A handheld air sampler keeps watch for airborne viruses at a Cambodian market. The device can also be loaded onto a drone or toy car to reach inaccessible spots.

INFECTIOUS DISEASES

Virus hunters test new surveillance tools

Ropes, drones, insects, and dust cloths could make monitoring faster, safer, and cheaper

By **Jon Cohen**, *in Phnom Penh, Cambodia*

On a Friday morning in September last year, Erik Karlsson visited the sprawling Orussey market here, where vendors hawked pots and pans, phone cords and radios, hats and dresses—and myriad types of Southeast Asian food. Dozens of orange-colored, whole roasted pigs hung on hooks, crabs the size of two fists filled buckets, and stacked fruit and dried fish formed mountains on tables.

Karlsson had come for the live poultry, but not because he was planning a dinner. As an epidemiologist at Cambodia's Pasteur Institute, he was hunting for potentially dangerous pathogens, both known and unknown. He had nothing with him but a cellphone in one hand and, in the other, a device that resembles a portable credit card reader, which he held close to men who were slaughtering, boiling, and defeathering chickens. The AeroCollect, as it's called, has a chip that uses an electric field to suck in and trap air in microscopic chambers. Later, back at his lab, Karlsson flushed each chamber's content with a water rinse and ran a polymerase chain reaction (PCR) to amplify any viral RNA in the air.

Karlsson is one of many researchers looking for simpler, faster, cheaper, and safer ways to find viruses—including the ones that could cause the next pandemic. If the AeroCollect works well, he won't need to don masks and gloves and get permission to draw chickens' blood and swab their behinds. Karlsson also plans to test flying the device on a drone into bat caves—which are known viral hotbeds—so researchers don't have to enter them.

Other scientists are tapping advances in nanotechnology, sequencing devices, artificial intelligence, and robotics to improve virus hunting and provide a more timely, deeper understanding of potential threats. Programs to monitor SARS-CoV-2's spread and evolution using wastewater (*Science*, 11 March 2022, p. 1100) have shown how new methods can help.

Many of the projects, including Karlsson's, are at the proof-of-concept stage, but Maria Van Kerkhove, who heads the emerging diseases and zoonoses unit at the World Health Organization, welcomes them. "I'm really excited about the stuff Erik is doing," says Van Kerkhove, who sampled birds at Orussey market herself more than a decade ago as part of a Ph.D. project on bird flu. "Air sampling is leapfrogging in technology."

But new surveillance technologies must not lead to what Van Kerkhove calls "a stamp collecting exercise": simply identifying the hundreds of thousands of viruses in wild and captive animals. "Detection is only one element," she says. "Does the virus have the ability to spill over from animals into humans? And if so, what does that cause? Is it a dead end or does it have an epidemic outbreak risk?" Just such a criticism has dogged the Global Virome Project, an ambitious scheme that promises to find the vast majority of pathogens that threaten humans but has struggled for many years to find the funding to launch (*Science*, 23 February 2018, p. 872).

To properly gauge the risk of outbreaks, researchers have to conduct repeated surveillance for viruses in spillover hot spots such as live-animal markets, farms, and bat caves, and at the clinics near them, says epidemiologist Christine Johnson, who directs the EpiCenter for Disease Dynamics at the University of California (UC), Davis. "I don't think we just want to do a snapshot in time," she says. "It has to be fundamentally more of a long-term, watching things evolve to really gain any true insight or understanding."

Still, most virus hunters agree a bit of ingenuity and creativity can make identifying threats a lot easier. Here are some of the avenues they are exploring.

JOHNSON'S TEAM is giving wild monkeys something to chew on—literally. It is exploring whether scientists can sample the animals' saliva by giving them treats on ropes.

PHOTO: J. COHEN/SCIENCE

The project grew out of the U.S. government's PREDICT, a 30-country effort launched in 2009—and run by UC Davis—to identify viral threats. Sampling wild primates traditionally requires trapping and anesthetizing the animals. That raises ethical and practical issues, however, so some scientists have simply collected feces or urine instead. But those approaches have drawbacks as well: They often require direct observation or accessing the animals' nests in trees, and neither is ideal for viruses that primarily shed through the mouth and nose.

These limits led Johnson and veterinarian Tierra Smiley Evans to test ropes laced with treats such as strawberry jam or banana baby food that monkeys chew on, leaving behind gobs of saliva. In proof-of-principle studies in Uganda and Nepal, they found simian foamy virus, parainfluenza 3, enteroviruses, and herpesviruses. The group has also collected plants chewed and discarded by Uganda's endangered mountain gorillas, most recently to assess whether people were infecting them with human herpesvirus-1 (HSV-1), which has sickened and killed captive lowland gorillas. There were no signs of HSV-1 in the plant discards from 294 gorillas, the team reported in October 2022 in the *American Journal of Primatology*, but they did contain gorilla-specific herpesviruses.

