

PATHOGENS ARE THE BEST TRAVELERS OF ALL

BY ELAINE VITONE

NO BORDERS

When Don Burke moves into a new space, he decorates the walls with maps. At Walter Reed Army Medical Center, where he trained as an infectious disease specialist and served for 22 years before retiring at the rank of colonel, he mounted maps of Thailand and Southeast Asia on his office wall. At Johns Hopkins University, where he directed the Center for Immunization Research for nine years, he hung maps of Cameroon and Africa.

But when Burke settled into his new digs at the University of Pittsburgh, where in 2006 he signed on as dean of the Graduate School of Public Health (GSPH); associate vice chancellor for global health, health sciences; and director of the Center for Vaccine Research (CVR); he had an idea for a slightly different conversation piece. It started with the round table he found just inside the door of his new office.

"You like that?" he says on a clear day in November 2009. He points to a photograph of Earth, enlarged to poster size and placed under a round sheet of glass on the tabletop. He laughs. "It's a subtle statement of my job. And speaking of 'no pressure,' here are some of our forebears at the University."

This puddle of rainwater in western Rio de Janeiro is a potential breeding ground for mosquitoes that carry dengue fever, also known as breakbone fever.

© GETTY IMAGES

Burke points to two framed *Time* magazine covers—a 1936 issue featuring then U.S. Surgeon General Thomas Parran Jr., who would later become the first dean of GSPH; and a 1954 rendering of Jonas Salk, the Pitt virologist who, with Pitt's Julius Youngner and others, developed the killed-virus vaccine that conquered polio. (Burke holds Salk's namesake chair in global health at the University of Pittsburgh Medical Center.)

Among his many responsibilities, Burke considers his directorship of the Center for Vaccine Research "the jewel in the crown." The CVR's mission: prevention, intervention, and therapy for infectious diseases, the leading cause of death worldwide.

No pressure, indeed.

Burke's codirector at the CVR, Pitt professor of microbiology and molecular genetics Ronald Montelaro, has a sobering take on the legacy they've inherited. For example, compare the poliovirus to the virus of the common cold.

"Worldwide, there are only three serotypes of polio—three to inactivate, combine, and put into a vaccine, and that's exactly what Jonas Salk did," says Montelaro. "But for the common cold, there are more than 130 different serotypes—and they're evolving."

Researchers in this field have a saying: All the easy vaccines have already been made.

Burke leads with what Montelaro calls a "bush-to-clinic mentality." He has conducted fieldwork in Thailand, India, China, and throughout Central Africa. He has used computational models to predict outbreak patterns and recommend vaccination strategies, establishing the University as a national center for disease-emergence work. Years ago, at Walter Reed, he was part of the team that produced the first hepatitis A vaccine, which has since been used to inoculate hundreds of millions of people. He was the fifth person in the world to receive it.

"These were the days when you volunteered to be your own test subject," Burke says.

The reason Burke has piled so much onto his plate is because he believes it will take a broad, all-encompassing vision to put resources to their best possible use in the years ahead. He's thinking big because he has to.

The Center for Vaccine Research is part of a network created by the National Institutes of Health (NIH) in the wake of 9/11 and the anthrax panic that followed. In those terrifying months, it became all too clear that there were a number of deadly pathogens in the world we didn't know much about and few laboratories that were properly equipped to study them.

Further, the federal government realized that this country could no longer afford to be so parochial as to limit research efforts to the pathogens that made themselves at home in our own subways, classrooms, office buildings, and shopping malls—nor could any other nation.

In this era of globalization, pathogens are the best travelers of all. For whatever microscopic saboteurs might float in the sneeze of a farm pig in Mexico, flow in the blood of an African bush-meat hunter's fresh kill, or flare up from the bite of a mosquito in Southeast Asia, each citizen of this borderless new world is just a plane ride away from becoming a potential new host.

10-minute Commute

In 2003, the NIH awarded the Center for Vaccine Research and 12 other institutions construction grants to build specialized labs called Regional Biocontainment Laboratories (RBLs). In summer 2008, Pitt's $33 million RBL became the second of these labs to begin studying live pathogens. The lab investigates more than a dozen highly infectious disease agents, with special focus on tuberculosis, influenza, and dengue fever.

