete metamorphosis, eggs, larva, pupa, adult, and then among the larvae you have four different instars and the larvae molt. They have an exoskeleton because they are arthropods and they molt and they get bigger until their final molt they become a pupa and then they become an adult and then the adult, whoever did the exercise, what sex did you have emerge first? Male. So, the males emerge first because they have to allow their terminalia to rotate so they can mate with the females and then they will mate with the females right away, the female goes host seeking looking for blood and then she lays her eggs. So, we have to think about this as a cycle that we want to interrupt if we want to control mosquito-borne diseases without vaccines. We can interrupt it at all different stages and Robin will talk about that a little bit in her presentation. The other thing we have to think about with mosquitoes -- my other take home message beside cycles; we think about disease transmission cycles and mosquito cycles -- is that there are lots of different species of mosquitoes. Does anybody have any idea how many different species there are? Give me a number.

[STUDENT:] More than 1000.

[DR. KRAMER:]More than 1000. There are about 3000 species of mosquitoes and they are all different. They have different microhabitats, different macrohabitats, some live in cities, as we talked about yesterday dengue is an urban disease so the vector lives in the city, breeds in the city, and then we have more rural residential diseases and then we have forest diseases like yellow fever, for example, can be forest or urban.

2. Mosquitoes that transmit dengue virus (01:56)

But, one of the mosquitoes we will be talking about today is *Aedes albopictus*. So, we saw with *Aedes aegypti* the dengue vector that they like these containers right outside people's houses. *Aedes albopictus* is also a dengue vector but it likes tree holes as you see in the upper right. That's where it breeds in Asia but when *Aedes albopictus* came to the U.S., it's an invasive species, it adapted to tires. Tires are sort of like tree holes. They are dark. They are rough. You know, rain water falls in them and they lay their eggs. So, they are more of a generalist than *Aedes aegypti*. *Aedes aegypti* only likes clean rain water in barrels, and there's lots of studies going on. Are they small containers that they prefer or large containers, are they plastic, are they metal, and there is actually people studying that. *Aedes albopictus* is much more of a generalist that will breed wherever it finds that's sort of like a tree hole. But then we see on the bottom right that ugly green stagnant water, and that's where *Culex* like to breed, certain species of *Culex* like *Culex pipiens* which is the vector of West Nile and we'll talk more about that. Mosquitoes don't often breed in this pristine beautiful standing water but some *Anopheles* species do and *Anopheles* is the vector of? Malaria, right. Okay. So, here we have our dengue transmission cycle. It's another cycle that we want to break just like we want to break the mosquito developmental cycle, and there are two important vectors -- as I said *Aedes aegypti* and *Aedes albopictus*, and we distinguish them by their markings, and this virus, dengue, is transmitted directly between mosquitoes and humans. It doesn't need any other host and because of that in cities, particularly in poor areas where people are living very densely, the virus gets transmitted very efficiently. The mosquitoes are there in the houses, they're breeding in the containers outside the houses, so you get very rapid transmission and it actually should

not be hard to break this cycle because it's just two hosts, the mosquito and the human. So, *Aedes aegypti* -- there are two vectors, but *Aedes aegypti* is by far the most efficient vector and the reason why is that they feed frequently on blood. So, if you remember the mosquito life cycle, generally a mosquito will take a blood meal, it will digest that blood over several days, lay eggs, and then go and feed again, but *aegypti* take blood meals every day. So whereas other mosquitoes may feed on sugar in between their blood meals, *aegypti* feed on blood. So each time they are feeding what are they doing? Their saliva which is filled with virus is going into the human that they bit so they are transmitting virus, so they are very efficient. Most of their blood meals are humans but 95% of *Aedes aegypti* blood meals are humans whereas *Aedes albopictus* which is this more generalist ecology has more general ecology, more of a generalist mosquito, it will take human blood meals, it will take cow blood meals, dog blood meals, whatever is there, whatever is convenient; it doesn't care. They are large populations, so each female mosquito over its lifetime will lay over 300 eggs. That's about three oviposition cycles. So you can think of each of those 300 eggs becomes a mosquito and so half of them are female, so you are getting this exponential growth of the population and *Aedes aegypti* are very hardy mosquitoes, so they lay lots of eggs, they survive well, and the virus grows very effectively in the mosquito so there's lots of virus replicating in those salivary glands being ejected with the saliva.

3. Dengue vector *Aedes aegypti* in the U.S. (05:35)

In the United States, we don't have a lot of *Aedes aegypti*. Here you can see in red where we have *aegypti* in Florida and Texas and in Arizona and this is where we find dengue. Wherever we have *aegypti*, we'll find dengue. Although it's not often transmitted within the U.S., until recently in 2009 and 2010 we had an outbreak in Key West, Florida so there is about approximately 60 cases in a very small region of Key West called Old Town. There's about 6000 residents, we've had 60 cases. We haven't been able to control it yet, so think about that. Here's a, you know, a contained area. Key West is not mainland Florida and we have not been able to control the virus. So, how are you going to control it in Nicaragua, a big country with lots of people? We're talking about 6000 people, but there have been outbreaks in Texas on and off throughout time and why do you think-what do you notice about those states where there's *aegypti*?

