

Researchers advance low-cost, low-tech Zika virus surveillance tool

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Aedes aegypti is the primary mosquito that carries Zika virus. To combat potential Zika virus outbreaks, some countries have considered using pesticides so strong that they are banned elsewhere. Credit: John Eisele/Colorado State University

To combat potential Zika virus outbreaks, some countries have

considered using pesticides so strong that they are banned elsewhere. But what if you could quickly determine that mosquitoes were not carrying the Zika virus? The use of harmful pesticides could be avoided, as well as the cost of widespread spraying for mosquitoes.

That's the premise behind a new study published May 3 in *Science Translational Medicine* and authored by Assistant Professor Joel Rovnak, graduate student Nunya Chotiwan and Research Associate Connie Brewster in Colorado State University's Department of Microbiology, Immunology and Pathology.

The CSU team is using an existing technology in a new way: they have demonstrated a method of biosurveillance that quickly indicates whether Zika virus is present in local mosquito populations - thus informing decision-making about spraying and other disease-prevention methods.

Using the existing technology, loop-mediated isothermal amplification, or LAMP, the research team found that they could easily detect Zika virus in human and mosquito samples from the United States, Brazil and Nicaragua.

LAMP lights the way to virus detection

CSU Research Associate Connie Brewster had been using LAMP, which was developed in Japan in 2001, to detect pneumonia in bighorn sheep through a project with Colorado Parks and Wildlife and the Quackenbush Lab at CSU. LAMP is similar to the technology known as polymerase chain reaction or PCR, which provides a very sensitive analysis of DNA samples. But LAMP can be used in the field, instead of in the lab, and it's also less expensive.

"With LAMP, you don't need the sophistication of a machine," Rovnak said.



These are researchers in Colorado State University's Department of Microbiology, Immunology, and Pathology collaborate with scientists from the Arthropod-borne Infectious Diseases Laboratory, also at CSU. Credit: John Eisele/ Colorado State University

To use the PCR method to analyze viruses, researchers first need to extract the RNA or ribonucleic acid from the sample. But with LAMP, the process is much simpler than using PCR.

Using LAMP, researchers start by squishing a mosquito in water. Rovnak said he then takes two microliters of water (about the size of the head of a pin), puts it in a tube and heats it up using a few chemicals or reagents. The sample then becomes cloudy and the color of the solution

changes. In the CSU-led study, researchers saw a change or signal within about 30 minutes, though it can take up to one hour.

"Using LAMP to detect Zika virus would be much less expensive for developing countries," said Chotiwan, a researcher in the Perera Lab, which is part of the Arthropod-borne Infectious Diseases Laboratory at CSU.

"The majority of the countries involved in the current outbreak are not rich," she said. "It's important for us to try to develop low-cost surveillance methods that might one day be used in these countries."

As a rough cost estimate, Rovnak said a heating device or heat block, as it is called, for LAMP costs \$250 or less. Real-time PCR machines cost between \$15,000 and \$25,000.

In the study, the research team focused on detecting Zika virus strains from Asia and Africa. Zika virus was first detected in Uganda in 1947 and is predicted to be heading back to the African continent, Chotiwan said.

The Centers for Disease Control and Prevention recently reported local transmission of Zika virus in Cape Verde, off the coast of West Africa. CDC has also issued Zika virus travel advisories for some countries in Africa. In the United States, Zika virus transmission has been reported in Florida and Texas.

ZIKA virus is a *mosquito-borne* pathogen that can cause symptoms of:

Fever

Eye Inflammation

Joint Pain

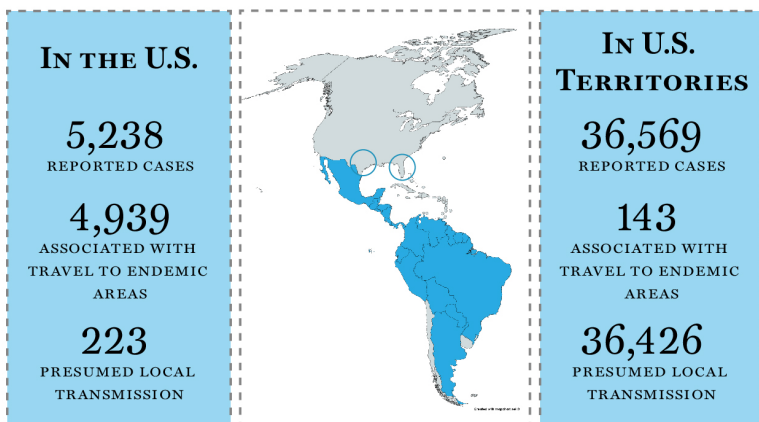
Rash

In some cases, Zika virus infections have been associated with Guillain-Barre syndrome, an autoimmune condition leading to muscle weakness and paralysis.



Infection during pregnancy has been linked to birth defects in infants including **microcephaly** (or smaller than normal head size) and eye abnormalities.

Cases of Zika virus infection have been reported in 49 states in North America, mostly associated with travel to endemic areas.



Local transmission of Zika virus has been confirmed in **TEXAS (6 CASES)** and **FLORIDA (217 CASES)**, as well as throughout **CENTRAL** and **SOUTH AMERICA**.

Zika virus is typically transmitted by mosquito species in the genus *Aedes*, but some cases of sexual transmission have also been documented.



Science Translational Medicine  AAAS

Statistics courtesy of the CDC [<https://www.cdc.gov/zika/>]
(As of April 19, 2017)

Infographic showing Zika virus prevalence in the Americas. Credit: Carla Schaffer / Chotiwan et al. / AAAS

No one really knows the extent of the original Zika virus in Africa, according to Rovnak. But being able to distinguish between the different strains is important due to the association of the Asian-linked virus with microcephaly, a congenital condition associated with incomplete brain development among newborns.

Brian Foy, CSU associate professor in the Arthropod-borne Infectious Diseases Laboratory, discovered that Zika virus may be sexually transmitted in addition to being spread by mosquitoes. With two known transmission routes, the virus is an even more formidable foe to public health.

To expand on the early results and use of LAMP to detect Zika virus, the scientists received samples from researchers in Brazil and from a pediatric hospital in Managua, Nicaragua. They also obtained samples from the CDC's Division of Vector-Borne Diseases in Fort Collins.

Next steps include hospital-based tests

Moving forward, clinicians at the pediatric hospital in Managua will test LAMP side-by-side with current, more sophisticated tests. The CSU research team will also test LAMP versus PCR using mosquito and wildlife [virus](#) samples from Puerto Rico obtained through a project with the U.S. Department of Agriculture's Animal and Plant Health Inspection Service.

How soon could LAMP be made available for more widespread use?

"For now, it's what would be considered a long slog," said Rovnak.

"The human diagnostic side of things presents a much more significant challenge," he explained. "It's going to take a lot of time and a lot of data before people will allow regulatory agencies to OK LAMP as a bonafide test for a person that's sick in the hospital."

More information: "Rapid and specific detection of Asian- and African-lineage Zika viruses," *Science Translational Medicine* (2017). [stm.sciencemag.org/lookup/doi/ ... scitranslmed.aag0538](http://stm.sciencemag.org/lookup/doi/.../scitranslmed.aag0538)

Provided by Colorado State University

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