Kelly Cole, associate director of the RBL and associate professor of immunology at Pitt, leads the RBL staff, a group of meticulous, detail-oriented types who are obsessive when it comes to pathogens. Plushy toy likenesses of bacteria and viruses decorate RBL staff member offices. They run contamination scenarios in their sleep.

"I dream of little bugs running after me at night and wake up wondering, *Did I do this today? Did I do that?*" Cole says with a self-deprecating laugh. "If you don't have those nightmares periodically, then you shouldn't be working in a BSL3 anymore."

BSL stands for biosafety level—that is, the level of biocontainment precautions required of a facility to safely study biological agents. The scale runs from BSL1—minimally hazardous bugs like the nonpathogenic strain of *E. coli*, which only call for a pair of gloves and maybe a surgical mask—to BSL4—the pathogens that cause fatal diseases for which we have no effective treatment, like Ebola.

As a BSL3, Pitt's Regional Biocontainment Lab is equipped for the class of airborne pathogens that can cause diseases that are fatal if left untreated (but they *are* treatable). Carefully screened and trained researchers wear facility-dedicated scrubs and shoes, face masks, respirators, double gloves, and disposable Tyvek suits. Showering-out is mandatory. Between the locker room and the lab suites just down the hall, it's a 10-minute commute.

When Cole accepted this job (she'd previously run her own research lab and two other BSL3 labs), she did so on the condition that she'd be able to hire full-time staff members for four areas of responsibility: research, biosafety, operations, and veterinary science.

The unprecedented RBL staff investment has paid off. Pitt runs a tight ship, and other labs have taken notice. Cole and her staff were invited to lead the inaugural national meeting of RBL staffers last May, and recently an NIH representative asked Cole to lead the meeting next year.

"She said, 'You guys are the model—let's be real here.' And I kept hearing that our management structure is the standard that all the RBLs are using. Everyone was calling us [for advice]."

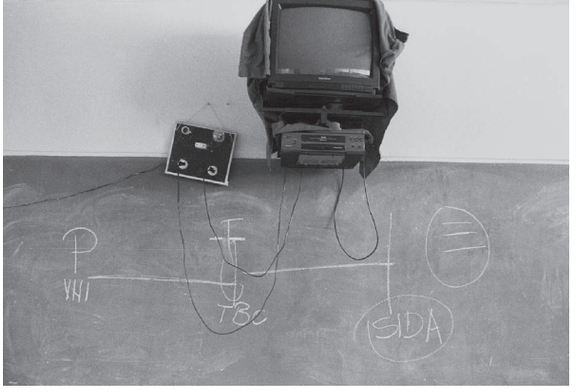
Bug Borne

Ernesto Marques, Pitt associate professor of infectious diseases and microbiology, moved from Johns Hopkins University to the CVR last August. In the past several years, he's conducted extensive field research in his native Brazil and built relationships between the country's government agencies and researchers here in the States. His goal: to get to the bottom of Brazil's recent outbreak of a debilitating mosquito-borne illness, dengue.

Dubbed breakbone fever, dengue causes joint and muscle pain, headaches, intestinal distress, and a bright-red skin rash. Once a disease of Southeast Asia, in recent years dengue has spread to more than 150 countries. Last fall, dengue returned to the United States for the first time in 50 years.

Climate changes and other factors have brought a reemergence of the disease's carriers. In Brazil, mosquitoes were all but eliminated in the 1950s in response to a yellow fever epidemic. But in the mid-'80s, a new government dropped the mosquito eradication program, giving the pesky pathogen-passers free rein over a totally unprotected population. Cases









© GETTY IMAGES

TOP LEFT: **Former mosquito control workers in Brazil protest a slow government response to a recent dengue fever outbreak.** BOTTOM LEFT: **The *Aedes aegypti* mosquito carries dengue.** TOP RIGHT: **Teaching about AIDS in a classroom in Peru.** BOTTOM RIGHT: **Center for Vaccine Research faculty are studying avian flu among other influenzas, including H1N1.**

in Brazil have grown steadily since then, with major upticks in 1998 and 2002.

Marques' first step was to establish a cohort of patients for the study in the city of Recife, the densely populated epicenter of the outbreak, but that was a lot easier said than done. His team couldn't find enough children to represent the range of ages that dengue has been known to affect in other parts of the world. Figuring there must be a lot of undiagnosed pediatric cases, Marques and his colleagues at Hopkins launched an effort to increase pediatricians' awareness of childhood dengue and to train them in using appropriate diagnostic tools.

