Loop-Mediated Isothermal Amplification Assay to Detect Invasive Malaria Vector *Anopheles stephensi* Mosquitoes

[Announcer] This program is presented by the Centers for Disease Control and Prevention.

[D. Peter Drotman] This podcast series is brought to you by *Emerging Infectious Diseases*, often referred to simply as EID. I'm Dr. D. Peter Drotman, Editor-in-Chief. EID is an open access, high impact, peer reviewed scientific journal published monthly by CDC. EID publishes articles on new and reemerging infectious diseases that occur anywhere around the world so as to improve the understanding of factors involved in disease emergence, control, and prevention.

[Candice Hoffmann] Welcome to the *Emerging Infectious Diseases* podcast. I'm Candice Hoffmann. On this episode, we'll be talking about a new test to detect *Anopholes stephensi* mosquitoes. These invasive mosquitoes pose a major problem because they spread malaria, and some of the proven methods to stop similar mosquitoes don't work with this type.

Fortunately, a team of scientists, including experts from CDC, interns with the Public Health Entomology for All program, and the Kenya Medical Research Institute have developed a rapid, easy to interpret test to detect these mosquitoes. This test will help countries know where best to focus efforts to stop the spread. They described their work in the September 2024 issue of EID in a paper titled "Loop-Mediated Isothermal Amplification Assay to Detect Invasive Malaria Vector *Anopheles stephensi* Mosquitoes."

First, we'll hear from the lead author of this paper.

[Cristina Rafferty] I am Cristina Rafferty, and I am the PMI molecular biologist in the entomology branch at the Centers for Disease Control.

[Candice Hoffmann] Cristina Rafferty is with the President's Malaria Initiative, or PMI, in the Division of Parasitic Diseases and Malaria within the National Center for Emerging and Zoonotic Infectious Diseases at CDC. Her team works to combat malaria, a serious disease caused by a parasite that infects a certain type of mosquito.

To set the stage, let's hear why malaria is a serious threat, and how a specific species of mosquito, known as *Anopholes stephensi,* is creating new challenges in controlling it.

[Cristina Rafferty] Malaria is still one of the leading causes of death in the world, especially in sub-Saharan Africa. Ninety-five percent of malaria deaths occur in the continent. And it is a big problem because even though we've made some strides in the last few years with interventions like nets and IRS, we've seen those gains kind of plateau, right? And one of the reasons that it is plateauing is this invasive species, *Anopheles stephensi*, which came from Asia. The type of malaria they transmit is the same. There are two main plasmodium or parasites that are linked to malaria, *Plasmodium falciparum* and *vivax*. And this mosquito is capable of transmitting both very, very effectively. That is part of why it is such a threat. It is well adapted, and it transmits malaria very readily.

[Candice Hoffmann] *Anopholes stephensi* mosquitoes are showing up in new places and they have some unique characteristics that make them better at spreading malaria. It is estimated that

as these mosquitoes spread, an additional 126 million people in urban areas are at risk of malaria.

[Cristina Rafferty] Traditionally, malaria in Africa is transmitted by mosquitoes, *Anopheles* mosquitoes that are found usually in urban areas. The *Anopheles* mosquitoes in Africa are traditionally rural mosquitoes. And so, malaria traditionally has been a rural disease. *Stephensi* is very different because it has very urban tendencies. So, it breeds in urban settings—tires, man-made holes, cisterns, things like that. So, all of our interventions normally have been aimed towards rural settings. Cities are usually not big places where malaria occurs, but yet *stephensi* is going to be settling in mostly cities.

The other thing that is very different is that traditional malaria mosquitoes are seasonal, so they only come with…because they breed in usually puddles and things that come with rain, you'll see malaria also have seasonality, right? So, after the rainy season, malaria will go up and then as it dries out, it'll go away. Because *Anopheles stephensi* like to breed in manmade containers, they are actually around all year long. And then lastly, and most importantly, they are a mosquito that lives outdoors, it feeds during the day. And so, interventions that we've had up to date like nets and indoor sprays are not going to target it because obviously those are interventions that aim to eradicate mosquitoes that feed indoors and feed at night. So, it poses a big risk.