[STUDENT:] - - .

[DR. KRAMER:]Pardon me?

[STUDENT:] - - .

[DR. KRAMER:]They are all in the south and they're bordering on Mexico, so when there is a lot of activity in Central America and Mexico, it moves up into the U.S., which is why in 2009 we probably had dengue become established in Florida because there's been so much activity in Puerto Rico and in other places outside of the U.S.

4. Dengue vector *Aedes albopictus* in the U.S. (07:01)

So *Aedes albopictus* is the other vector and what I didn't mention on that map is there was another outbreak which surprised us besides the one in Florida, and that was in 2001 in Hawaii. So, people went to Tahiti to go skin diving, they came back to Hawaii and they were infected and then there started to be an outbreak which was not *aegypti*-transmitted but *albopictus*-transmitted so *albopictus* is another important vector although people question whether it's really involved outside of Asia in dengue transmission but we do worry about *albopictus* because look at this map. I mean look at the difference between this and what we saw with *aegypti*, much more dense population, much more expansive population. *Aedes albopictus* was brought to the United States in the late-'70s when we started importing

used tires from Asia, particularly Taiwan and Japan. Those tires I showed you are perfect breeding grounds for *albopictus*. Then in 1985, there were large breeding populations in Texas and then throughout after 1985 to about 1988, we found *albopictus* wherever there were tires east of the Mississippi River, so they've really spread but you'll notice they didn't go very far north and that's because they are temperature sensitive. They don't do well in cold temperatures and that's why some of you had trouble with your projects because the temperatures were a little cold for the mosquitoes to develop. So you can see, we have a problem with *albopictus* in this country so we worry about it for dengue and other viruses. There is another encephalitis virus, La Crosse encephalitis, which grows very well and *Aedes albopictus* transmits well and people are concerned about that. And then outside of the U.S. there is a virus called Chikungunya, which is one of my favorite virus names, that the virus has mutated so that it's very rapidly spread by *Aedes albopictus* and that's causing havoc in some parts of the world.

5. Result of student mosquito-rearing activity (09:01)

So this was your experiment. These are the results – using *Aedes albopictus* or the Asian tiger mosquito. So, in blue you see when you had your first larvae appearing, so they can appear as early as one day but because the temperatures were cool generally we'll see the eggs hatch within 48 hours but because the temperatures were cool some of you had larvae kind of there was a protracted hatching period, so some people didn't see any larvae until day eight. And then the first pupae appeared about day six, which is about right. I mean, that's what we would see at a normal tropical temperature, so that worked very well and the adults started appearing day nine. So, it's a little bit slower than you might find in a tropical area, your adults might come up about day seven, but actually the experiment worked quite well, better than I expected I have to say at the cool temperatures that we had. So, here's the summary of the results. First larvae, three days, first pupae, about nine days, and first adults twelve days. But what we saw also was that we didn't get 100% hatch. We only got 61% of the eggs hatching. That's partly because the eggs may have been too dry, *Aedes albopictus* does not survive well during desiccation whereas *Aedes aegypti* can be desiccated and they will survive for a very long time. The other thing that happens is sometimes the eggs have to be multiply immersed in water so it rains because their eggs are laid above the water and it rains, they get wet, and some hatch and some don't. That's a survival mechanism of the species. Then we got about 42% becoming pupae and about 33% becoming adults. So that doesn't seem like it was really successful, but when you consider the populations of mosquitoes out there, 33% is pretty good.

6. West Nile virus and *Culex* mosquitoes (10:48)

So now we're going to switch to a real temperate area virus. So we've been talking about dengue mostly and that's tropical but West Nile is a temperate virus. It came to the U.S., we're a temperate area. And as also the other take home message is, besides there are all these different mosquitoes that require different habitats to lay their eggs, habitats in where they feed, and what they feed on, we have different kinds of cycles, and this is an enzootic cycle. It's a zoonotic virus with an enzootic cycle. So the virus is amplifying as it is transmitted between *Culex* mosquitoes and birds. Birds are the amplifying host for this virus and you can also see in this photograph *Culex* lay egg rafts on the water so they can't desiccate. They need water there for them to develop, so if there is no water the eggs can't dry. If they dry they die. But what happens is that the virus builds in this cycle. More and more mosquitoes become infected and more and more birds become infected and it spills over to horses and humans which get very low titers of virus, low concentrations of virus in their blood but they get very sick. They get encephalitis and they die or they recover or they are asymptomatic just as we saw with dengue but the virus does not go back when mosquitoes feed on humans. It doesn't matter. They are not going to get infected. And then we have the other phenomenon like with American crows which were sort of the sentinel for West Nile where people saw dead crows, there was West Nile being transmitted, and crows

are not fed upon that much by mosquitoes. They are fed upon by mosquitoes but they can also get infected because they eat carrion. So, if a bird dies that's infected or an animal dies that's infected and the crow eats it, the crow will become infected but the crow gets very high concentrations of viruses. It's very, very susceptible to the disease, and it dies and so we see dead crows.