They got their childhood cohort, all right. And they also got their work published for addressing this pediatric health concern. But their efforts were just beginning.

Next, Marques' team used NIH funding to design a centralized, open-source database that streamlined record-keeping in area hospitals and made data collection much easier for the study. This effort made ink in a few journals, too. It also won the support and cooperation of local doctors, who were grateful to Marques' team for significantly reducing their paperwork.

The cohort of 450 patients in place, Marques' team began mapping immune responses to dengue. The resulting knowledge base has provided a solid foundation for vaccine development.

There are four types of dengue virus. Once you develop the response against one, your immune system wants to respond the same way to the next type it encounters. Consequently, dengue tends to be far more severe the second time you're infected. (Credit Burke's epidemiological research in Southeast Asia for this discovery.)

Marques' data have shown that, as people age, they're more likely to be exposed to a second strain of the virus and fall ill. It follows that the inverse is true for the young—no wonder it was so hard to find children for his cohort.

However, he realized that as each of the four strains continue to spread, exposures won't be so scattershot anymore. Secondary infections are likely to occur in younger and younger people. (Note: Before we went to press, Marques checked back with his colleagues abroad; so far, it appears he was right.)

Husband-and-wife team William Klimstra and Kate Ryman, Pitt associate professors of microbiology and molecular genetics, also specialize in mosquito-borne diseases. They joined the CVR in December 2009.

Ryman was recently awarded NIH funding for her work with chikungunya, a disease very similar to dengue that's pandemic in nations along the shores of the Indian Ocean. Originally, chikungunya was a disease of monkeys, but an adaptation enabled the virus to be carried by another species of mosquito—one that feeds on humans.

The possibility that the same could happen with other viruses worries Klimstra and Ryman. They've been monitoring, for instance, eastern equine encephalitis. Though for now human cases of this horse disease are still rare, they are on the rise. The Centers for Disease Control and Prevention estimates the fatality rate in humans to be 33 percent; others estimate it as high as 70 percent.

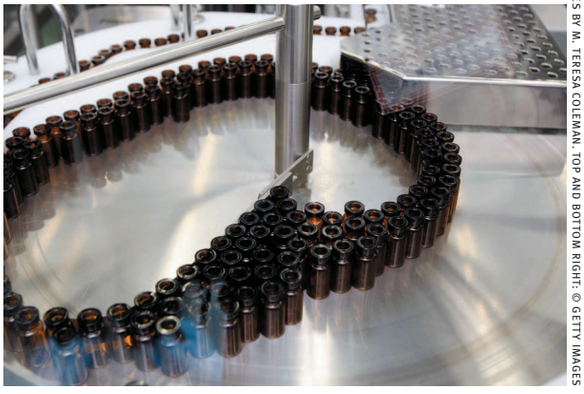
In their studies, Klimstra and Ryman are examining what happens right after a virus enters a host, hoping to identify steps in the infection cycle that might be blocked by antiviral drugs. They're also exploring ways of better targeting antigen-presenting cells—the immune cells that initially bind to the virus and haul it into lymph nodes, enabling the immune system to recognize it and sound the alarm.

Model Behavior

Tuberculosis (TB) infects about one-third of people on the planet. Pitt professor of microbiology and molecular genetics JoAnne Flynn explains that 90 percent of people infected with TB have the latent, or asymptomatic, form of the disease. Although they remain TB-positive for the rest of their lives, they're







LEFT: RECONSTRUCTION OF PET/CT IMAGES BY M. TERESA COLEMAN. TOP AND BOTTOM RIGHT: © GETTY IMAGES

LEFT: Pitt researchers came up with a new way to image TB over time, showing both the structure and metabolic activity of granulomas (see red and yellow spots in lungs). TOP RIGHT: TB has always been difficult to study in the clinic. In 1957, people of Glasgow, Scotland, line up for chest X-rays during an epidemic. BOTTOM RIGHT: Work at Pitt may help produce more-effective TB vaccines.

totally unbothered by the bacteria in their lungs, which their bodies contain in ball-like masses of immune cells called granulomas—the hallmark of TB.