One of the reasons it's so important to detect them quickly is that if you look at, for example, the case in Djibouti or even in Ethiopia, Djibouti was close to elimination before prior to 2012, then they found *Anopheles stephensi*, and now they are actually back to endemic status, having more cases than they've seen in decades and they are all being linked to *Anopheles stephensi*. So, because this mosquito is such a good malaria transmitter, it behaves so differently and it's not really touched by the existing interventions that we've deployed, it poses the risk to put a lot of people in danger of getting malaria, people who were never exposed before.

[Candice Hoffmann] To find where these mosquitoes are spreading, first you have to collect mosquitoes. There are many ways to do this.

[Cristina Rafferty] Mosquitoes get collected in all sorts of different ways. We have light traps, which are actually, they are called the CDC light traps, and they use a carbon dioxide source. Mosquitoes get attracted to them and then they have a bag. That's probably the most common way for mosquitoes to get collected in big numbers. Some countries are still using what are called human landing catches, where basically people at night sit around and kind of wait for mosquitoes to almost land on them and then collect them that way. And of course, now with *Anopheles stephensi*, there's a lot of larval collections as well. So, people will go out to cisterns or wells, or even rice fields is a big place where people do a lot of larval collections and do dipping, collect mosquitoes and then take them back to the lab, rear them and then be able to identify them as adults.

[Candice Hoffmann] Identification of these mosquitoes is important, but it isn’t always easy. One method is morphological identification, in other words, using a step-by-step key to look at mosquitoes’ features and identify them by eye.

[Cristina Rafferty] Morphological identification of mosquitoes is a difficult skill anyway. If you can imagine, they're very tiny. The thing that sets them apart are tiny little, you know, how bands are spread out on a wing or an antenna, that kind of thing. And so, that morphology requires high level training. But more importantly, the tools to really identify all these mosquitoes tend to be

molecular. So, it requires people to be in a lab, to be able to run something like PCR, sometimes even have to do high level molecular work like sequencing. And so, the quicker we can detect a mosquito as it moves into a population or a new place, the faster you can switch interventions to be able to target it, right? And so, for *Anopholes stephensi*, probably the main intervention that we have is larval management, just because since they are daytime and outdoor feeders, there's really very few ways that you can control a mosquito that's just outdoors all the time. And so, you have to target it before it emerges in the larval stage. And so, if you think about being able to do that as it first gets introduced in a country, it's a lot easier to control it that way than if you wait until it's everywhere and it's already settled.

[Candice Hoffmann] Cristina's team wanted to find a simpler and faster way to detect the presence of *A. stephensi* mosquitoes, so countries know where to focus their efforts to stop them.

[Cristina Rafferty] One of the things that we really wanted to do is give countries a tool that they would be able to utilize to screen large numbers of mosquitoes as they collected them as a first step to be able to get to the confirmation of the presence of *Anopheles stephensi* in a country. Because usually when countries go out and collect mosquitoes in the field, they'll collect thousands of mosquitoes. And you'll do a morphological identification and then this gets sent to a lab, and there's a lot of bottleneck, a lot of time that you have to wait, reagents that have to be used, et cetera, to be able to get to an answer. And so, the idea was to try to give…to come up with something that would be quick, easy, and easy to interpret so that if there was a sample that then needed to be followed up for testing, it wouldn't have to be all thousands of them, but maybe only a few. And so, that was kind of in the back of our mind when we were trying to come up with an easy way to do this and a way that would be friendly for people to be able to use in the lab and the field.

[Candice Hoffmann] The team created a new assay, or test, that is described in the paper using the acronyms CLASS and LAMP.

[Cristina Rafferty] CLASS is basically just the colorimetric LAMP *Anopholes stephensi* species ID assay. And that was just after when we were writing the paper, it was becoming very cumbersome to say colorimetric LAMP *Anopholes stephensi*. And so, we just, you know, we were like, we need a catchy name, that's what CLASS stands for. That's what it was.