7. Monitoring infection among mosquitoes and birds (12:48)

We can monitor infection in mosquitoes and we do this because we want to know how much virus is out there. We can trap host seeking mosquitoes that are attracted to CO2. We have after they get their host blood meal they are engorged, that means they have blood in them and we collect them with these backpack aspirators and that's very labor intensive. You can spend all day and maybe get three mosquitoes and you feel good like I got three mosquitoes, or you can find a location where they are all sitting and get a lot of mosquitoes. There are certain culverts where they may go sit. And then we can also after they take their blood meal and rest, so they're different from *aegypti* which take blood meals every day, they rest afterwards, and then they go and lay their eggs and we then can collect the egg-laying mosquitoes or the gravid mosquitoes, the mosquitoes that digested their blood and have eggs. We can also look at the birds, so we can monitor humans as you do with dengue or we can monitor-we're hoping to interrupt the cycle before it gets to humans-monitor birds, and then take control efforts. So, we mist net the birds and there is a bird in the net and then we're taking a blood sample from that bird to see whether the bird has antibodies or has virus. So, we'll know what's the level of activity in the area...

8. Video: West Nile virus field research (14:03)

...and if you could roll the video. This is a project that we're doing in collaboration with Marm Kilpatrick.

[DR. KILPATRICK:] So the overall thrust of our research is really just to try to understand what are the factors that drive West Nile virus transmission. So we basically want to know both in space, so at different sites why certain sits have more disease than others, and then why do certain sites have more disease in some years than others. We examined West Nile virus transmission in both mosquitoes and birds at a set of sites that spans a gradient from intact forests to downtown urban areas, so basically we have some sites in pretty nice intact forested areas, some sites in kind of parks surrounded by residential areas, and then some sites in kind of downtown city areas, so both in Washington, D.C. and in Baltimore, and we're able to actually look at and measure West Nile virus transmission in both mosquitoes and birds across that gradient in those sites over a number of different years.

[DR. KRAMER:]So what he is showing here is when we put up the mist nets to catch the birds to net the birds, we are putting them up in known flight paths of the birds. So you observe the area, you decide where to put your net, you have to learn how to take birds out of nets because they get all entangled but it doesn't hurt them. Then, we go and we band them, measure them, sex them, age them because it's different if you detect the antibody in a newly-hatched bird, it means that bird got infected this year whereas if you detect antibody in an adult it could have gotten infected any time and then we release the bird. This is dry ice that gives you CO2 to attract mosquitoes. Those are the host seeking mosquitoes. It has a light also which attracts mosquitoes and it's being raised up into the canopy, so it's going all the way up there which is kind of a trick to get it up there but we can talk about how we did that later if you're interested in that. So that will look for mosquitoes that have emerged and are looking for a blood meal. Here he is looking for resting mosquitoes with a backpack aspirator and then those we're going to test not only we can test them for infection, but we can test the blood that's in them so we know what they fed on, so we know who the important bird is or mammal or who the important host is and he is looking near water because mosquitoes like to be near water. This person is talking about a gravid trap so there the mosquito is going to come to the water to lay eggs and it's going to get drawn up into the

trap. And we also sometimes, when the mosquito comes sometimes it will lay egg rafts before it gets caught up in the trap and we can use those egg rafts for experiments in the lab to look at the competence of the mosquito, how good a vector are they.

9. American robin is an important West Nile carrier (16:32)

So when we did that study, there are lots of different birds in urban areas so this is Washington. It's Baltimore to Rock Creek Park looking at the urbanization gradient. You get lots of different species of birds -- robins, starlings, doves, and what we found and just looking at this very simply if you look at the colors, on the left is your percent of birds in that environment. The percent of the total population and what you see mostly here this is National Mall so we consider this an urban area that mostly there are house sparrows. But then, what are the mosquitoes feeding on? Well, the null hypothesis would be that the mosquitoes feeding on the most prevalent bird but we don't see that. We see that even though American robins make up about 2% of the population at the National Mall, you can see that about 30% of the blood meals are coming from robins. Similarly, in Bethesda, which we consider a residential area, again, house sparrows, largely the dominant bird, American robins -- they make up a little bit more or maybe 5% of the population, but about 45% of the blood meals are coming from robins. So, other birds are also being fed on. Mammals are also being fed on but we feel that the American robin then is the driver of transmission in this area in the enzootic cycle. So, it's the most important West Nile host in terms of amplifying the virus in the northeast and Atlantic region.

10. Why do mosquitoes not feed on rock doves? (18:03)

So I'm stopping there and you can ask questions and then Robin Moudy will speak. Yes?

[STUDENT:] I noticed that your data, the rock dove, was not fed on by the *Culex*. Do we know why that happens?

[DR. KRAMER:]Well, so, the question, you know -- I'm talking about the mosquito's preference for a certain bird species, and "preference", I should put in quotation marks. It may either be that the bird does not tolerate the mosquito feeding so it moves around all the time, you know, and so the mosquito can't get a good blood meal so when we're testing blood meals we're not finding rock dove. The rock dove is an interesting bird because it gets infected, it becomes immune, it can get encephalitis but it doesn't die. It doesn't even get sick even though it has an encephalitis in the brain. So we think of it sort of if they were to feed on rock doves it would sort of dilute the virus because it would be a wasted blood meal for the virus since the bird is immune. But they do feed. I mean these are two locations. There are other locations where they will feed but it may not be that the mosquito doesn't like rock dove blood; it's just the rock dove doesn't allow it.