But for the unlucky 10 percent of those infected, at some point their TB will progress to active disease, causing chest pain, bloody coughs, fever, chills, fatigue, and shrinking appetite and weight. Worldwide, nearly two million die of TB each year.

For 30 years, the standard treatment for TB has remained the same: a combination of four antibiotics taken over six months. Particularly in the developing world, the expense of this unusually long course of treatment is a problem. Many people stop treatment before it's complete, which gives rise to drug-resistant TB.

No one knows why this particular drug combination works, why it takes so long, or what exactly happens inside the lungs to cause latent infections to become active. Clinical data are virtually nonexistent because people with latent TB live healthy, normal lives and are not too keen on signing up for trials. Animal data, too, have been sorely lacking. Traditionally, the only way to study granulomas in the lab has been to sacrifice the animal.

"Not only is that a humane issue, but then you're also stuck with one time frame," says Flynn. "You don't really know what happened before or after that."

The thinking has always been that different drugs in the TB treatment regimen must target different subtypes of granulomas—and there are a lot of them. In the same set of infected lungs, you might find granulomas both large and small, that are "hot"—meaning metabolically active—or "cold." And what's more, every one of these types of granulomas is common among both latent and active TB cases.

With diligent work over the past decade, Flynn has developed a model that mimics the huge spectrum of TB found in humans. And with a new imaging tool, she has struck a gold mine of data. In 2008, she was awarded a two-year, $11.4 million grant from the Bill & Melinda Gates Foundation's Drug Accelerator Program to create a unique TB imaging system. (She's just been renewed for a third year.)

Flynn teamed up with Pitt's Jonathan "Eoin" Carney, assistant professor of radiology, and Brian Lopresti, research instructor in radiology and head of preclinical research at the University's PET Facility. Carney and Lopresti devised a way to make two separate scanners work with one another and overlay two types of scanning technologies in the same image. In one layer, a CT (computed tomography) scan paints a clear picture of the structure of a granuloma. In the other, a microPET scan (the small-scale version of a PET scan, or positron emission tomography) shows the metabolic activity in the animal's granulomas.

Now, her team can follow each granuloma over time. They're starting to ask the really nagging questions: Which kinds of granulomas does a given drug target, and how fast? What kinds of vaccines might prevent the bacteria from spreading from person to person or latent TB from turning active? And what causes latent TB to activate, anyway?

Flynn's team is finding what appears to be a huge spectrum of latent disease. Though it's too early to say for sure, she suspects that different types of latency may have different triggers, and latent people with the most metabolically active and widespread granulomas could well be the most prone to reactivation. She's just beginning to sort it all out, though, working with collaborators around the world—among them Clifton Barry, chief of the Tuberculosis Research Section at the NIH, who is conducting a PET study based in South Korea on people with drug-resistant TB.

AIDS is another disease for which the model studied could make all the difference. Consider the work of Cristian Apetrei, Pitt associate professor of microbiology and molecular genetics, and his wife, Ivona Pandrea, Pitt associate professor of pathology. They joined the CVR last summer after having worked in Gabon and at Tulane University.

Macaques infected with simian immunodeficiency viruses (SIV) are often used to simulate HIV infection in preclinical studies. In the wild, SIVs naturally infect more than 40 African monkey species, each carrying a specific SIV. Interestingly, although up to 60 percent of the monkeys in Africa are infected with

SIVs, they generally do not end up with AIDS. When these viruses are transmitted to non-natural hosts, however, the new host may end up with an infection that progresses to AIDS. By comparing nonprogressive to pathogenic and controlled infections, Apetrei and Pandrea hope to understand how some species are able to fend off AIDS and what cues humans might take from the ones who stay healthy.

The First Test

On the day the H1N1 story broke last April, Ted Ross—Pitt associate professor of microbiology and molecular genetics and a CVR faculty member—was in France, at an influenza conference full of vaccine manufacturers and researchers.

"People started fleeing the meeting to fly back home," he says.

Ross was immediately telephoning, Skyping, talking with Burke and others at the CVR. The Centers for Disease Control and Prevention had contacted the CVR and other labs like it, and they wanted to know: Could they ship out samples of this virus for testing the next week? Could these labs help government officials get a sense of what we were up against?

