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Virus Hunters: Catching Bugs in the Field

Densely populated areas in rural China require constant vigilance and state-of-the-art technology to stop new pandemics in their tracks. Hurdles are not only scientific in some parts of the developing world.

For more than a decade, farmers in rural China have suffered from a lethal infection that causes high fevers, reduces platelet levels in blood, and sometimes triggers massive hemorrhages. Based on its clinical presentation, doctors assumed the disease was anaplasmosis, caused by the bacterium *Anaplasma phagocytophilum*. But in 2009, Xue-Jie Yu, researcher at the University of Texas Medical Branch and visiting scientist at the Chinese Center for Disease Control and Prevention (CCDC), noticed something unusual. Anaplasmosis causes few deaths in the Western world but was killing nearly three out of ten patients in China.

Yu is a microbiologist who specializes in culturing bacteria. The CCDC had invited Yu to help isolate the anaplasma bacterium, but following his hunch, Yu decided to look for a virus. He inoculated DH82 dog-derived cells with white blood cells from patients to generate a viral culture. He was lucky; viruses are picky about their hosts and only show up in certain backgrounds. When he saw diseased cells, he thought, “Wow, we’ve got a virus.”

This was firm proof that the outbreak was not anaplasmosis. Once Yu had enough viral particles, he looked at their morphology under an electron microscope and then sequenced the genome. He found a novel *phlebovirus* called severe fever with thrombocytopenia syndrome virus (SFTSV) belonging to the *Bunyaviridae* family, published this year in the *New England Journal of Medicine*.

Since Sept. 2010, 171 patients have been diagnosed with SFTSV, and 36 have died.

The case has been hailed for the comparatively short time that it took Yu and his CCDC colleagues to follow through on his hunch and characterize the virus. He had technologies at this disposal that, a decade ago, would have been difficult to find in China. And such technologies remain hard to find in much of the developing world, leading to a push for better and cheaper diagnostics to hunt for emerging viruses.

In the 1960s, flush from the successes of drug discovery and sanitation, public health officials had declared mastery over infectious disease. But over the past three decades, about 87 new pathogens causing human disease, most of which are viruses, have emerged, and nearly 75 percent of these have been zoonoses. Because environmental changes are occurring most rapidly in the developing world, where people and wildlife live in close proximity but where access to diagnostics is limited, cheaper and better technologies are of vital importance to prevent the global spread of diseases.

A Fishing Expedition
Malik Peiris, microbiologist at the University of Hong Kong, and his colleagues, widely credited with discovering the SARS coronavirus, have been sequencing the viral background of bats. They have found several new coronaviruses, relying on sequencing machines to hunt for novel bugs. Bats are excellent reservoirs for potentially lethal viruses such as Nipah, which causes influenza-like illness in horses and humans. Ecologists capture bats and stick oral swabs into their mouths to get a sample of microbes and send these to Peiris’ lab, where scientists use either multiplex PCR using family-wide primers or direct sequencing to find novel bugs.

If the sequence matches something in the database, the computer gives a percent homology. Researchers then need to show to their best ability that the isolated virus is indeed causing the disease and fulfills Koch’s postulates. The postulates state that the virus must be present in all patients; the virus derived from patient samples should be isolated and cultured; if someone injects the culture...
into a healthy patient or animal, the virus should cause original disease; and when these new patients are tested, their disease should be traced to the original bug.

Among viruses causing human disease, deep sequencing has helped scientists identify pathogens, including the hemorrhagic fever Lujo virus from South Africa and the Dandenong virus that causes disease in patients receiving organ transplants.

Still, high-throughput sequencing needs to be used sparingly, Peiris says.

“Applying deep sequencing for pathogen discovery is extremely intensive and extremely expensive, and if you do it on every single specimen, it is not productive,” he says. Before sequencing, researchers need to eliminate possible known bugs to establish that they are indeed dealing with something new. And even before that, front line responders need to recognize a disease is unusual and order tests.

“It’s a fishing expedition,” says Leo Lit Man Poon, assistant professor of microbiology at the University of Hong Kong and part of Peiris’ team. “You try to sequence as much as possible hoping that you will be able to identify some of the sequences which do not belong to the host.”

Often, sequences from a patient sample don’t match anything human, bacterial, or viral in the database.

“This is the black box,” says Poon. Although sequences are being added to the database at record rates due to the genomics revolution, the majority of the world’s organisms—and especially viruses—have not been sequenced. In these cases, bioinformaticians use various cryptographic methods to figure out patterns—the frequency at which some di- or trinucleotides appear, for example—in the dark matter.