11. Do all mosquito males not take blood meals? (19:21)

Yes?

[STUDENT:] My question is you were saying how males don't eat and that females feed on blood, and I was wondering is it just males in general, of all the mosquito species that don't eat or just specifically to the *aegypti* and the *albopictus*?

[DR. KRAMER:]Yeah. Male mosquitoes of all the species do not take blood meals and the blood is needed by the female for the protein to allow the eggs to develop so they've evolved to take blood. There is one species of mosquito, the *Toxorhynchites*, where the females do not take blood.

12. Why are the dengue mosquitoes in Texas and Florida? (19:54)

Any other questions? Yes?

[STUDENT:] It said on the graph, picture you showed that Texas and Florida have the most. Is there an ecological reason as to why that would happen or is it because since climate's changing so much that because of it they are becoming more successful to living in that area.

[DR. KRAMER:]So the reason Texas has a lot is because you're right next to Mexico and just across the border there is a lot of dengue and there have been studies looking at Brownsville, Texas, and right across the border is Matamoros. There is a lot of dengue in Matamoros. There is some in Brownsville, but when you look at the mosquito populations in the two locations, there is much more dengue in Matamoros because there is more *Aedes aegypti*. There is more containers holding water and there's no screens that we talked about and there is a lifestyle difference. There is also a population density difference, people living in much closer contact although you do have some in Brownsville. So, I mean, that's telling you why there is less in Texas than Mexico, but there is more in Texas than in other states because of that proximity to the border. But Florida is interesting. I mean, Florida you would expect more dengue because of people going back and forth and there really hasn't been or hasn't been reported. The dengue outbreak in 2009 is after about 70 years of no dengue being reported, no transmission in the state being reported in Florida, and it's kind of something everybody's been waiting for and it hasn't happened. I think what's interesting is with all the *albopictus* that are in the U.S. that we don't see more dengue transmission and there are people who don't think *albopictus* is really an important vector for dengue. I don't know. I mean there have been *albopictus* outbreaks in southeast Asia.

13. How would knowing the bird hosts help us control the disease? (21:42)

Yes?

[STUDENT:] Since we know the birds that mosquitoes prefer to feed on, how would that help us to control the spread of viruses?

[DR. KRAMER:]Well, it helps us determine risk. So, we did a study that, again, Marm Kilpatrick kind of headed. First we did it in the lab. There is a vaccine for dengue that's a DNA vaccine that requires a single dose, and so we in the lab inoculated robins that we brought in to the lab and saw that they were protected and then challenged them, and saw they were protected against dengue, so then he went out in the field -- sorry, West Nile. No dengue. Birds do not become infected with dengue. I guess it's just dengue on the brain. So then he went out into the field and they took nestlings. They had four different areas with a control plot and a study test site and took all the robins and vaccinated them in the test site with dengue -- West Nile -- and god. And in the areas where they were vaccinated, transmission dropped tremendously, but so is a veterinary vaccine a useful tool? It's been used for rabies and it's been used for other things but, you know, the trick would be how to get that vaccine into the robins naturally. And robins are not the driver everywhere. It's regional but it's actually looking like it goes into Colorado and the Midwest and Connecticut as well that robins are drivers of West Nile, not of dengue. But, your point is really good. I mean, so why do these basic research studies? If we can find a way to determine risk for humans, then the control efforts are very expensive. It takes a lot of people to go out testing mosquitoes, you know, doing all of this and then putting in BTI or spraying; whatever they are using for control, so if they can become targeted, so if we know that where there's robins and we get a certain infection rate in the mosquitoes, people are at risk. Then we can target control to that area and not just be wasteful and in these times particularly we need to figure out a good way. Zoonotic diseases are very difficult. We're never going to get rid of them because of this amplification in the wild cycle. We're not going to get rid

of all the robins and it wouldn't make a difference anyway. They would just go to mosquitoes would feed on another bird. But it's really, we're really trying to come up with a good formula for risk.

14. Methods of controlling mosquito-borne diseases (24:37)

[DR. MOUDY:] I know we've talked a little bit about different ways that you can control mosquitoes, so I mean there is the traditional mosquito control things where you remove standing water like when Eva was talking about with her cool little dance which I'm not going to repeat, or you can use larvacides which she said is very problematic because you're putting chemicals in your drinking water. There's adulticides which is like giant spraying. I don't know if you guys have the big spray trucks that come down your streets like we have? And it's, yeah. So, you know. But then you can also reduce mosquito-human contact. Again, like Eva was saying, window screens. It's huge. It's really helped, you know, get rid of dengue and yellow fever to an extent in the U.S. but there is also insect repellants when you put DEET on if you're going hiking. That helps get rid of them or at least keep them away from you. And, you know, you may have heard about insecticide-treated bed nets for malaria and those can be very important to help reduce human contact with mosquitoes. But then there are also ways that people are looking at in the lab to help reduce the mosquito vector competence which is just how good a vector for this specific thing, that specific mosquito is. That's all vector competence is. So, one way people are trying to do that is by genetically engineering mosquitoes so that they are -- they call them transgenic mosquitoes. There is lots of different ways they can do it and there is all different kinds of things that you can put genes into a mosquito to make them so that they are not as susceptible to infection if they bite somebody, or if they get infected they can't actually transmit the infection, or you can make it so that if they get infected they'll die. Ant then, another way is to make them so that the mosquito, if it lays its eggs, the eggs either don't hatch because the embryos die or they'll hatch but they never reach adult stage, so they die during larval stage generally. The World Health Organization has made several recommendations for people that are thinking about doing open field trials and most of the recommendations really have to do with getting public comment and public consent. It's like Eva was talking about, the top down versus bottom up models of public health programs. They really want to help get people involved at the community level and get people kind of buying into whatever intervention they are proposing. So that's really what most of these are. With genetically modified organisms that can be a problem because a lot of people are kind of scared of genetically modified organisms possibly for good reason.