UC Davis has more futuristic projects in the works. A team is exploring whether a sandwich-size air sampler can identify active viral infections in humans from patterns of volatile organic compounds (VOCs) in their breath. Several respiratory diseases, including influenza, tuberculosis, and COVID-19, come with specific molecular breath signatures, researchers have found. In a December 2022 paper in *Communications Medicine*, a UC Davis team led by Mitchell McCartney and Cristina Davis even reported distinctive breath markers during the spread of the Delta and Omicron variants. The group has also tested the air sampler's ability to detect disease signatures while attached to a person's hip or flown on a drone.

The "breath biopsy" field is still in its infancy, despite a surge of interest during the pandemic in room air samplers and breathalyzerlike devices. (The Netherlands used SARS-CoV-2 breathalyzers for widespread COVID-19 testing, but officials soon deemed them unreliable.) In their attempt to make "breath the new blood" for diagnoses, Davis and an international group of colleagues last year launched the Human Breath Atlas, an effort to conduct large-scale investigations into the rare VOCs people exhale when sick.

IN THE NETHERLANDS, veterinarian Marion Koopmans and colleagues have been placing electrostatic cloths in chicken barns to collect dust that can be analyzed for viruses. "There's remarkably little high-quality information on viromes of farmed animals," she says. "We're exploring ways to make surveillance more accessible."

There are 22 billion chickens in the world, according to a 2017 estimate, and they harbor dangerous viruses—H5N1 influenza is the most famous one—that occasionally spill over into humans and might cause the next pandemic. Earlier this year, Koopmans and colleagues repeatedly visited three farms and compared dust snagged by the cloths with chicken feces scooped up by hand, the usual way to look for avian viruses. They didn't simply test the samples for known viruses, but used metagenomics to sequence any genetic material in the sample. Samples from both sources yielded similar results—viruses from four families—confirming the "electrostatic dustfall collectors" are a good alternative to sampling poop, the group reported in *Scientific Reports* last year.

Scientists analyzed viruses left on this rope after a monkey ate an attached treat.

PHOTO: TIERRA SMILEY EVANS

Koopmans hopes the technique can help scientists forecast where trouble is brewing. "Right now, the way our surveillance works is really very reactive," she says. "How can we move surveillance forward so we pick up things before you see human disease?"

SOME VIRUS HUNTERS want to send in an army—of ants. Voracious army ants prey on many insect and vertebrate species and can travel about 1 kilometer a day, often in and out of dense forests that researchers cannot easily enter. A team led by plant virologist Philippe Roumagnac at the University of Montpellier wondered whether the ants pick up the pathogens infecting their meals along the way. In Gabon, Roumagnac and his colleagues captured 209 army ants from 29 different colonies of the genus *Dorylus*, ground them up, and conducted a metagenomic survey of the DNA inside them. Sequences from 157 different viral genera popped up, the group reveals in a preprint posted last month on bioRxiv. Metagenomic analyses can also reveal what species the ants ate, helping tie specific viruses in a sample to what they may have infected. Using ants avoids focusing on animals that humans think pose the greatest risks, Roumagnac says: "They have no sampling bias."

To validate the method, the team plans to sample bat guano in Gabon for viruses and compare them with what they find in army ants feeding on the same guano. Roumagnac is optimistic: "Army ants could be one of the key players that could give clues about the bigger picture of the ecology and evolution of viruses in a single ecosystem."

KARLSSON'S PHNOM PENH OFFICE is filled with what he calls "toys" that might expand the AeroCollect's surveillance powers. A drone could be used to sample bat caves—even ones that only rock climbers can now reach. Karlsson recently purchased a remote-controlled, pint-size car that can hold the device—he plans to drive it around farms to collect samples from a distance. Battery-powered, downsized PCR devices and portable sequencing machines will accelerate the analysis. "We want to bring the lab to the field," Karlsson says. "If we're at an outbreak, we can get ahead of it faster."

The Pasteur team that Karlsson joined 5 years ago has a long history of working to improve viral surveillance in Cambodia, focusing on how bird flu evolves and spreads to humans. The team also understands the challenge of translating its findings into actions that protect people—a challenge underscored by PREDICT, which, despite an investment of more than $200 million, did not identify the threat of SARS-CoV-2. Veasna Duong, who heads the Pasteur Institute's virology division, adds that preventive measures require political will and acceptance by local people who often don't accurately perceive the risk.

That's no reason not to try, Karlsson says. "Can we prevent pandemics?" he asks. "I think we can be better prepared for the next one, that's for sure." ■

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