The Virus World on a Chip

Nucleic acid-based microarrays are becoming so robust and dense that virus hunters can identify bugs quicker and cheaper than through high-throughput sequencing. Even the best sequencers today take about a day to work through data, says Ian Lipkin, professor of epidemiology at the Mailman School of Public Health at Columbia University.

“Given the price of arrays, the density of arrays, the fact that you are probably going to be able to survey for all known viruses for $15 or $20 in the not-too-distant future, and do it in a couple of hours, is really quite extraordinary,” he says.

Eight to ten million spots can be placed on a single piece of glass, more than enough to cover the entire viral world, says Lipkin. He is collaborating with Tom Slezak at the Lawrence Livermore National Laboratory on a Department of Defense-funded project to generate universal bacterial and viral microarray systems that are flexible enough to detect emerging and variant viruses.

The idea is that, as long as the probes are well designed, scientists can pick out an unknown virus whose identity is suspected at the level of the genus or family. The microarrays’ first success was the SARS coronavirus. Whereas Peiris’ team worked painstakingly to characterize SARS the old-fashioned way in Hong Kong, a young researcher named Joseph DeRisi at the University of California San Francisco decided to use a microarray. Without culturing the virus, he applied respiratory samples from patients onto the array. Within 24 hr, he got a positive result corresponding to the coronavirus family, which was further confirmed by sequencing results. DeRisi’s “ViroChip” array is now a go-to for virus hunters. Charles Chiu, also at UCSF, has used the ViroChip to identify a novel adenovirus in monkeys and humans and, most recently, a novel hemorrhagic fever virus that is yet to be published.

The trend is toward multiplexed systems on chips, microspheres, and nanowires. There is talk of further compaction, with an array hosting all of the steps of pathogen discovery, including sample preparation, PCR, hybridization, and sequencing.

The upshot is that microarrays will one day become more portable, cheap, and easier to use, all of which are needed in the developing world, where emerging viruses typically crop up.

“All of these things are going to come together; they have to, because we need to have some way to do discovery work in the field,” says Lipkin.

Virus Hunting on the Ground

Most of these sequencing and microarray technologies are not available beyond a handful of laboratories concentrated in the West. In the emerging disease hot spots of the world, including South East Asia, India, and parts of Africa, basic PCR tests are the norm if they are performed at all. Though PCR can help detect known viruses, the test is too specific to detect novel bugs.

“Right now, the big issue in the developing world is there’s very little surveillance being done for any disease,” says Chiu. “Even for large diseases like malaria or TB in Africa, there are some cheap assays, they are not very sensitive, they are not very specific. There is nothing there right now.”

Doctors typically diagnose diseases on the basis of clinical examinations, and they do not perform virological tests to back up their ideas. Most viruses go undetected. “A clinical syndrome has to be diagnosed, has to be noticed, has to be reported, specimen has to be collected, specimen has to be sent to reference laboratories often in Europe or the United States, which takes a lot of time,” says Heinz Feldmann of the National Institute of Allergy and Infectious Diseases. Even then, there may not be enough quantities of the specimen to apply on high-throughput technologies, he says.

Exporting biological samples has also become challenging following the 9/11 anthrax scares in the United States. Since the SARS outbreak, the Chinese CCDC has coped with this barrier by collaborating with researchers like Yu, as well as experts from the U.S. Centers for Disease Control and Prevention and elsewhere. Their national labs are equipped with state-of-the-art high-throughput diagnostics, including sequencers and microarrays found in the West.

But given China’s position as an emerging disease hot spot due to the proximity between wildlife and people and its enormous population spread out over a large area, virus detection is still challenging. The weakness is in the hospital system, where diagnostics remain rudimentary, lab technicians have less training, and doctors rarely try to identify bugs causing disease, says Jay Varma, scientist with the New York City Department of Health and Mental Hygiene, who helped the CCDC revamp their system. The expert virus
hunters at CCDC are distanced from the ground realities of disease in the population, he says.

In other parts of the developing world, corruption and patchy water and electricity supply can doom the efforts of virus hunters. Lipkin recalls the time his graduate student attempted to set up a basic multiplex PCR system in Sierra Leone as part of a philanthropic Google.org project to help with surveillance in the developing world. The effort was meant to find novel viruses, as well as put in place a system for rapid response to outbreaks. But parts from the instrument were repeatedly stolen.

“When people come in and they want a bribe, and they take the wires from the instrument, there’s not too much I can do about that,” says Lipkin. “That’s not a failure of technology.”

ACKNOWLEDGMENTS

Wen Xue at the Koch Institute for Integrative Cancer Research of MIT kindly provided the Chinese translation of this article with the assistance of Helena Yang at Cancer Cell. The translation is available at http://www.cell.com/virushunters-chinese.

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London, UK
DOI 10.1016/j.cell.2011.11.037