15. Transgenic mosquito trial on Grand Cayman island (26:52)

The reason we're talking about this today is I don't know if you guys have seen the article that I think was in your goody bags. This article just came out, gosh, two to three weeks ago, and what's since come to light is that a company from England has basically, they make transgenic mosquitoes. That's what they do and they are making them to help disease control. So what they decided to do was to do an open field trial in Grand Cayman. It's been said, it seems to be said that it was a secret trial. Supposedly it wasn't secret. They were working with the government of Grand Cayman so the government knew whether or not the people that they were actually really kind of testing on knew is up for debate and nobody really knows. So what they really did is they released three million male mosquitoes in an area that is less than a tenth of a square mile, so it's a pretty small area, a heck of a lot of mosquitoes. It basically makes it out to ten modified male mosquitoes for every one wild male mosquito that was in this little area, and what happens with these specific mosquitoes is that when they mate with a female they pass on the genes to the offspring. It's a dominant gene and it makes it so the larvae have this enzyme that's building up in them and it kills them before they emerge to adults. The only way they can raise these mosquitoes in the lab is to raise them on tetracycline, so if they have tetracycline they can live, they can grow to adults, and they can grow them up, and they can make big colonies of them and it's great. If they don't have tetracycline the larvae are going to die, so basically every time a male

modified mosquito mates with a female in the wild, their offspring won't live. So, what ended up happening is that immediately after the study they did a test to see how many mosquitoes were in the test area versus a control area and they found that basically they had an 80% reduction in the number of *Aedes aegypti* in the test area as compared to those in the control area. But, because this was a potentially sort of secret study, nobody in the international community including other collaborating researchers that are working with the same company knew about this, there has been a lot of outcry against it. One of the reasons is because, you know, the main recommendation is that you need to inform the public to get public comment on things and the way they informed the public is they relied on the Grand Cayman mosquito control unit to do it, and the way they decided to do it was they sent a press release like basically information out to all the papers in the local area and they put a video about it on YouTube. Whether or not most people got that information is up for debate. You know, we don't really know. They didn't seem to hold any town hall meetings. They didn't hold a big public comment session, so as you can imagine, there are different ways to get information out to the public. If you're doing a public health intervention and you really have to think about where your population is, how technologically savvy they are -- you know, do the population of Grand Cayman get on YouTube all that often, would they even think to go on YouTube and look up mosquito control projects? I mean, I don't know that I would think to get on YouTube and look up mosquito control projects. Would you guys? But, you know, if you go to YouTube, you can actually see this video that they've put out. I've seen it. It's interesting [laughter].

16. Carefully-regulated transgenic mosquito trial in Malaysia (29:57)

So, with another interesting thing about this is that they are doing another test site in Malaysia, and this was the original study that they wanted to do next because they've done kind of caged field trials in Malaysia where basically they build this big mosquito cage and they put the mosquitoes in it, and so it's out in the field. It's not in the lab but it's still in a cage so the mosquitoes aren't going to be flying out and just wreaking havoc on the population. So, in this one they are looking at a completely different system so they are trying to see how well these mosquitoes survive in the wild because if the mosquito adults survive as well as the nontransgenic mosquitoes, that would be bad because they are never going to be able to mate with the females because all the wild ones are going to do it instead. So, they are going to release a lot fewer mosquitoes -- as you can see, four to six thousand as opposed to three million. They're going to recapture them and then at the end of the study, which is about a month, they are going to completely spray insecticide over the entire area so that they'll kill anything, any other mosquitoes that happen to have lived past that month. So, I mean, as you can see there are a lot of things that were done differently about this particular trial. They had to get a lot more approval because Malaysia has a National Biosafety Board, Grand Cayman does not. They've got a draft biosafety bill that's in their parliament or legislature. I don't know exactly what their governmental system is, but it's in a draft form and it hasn't been finally approved so they don't have a national governing body that takes care of genetically modified organisms. Whereas in Malaysia, they have this type of thing so they had to get a lot more approval and that's part of the reason that this trial took so long to implement and the company ended up deciding to try to partner with another country to see if they could get it done faster. So that's part of the reason they did the trial in Grand Cayman first, because they didn't have to go through all of this type of thing. And as you can see, they also did a lot of public comment on this one, so this trial seems to have been designed a little better. At least this is my opinion and I'll say that right now but, but you know, I'm interested to hear what you guys think because as you can imagine there are a lot of ethical issues when you're doing research in a developing country, especially if you're coming from the developed world and bringing your technology to the developing country. You kind of have to think about a lot more things than you might think you would have to consider.