So, LAMP…loop-mediated isothermal amplification, that's what LAMP stands for. But basically, what a LAMP assay does is it is, in very layman terms, it's like a super robust PCR in the sense that the components in this reaction interact with each other and they create these loop structures within the amplification. And what that does is it very quickly…it creates a change in the reagent.

Our assay itself is a colorimetric assay, which means it is phenol-based. So, any change in that reaction basically causes a pH change, which then the phenol can detect. So, in our case, the reaction starts as pink, which is because of phenol red. And then when there's a change…which means it's positive, right? There's *Anopholes stephensi* in there. That reaction turns from pink to yellow. And so, it makes it very easy to be able to detect it because the change is a lot, it's fast and it's very evident when it works.

[Candice Hoffmann] So, instead of relying on complicated equipment or experts with specialized knowledge of morphology, the team created a test where you can see a color change that tells you if mosquitoes are *Anopholes stephensi* or not. The test requires a heat source to operate.

[Cristina Rafferty] You heat it to 65 degrees and that can be achieved in a number of ways, right? So, a lab can use a standard thermocycler that they have or a heat block, a water bath. Like I said in the paper, there have been instances of people doing LAMP assays where they have used hand warmers in a cooler to get to that temperature to achieve the 65-degree temperature and, you know, put the tubes in that cooler and the reaction takes place.

[Candice Hoffmann] Gloria Raise and JeNiyah Scaife were two interns who worked with Cristina on this project through the Public Health Entomology for All fellowship.

[Gloria Raise] I'm Gloria Raise, and I was an intern at the CDC under Cristina. And right now, I'm an application scientist at OPS Diagnostics.

What got me into entomology was actually the research that I was doing during my bachelor. I started doing research in forensic entomology, and that kind of opened up a new world to me because I didn't know anything about entomology and because of that research I started going to the Entomological Society of America conferences, and that's where I learned about the public health side of entomology and that's where I learned about the Public Health Entomology for All program, which I decided to apply. And that's how I got into the CDC as an intern. And what I enjoyed the most about the internship was the fact that every day I was learning something new, both on a technical level in the lab, but also like knowledge about malaria and mosquitoes, and also the fact that I was able to be part of making a change in the world for a better place.

[JeNiyah Scaife] I am JeNiyah Scaife. I was an intern at the CDC. I recently just graduated from Fayetteville State and I'm currently still looking for work.

So, I found out about the internship through my advisor at my university and I had all the qualifications that the CDC was looking for for the application, so I decided to give it a shot. I just was looking for more lab experience before I finished my degree and I fell in love with my internship. I didn't dread like getting up going to work every day. I was very eager to finish the project that Cristina had started for us and I'm glad that we were able to complete our project within our 10 weeks.

[Candice Hoffmann] During the internship, Gloria and JeNiyah got firsthand experience working on something new that could advance the field of public health. The moment the test worked was memorable and surprising.

[Cristina Rafferty] Let's see…I think the first thing that surprised me was, I mean, really to an extent is how it was really amazing to see it work. I think we can all attest to we remember the... because we tested a lot of different combinations of these reagents where there were kind of mixed results, but when it worked, it worked. It was like it worked and then we started testing it with different temperatures, different things, and it was just consistently working. And I think the fact that when it works, it works well, was really refreshing to an extent. As a scientist, sometimes, you know, you have to battle with optimization so much. But this particular assay just seemed, it seems pretty robust and that was surprising in a way, but in a very, very good way.

[Gloria Raise] I would have to agree with Cristina. I still remember vividly how it felt when it was actually working, because in the beginning it was just either not working or there was contamination, and it was just not good enough. But then when it worked, it was surprising. And at the same time, it was…I felt like accomplished, like, ok, so we are actually making progress here.

[JeNiyah Scaife] I can agree with Cristina and Gloria. I'm glad that all our hard work and all our research definitely made a difference in the science field.

[Candice Hoffmann] Cristina, Gloria, and JeNiyah have this advice for future researchers who want to get involved in similar work.