"I told Don and the others that there was bound to be an outbreak of something, be it man-made or natural," says Ross. "I said, 'This is our first test. Better that it's flu than an anthrax outbreak.'"

Ross pulled his entire team off of what they were working on to study swine flu. After six weeks of intensive preclinical studies, they were able to tell the CDC that H1N1 would spread a lot faster and further than the typical seasonal flu; however, this new disease, in its current incarnation, wouldn't be nearly as deadly as other influenza outbreaks—not like avian flu, which has a 75 percent mortality rate; not like Spanish flu, which killed upwards of 50 million people in the pandemic of 1918-19. Knowing this, the government could begin to form a vaccination plan.

H1N1 highlighted problems that Ross and his colleagues have worried about for decades: the cost, time, and uncertainty associated with the current, antiquated vaccine-production process of cultivating live virus in chicken eggs. Ross' team is using novel technologies that might alleviate the problem, including virus-like particles (VLPs)—man-made structures that mimic the outer shells of viruses, minus the genetic innards.

His team is currently working on vaccines for five different pathogens: HIV, influenza, West Nile virus, Rift Valley fever, and dengue virus. He has an NIH grant to study highly pathogenic (i.e. highly infectious) avian flu. He also shares with several other CVR faculty members a Department of Defense grant for a dengue-vaccine candidate, among other grants and contracts.

More than half of Ross' team is devoted to influenza because there's a lot to do: high-path influenza, low-path influenza, avian flu, and now H1N1.

"I think if, back in April [2009], the only places the government had to do this work were the CDC and the NIH, they wouldn't have been able to handle it. They were just dealing with a flood of samples coming in and needed to outsource to the network that had been established. This is exactly what this containment facility was built for." ■

THE SCIENCE OF SNEEZING

As we've all become keenly aware in the wake of H1N1, the most common means of passing an airborne pathogen is in the fine particles of a cough or a sneeze. However, most research has used animal models infected intravenously or via nasal drops, simply because the science of simulated sneezing—dubbed aerobiology—is so difficult to master.

To date, the University of Pittsburgh's biocontainment facility is one of only a few with dedicated aerobiology faculty members. The team is led by assistant professor of immunology Doug Reed.

His bosses like to brag that he's one of only two academic infectious disease aerobiologists in the country with his level of training. For nearly a decade before he joined the University, Reed was a principal investigator at the United States Army Medical Research Institute of Infectious Diseases (USAMRIID), the historical home of aerobiology research.

Appreciation for the discipline has come and gone over the decades, Reed says. The Persian Gulf War of the early '90s saw a resurgence as biological weapons became a very real concern—a concern that has heightened in the past decade.

"When I first got to USAMRIID in '99, we were using 40-year-old technology," says Reed. "It was all manually driven and very prone to operator error."

His close friend and ex-officemate at USAMRIID, a physicist named Justin Hartings, took the human out of the equation and put a computer in her place, making it easier to reproduce all the variables that can make or break an experiment: temperature, air pressure, and humidity, to name a few. Hartings has since founded his own aerobiology-device company—Biaera Technologies, based in Frederick, Md. Reed often beta tests these devices.

On his computer monitor, Reed pulls up photos of the aerobiology equipment. A large, sealed cabinet keeps contaminated air separate from the air in the room. The cabinet is topped by a large metal hood, which directs the air outside via a series of sanitizing HEPA filters.

"There's nothing getting out of that sucker," Reed says. "Viruses can do a lot of things, but they can't defeat physics."

The synthetic sneezer of choice for both the RBL and the Army is known as the three-jet collision nebulizer, a device that vaguely resembles a bicycle-tire pump with a glass base. Air is forced down to the bottom of the tank, into a pool of liquid, and through three jets. The jets shoot the liquid back out, and when the liquid hits the glass, it becomes aerosol.

This device generates particles of just one micron—the ideal size, says Reed. Any larger, and it won't penetrate the deep lung, as more severe disease tends to. Any smaller, and it could float right back out.

Reed is excited to be working at the CVR, a research facility with a grand scope that's not limited by what the military intelligence of the moment says might someday become a tool for bioterrorists.

"Take seasonal flu," Reed says. "The military doesn't consider it a threat. But it kills 36,000 people a year in this country alone. To me it's a more real-world disease." *—EV*