17. Can you target and kill only infected mosquitoes? (32:18)

[STUDENT:] My question -- the first thing I thought-I saw the article, it was in Science Magazine for this month and the first thing I thought of was if you could design the genetically modified mosquitoes to target only mosquitoes that are carrying the dengue or the specific whatever the virus is. Have they looked into that at all or anything?

[DR. MOUDY:] So do you mean targeting like the males so that they would only mate with the infected females or something like that or --

[STUDENT:] [interposing] Yeah. They only kill mosquitoes that have the dengue.

[DR. MOUDY:] I mean they've looked at mosquitoes or making transgenic mosquitoes that if they become infected they will die but in that case they'd almost have to make something with a gene where it would be driven into the population that's in the wild, so they would have to keep those just basically make it so that all the mosquitoes out there in the wild are now transgenic so that if they got infected they would die. With this type of technology, they have to continuously release more males because the offspring die so it's not like you're getting this gene in the population. So, somebody said yesterday one of your fellow students out there said or asked if it was essentially like a sprayless spray and that's kind of like what it is. I mean because you have to continuously do it, so in Grand Cayman, I mean they've eradicated *Aedes aegypti* numerous times and it always gets reintroduced so in a case like this even if they eradicate it completely on the island using this technology somebody brings a tire in and, you know, you get more *Aedes aegypti* or something so you're going to have a continuous reintroduction.

[DR. KRAMER:][interposing] And nobody is happy.

[DR. MOUDY:] Yeah.

[DR. KRAMER:]That's been the big issue with transgenic mosquitoes is what's going to drive that gene in the population and looking at transposons and *Wolbachia* and things like that, but it's complex to make it work.

[DR. HARRIS:] Because they're often less fit.

[DR. KRAMER:][interposing] Yeah.

[DR. MOUDY:] Yeah.

[DR. HARRIS:] There's often a price for introducing an additional gene and so even if it's not dramatic there's often they won't compete as well with the wild population.

18. Can you get dengue from a blood transfusion? (34:16)

Yes?

[STUDENT:] About dengue, could you say you had a blood transfusion or something from someone who has dengue but is not showing any symptoms. Would you then get dengue or does it only have to be mosquito-borne?

[DR. HARRIS:] Right. Yes. There is a concern and there has been a number of tests now for dengue in blood banks but dengue we only have this five day window -- you know, maybe a week or so where it's in the blood but there's enough that actually that they have been developing tests to check for blood and this is an issue with West Nile.

[DR. KRAMER:]With West Nile it's a major issue so as you looked at West Nile moving across the country after it was introduced in 1999 and about 2001 they saw people getting transfusion transmission West Nile and getting very sick and so they rapidly worked together just like countries worked together with SARS -- the companies and the FDA worked together to develop very, very sensitive nucleic acid tests for West Nile and that was good because in 2002 there was a huge increase in natural cases in the Rocky Mountains, in 2003 and the cases doubled and had they not stopped that transfusion transmission we would have seen a lot of it, so that's been instituted by the FDA. The Red Cross uses it regularly. There is a debate going on now that actually I'm partaking in next week with the FDA about implementation, how to best implement dengue tests -- you know, where do we want it, the dengue blood tests. So that's a good question.

[DR. MOUDY:] Uh huh.

19. How do you mark mosquitoes for recapture in the field? (35:59)

[DR. HARRIS:] Yes?

[STUDENT:] So you said after you release the mosquitoes into the wild you put them back into the cages. How does that work?

[DR. MOUDY:] Oh with the cage trials or --

[DR. HARRIS:] [interposing] The recapturing one you mean?

[DR. MOUDY:] The recapture one or the cage trial?

[STUDENT:] Yeah. Capture trial.

[DR. MOUDY:] Okay so with the capture one it seems like they are going to mark the mosquitoes somehow. We've done it in the lab before with fluorescent dye. You can actually kind of shake it and it gets in their scales and then when you capture them you can look at them under a fluorescent microscope or just a fluorescent light to see if the mosquito you captured is one of yours or if it's just a random free range mosquito I suppose so --

[DR. KRAMER:][interposing] Well, but with the *Aedes aegypti* they have genetic lines that have white eyes. There's the white eye line so they could use one of the genetic lines.

[DR. MOUDY:] Yeah, and I don't know if that -- I can't remember if that's what they used for this specific thing I think it might be. It might be the Higgs white eye strain --

[DR. KRAMER:][interposing] Yeah.

[DR. MOUDY:] -- so they'll be able to see whether or not it's one of theirs or the free range mosquitoes, and then for this particular study in Malaysia, the recapture study, they're going to have to do genetic testing on them because they are not just releasing transgenic mosquitoes; they are also releasing nontransgenic mosquitoes so normal mosquitoes but they are lab mosquitoes just to see how far they fly to compare the two. I mean, they can put out, you know, traps basically just to see how far they fly and we've done that just with trapping them although it might be a little more difficult because these males aren't going to be seeking hosts. So, the traps may have to be different.