[Cristina Rafferty] I would say, especially when it comes to learning about mosquitoes, don't dismiss, you know, I know that this is mainly a molecular assay and, you know, we're all over here, we're working in a molecular lab, but I think we have to remember that, first and foremost, that most of the valid interventions that have been done to fight malaria and all of the gains that have come linked to malaria or a big, big part of them in the last 15 years are tied to vector biology. So, IRS, nets, they're deployed because of the knowledge that we have about mosquitoes. And so, knowing the species of mosquitoes that are in a place is vital to inform decisions for interventions and to be able to find out not only a mosquito, not just at a general species level, but down to the subspecies is really, really important because of their difference in behaviors and biology. But tied to that is how important it is for the field to be well trained in morphology. I think morphology is a little bit of a lost art. It's getting kind of lost in the weeds because of advancements elsewhere. But I think it's still…that very first line of, it's kind of like the first line of defense, people who are able to identify those mosquitoes first so that then they can be confirmed in a lab setting, it's really, really important.

[Gloria Raise] Yes, so in addition to what Cristina said, I think an important thing, especially for people who are still studying, or they recently graduated, I think it would be to take an entomology class. There are many entomology classes online where you can learn more about insects and get a more general idea of what entomology is and how it's used in a work setting. And the other thing would be, I think, going to conferences where you can…entomological conferences, where you can meet new people, network and kind of get information that you need to get started in a work setting that is either with malaria or mosquitoes or other type of insects.

[JeNiyah Scaife] I would say definitely reading articles or just finding out different background information or even finding crash courses online or videos just to help someone understand those…the little, even like the verbiage that you may not understand, just little things like that.

[Candice Hoffmann] Students who are interested in following in Gloria and JeNiyah's footsteps may want to look into the Public Health Entomology for All, or PHEFA program.

[Gloria Raise] The PHEFA program is open to students that are going to minority serving institutions for them to get the opportunity to be part of the CDC in collaboration with the Entomological Society of America. And so, I think that is a great opportunity for students to learn more about entomology and public health.

[JeNiyah Scaife] I would definitely tell students to apply even if their major isn't public health or entomology geared because you don't want to turn down any opportunities because you never know what the outcome may be.

[Candice Hoffmann] Publishing in EID for the first time was an exciting moment for Gloria and JeNiyah.

[Gloria Raise] Well, it's, you know, when I learned that the article was accepted and it was about to be published, I was very excited. I remember texting Cristina, I was like asking, do you know when it's going to be published? You know, it's just great to know that it's kind of…it kind of makes it official to be part of the team that worked on that. So…and it still feels great to know that it's been published.

[JeNiyah Scaife] I can definitely agree with Gloria. It is just a fantastic feeling knowing that I was able to be a part of this and knowing that I was able to help out with the research.

[Candice Hoffmann] And whether you're a scientist working on infectious diseases, a student exploring career possibilities, or any other type of listener, we hope you'll become a regular reader of EID like Cristina Rafferty.

[Cristina Rafferty] I'm a regular reader and I get, you know, I subscribe to all the different groups for it. And I mean, a couple of reasons why we decided to publish through EID. I know that EID had previously published a few findings and papers on *Anopheles stephensi*. And so, we thought it was a good compliment to those stories. But, you know, on a separate note as well, I think, you know, it's a nice tie-in when the work happens at CDC, and EID is obviously closely affiliated with us. So, it just seemed like a good fit.

[Candice Hoffmann] Thanks for listening to our podcast. You can read the *Emerging Infectious Diseases* journal at cdc.gov/eid. You can also follow EID on X and Instagram @eidjournal, and on LinkedIn @eid-journal.

[Outro] The conclusions, findings, and opinions expressed by authors contributing to this journal do not necessarily reflect the official position of the U.S. Department of Health and Human Services, the Public Health Service, the Centers for Disease Control and Prevention, or the authors' affiliated institutions.

[Announcer] For the most accurate health information, visit cdc.gov or call 1-800-CDC-INFO.