20. Can you prevent mosquitoes from getting infected? (37:25)

[DR. HARRIS:] Uh huh?

[STUDENT:] I know you've talked about making so that mosquitoes don't transmit to the humans and like stuff all in like that part of like the whole cycle. Has there been any work in making it so like maybe like a drug or messing with the disease itself so that it can't get transmitted back in the mosquito in the human like let's say you get it and then you take a drug so even if the mosquito stings you and the disease either doesn't really get into the saliva glands or the disease just doesn't work or something like that?

[DR. KRAMER:]Well, that's a good question. With tick-borne diseases, so some cattle get so infested with ticks and it's not even really a disease transmission. They are using salivary compounds to make it so that when another tick tries to come and bite, they can't. They die. So they are manipulating the use of the tick's saliva so you could work on that angle to immunize people against the salivary secretions so that when the mosquito comes it won't bite; it won't take blood. I mean, that is an angle that can be explored and I think it is being explored using other types of vaccines; not against the virus itself but against the vector.

[DR. HARRIS:] Another, for instance, with dengue and this is just backing up slightly, but there's one approach which is to have mosquitoes that will destroy dengue virus once it is ingested so that it has essentially RNAi that will knock down the ingested dengue virus RNA so that even if the mosquito drinks up blood with virus in it it actually destroys the virus in the mosquitoes so that it wouldn't be there to transmit to the next and in Malaria there's some really nice transgenic mosquito ideas. Actually they have them with that SMA peptide that essentially won't allow the parasite out of the midgut and so there's like all kinds of kind of clever things. Again, the big question is is this really economically feasible; is this okay in the environment, is this okay with, you know, public perception, etc.

[DR. MOUDY:] And, the other issue is that they've done some mathematical modeling with dengue and the different types of transgenics so looking at basically like the sterile males like they released in the Caymans versus some of the ones that make it so the virus can't actually get into the midgut or can't escape the midgut or something like that and it seems like at least on the computer modeling which, of course, is not exactly how it would be in the real world some of those types of technologies so the reducing infection or reducing transmission might end up having an unintended cause where they could select for a more virulent virus which we also don't want --

[DR. HARRIS:] [interposing] Uh huh.

[DR. MOUDY:] -- so that's the other issue.

[DR. KRAMER:]The big difficulty with dengue, too, is that you know we were talking about *aegypti* feeding every day --

[DR. MOUDY:] [interposing] Uh huh.

[DR. KRAMER:]-- you don't need a lot of mosquitoes to have transmission occurring so it's not like with West Nile where the mosquito will feed and then it will rest. You could have one infected mosquito that's going to infect a household, you know a family.

[DR. MOUDY:] Yeah.

21. How can you make mosquitoes that die if they get infected? (40:42)

[DR. HARRIS:] There were a couple of hands over here. Yeah?

[STUDENT:] Since you said that transgenic mosquitoes will die if they have the dengue virus inside of them, how does that work and how many will actually become infected?

[DR. MOUDY:] I don't know exactly how they've done it. I think there are a couple of different groups working on it. Sometimes they are working on it where it's not even necessarily a transgenic mosquito but the mosquitoes are infected with a specific type of bacteria, it's called *Wolbachia* and there are different strains of *Wolbachia*. And so, some mosquitoes if they get infected with *Wolbachia*, then when they get infected with a virus it kills them so that's more what they've been working with but they could try to get *Wolbachia* basically driven into the entire wild population. It's similar to transgenics but not exactly the same thing.

[DR. HARRIS:] Uh huh.

22. Do *Aedes* mosquitoes survive in cold areas? (41:28)

Wait, I'm gonna do people who haven't spoken before. Yeah?

[STUDENT:] I was like wondering for the home activity you know how we were supposed to have like maybe a warm or hot temperature to grow the mosquitoes, so what are the chances of their survival if they are like in an extremely like cold area. Are they even going to survive or not?

[DR. KRAMER:]No. They're cold sensitive but what *albopictus* can do so *albopictus* range if you remember from the map goes further north than *Aedes aegypti* and that's because *Aedes albopictus* eggs are able to enter diapause state when as the temperatures are cooler and that's determined before it gets to the egg stage but as the days get shorter and the temperatures are cooler, the egg will go into diapause and that's how *albopictus* make it through the winter so you have lost of *albopictus* here and the eggs survive the winter. The adults don't survive the winter. So that's what they do to protect themselves and that's why they've been so successful in a temperate region like the U.S. whereas *Aedes aegypti* really is a tropical mosquito. So you see it, well it keeps on getting reintroduced from Mexico and further south but you see it, you know, in the southern states--*albopictus* goes further north. But they develop... the temperature... what happened in your experiments, or at least my house is cool, so my larvae didn't get totally stuck in the larval stage but they went very, very slowly and I had a very protracted hatching. I did eventually get adults but then when I brought one of the containers into the lab and put them in our insectary popped out the adults, so they wouldn't have been able to enter diapauses because of the way diapause works. They've been in the cool the whole time and they wouldn't have entered diapauses but they just were slowed down but they're protective mechanism is diapause.

[DR. HARRIS:] Okay.

23. Does the dengue virus affect mosquitoes adversely? (43:30)

Over here and then we'll come back to you. Yeah?

[STUDENT:] Does the dengue virus have any ill effect on the mosquito? Does it kill it or like?

[DR. KRAMER:]No. So, it used to be thought that there was no ill effect at all on the mosquito, by these viruses that replicate in them, but what we're seeing now is there are some minor pathologies, there

is apoptosis that takes place in the midgut which is the mosquito stomach and there is apoptosis in the salivary glands but it's over a long period of time. So whether the mosquito in the wild is going to live long enough for that to happen, I mean some mosquitoes will, some won't. There is other viruses like La Crosse encephalitis which is a California serogroup virus that I talked about that replicates and is transmitted by *Aedes albopictus*. That causes a real problem in the salivary gland so the mosquito has to probe more often to get a blood meal and that's good for the virus because just like with *aegypti* each time it's probing it's transmitting. Saliva is coming out which is an anticoagulant and then so the virus is going with that each time. We've seen in the lab we've done some studies with West Nile and *Culex pipiens*. We've seen some very minor effects on fecundity, the number of eggs that are laid in that first oviposition, but then after that it seems fine so there's minor pathologies but not major pathologies.

[STUDENT:] So it doesn't affect population?

[DR. KRAMER:]It does not affect survival but it affects the population in that first OV, you're cutting back on the population a little bit.

[DR. HARRIS:] It's really cool, though, because a lot of parasites have evolved to influence the behavior of either their vectors or their hosts even so like *Leishmania* is a parasite that I used to work on and it does the same thing. One of its molecules can muck up the salivary secretions so that it actually has to go to different hosts.

[DR. MOUDY:] [interposing] Uh huh.

[DR. HARRIS:] There is a crazy story about another parasite called *Toxoplasma* which actually eventually affects cats but it can actually change mice which was an intermediate host to essentially be less cat-averse. Yeah, I know. It's crazy so it will actually change. You know, you can put like cat urine in this and in this and then you can test these rats and mice that have been infected or not with *Toxo* and see, and apparently the *Toxoplasma* can like change the behavior of the mice so that they no longer are afraid of cats so presumably the cat will eat it and get the parasite.

[DR. KRAMER:]I remember that.

[DR. HARRIS:] That's crazy [laughter].

[DR. KRAMER:]There also was a study done with children who had malaria and they are more attractive to the *Anopheles* mosquito than healthy children so that's again the parasites kind of manipulating the host.

[DR. MOUDY:] Something with like their pheromones or the odorants.

[DR. KRAMER:][interposing] Or slight fever that makes them more attractive.

24. Is there an evidence of co-evolution in dengue virus? (46:24)

[DR. HARRIS:] Well this and one more and then we want to see our larva. Yeah?

[STUDENT:] Has there been any modification in like the dengue virus genes at all?

[DR. HARRIS:] Do you mean co-evolution type of - - ?

[STUDENT:] Yeah.

[DR. HARRIS:] So, not directly in dengue virus in relation to mosquitoes but it looks like there is a human flavivirus resistance gene and I don't think it's as much from dengue but I think that it's more probably from yellow fever which is very closely related to dengue in the African subcontinent because people of African descent are more resistant to severe dengue and so I think that there might be something because essentially for many hundreds of years people coexisted in Africa with yellow fever but when like the European colonialists came they all dropped like flies and actually - - so I think that essentially there might be a flavivirus resistance gene in the human population and it's kind of --

[DR. KRAMER:][interposing] But, in the vector. So, actually we talked about, or Joe talked about quasi species this morning, and what we see with West Nile in the mosquito is that heterogeneity of the virus is built up so that you have more of a population there where allowing for a virus that's more efficiently transmitted to be transmitted and then when it goes into the bird there is purifying selection so that the population narrows down again and what happened with West Nile and what also happened with Chikungunya virus is that there was a mutation in the virus and West Nile was in the envelope and Chikungunya was in the E1 gene. That's a different, it's an alphavirus that allowed the viruses to be transmitted more rapidly and that's what made the difference with Chikungunya spreading. Chikungunya got to Italy so again we have a tropical virus that, you know, became successful in a temperate area because it was a mutation that allowed *Aedes albopictus* to be a much more efficient vector and then the virus took off with *albopictus* which are invasive everywhere in the world and West Nile with *Culex pipiens*, you know, our lab has shown in the U.S. there was a mutation that allowed it to be transmitted more rapidly by *Culex tarsalis* another vector and *Culex pipiens*. So these changes do occur in particularly in the vector.

[DR. MOUDY:] And even in dengue there's that whole genotype difference between in dengue 2. There is the Southeast Asian genotype and the American genotype --

[DR. HARRIS:] Right.

[DR. MOUDY:] -- and the Southeast Asian genotype was found to actually be more infectious for mosquitoes and be transmitted better than the American genotype.

[DR. HARRIS:] In fact, we're testing that right now with we have a change, an evolutionary change in Nicaraguan viruses that Laura actually is testing in her lab with Nicaraguan mosquitoes that we brought up to see whether the clade of virus that essentially dominated in nature actually is able to transmit better in mosquitoes, so yeah, that is true. Good.

[MALE VOICE:] I think you should go ahead and --

[DR. HARRIS:] Okay. Now we're going to get to our larva and our pupae!