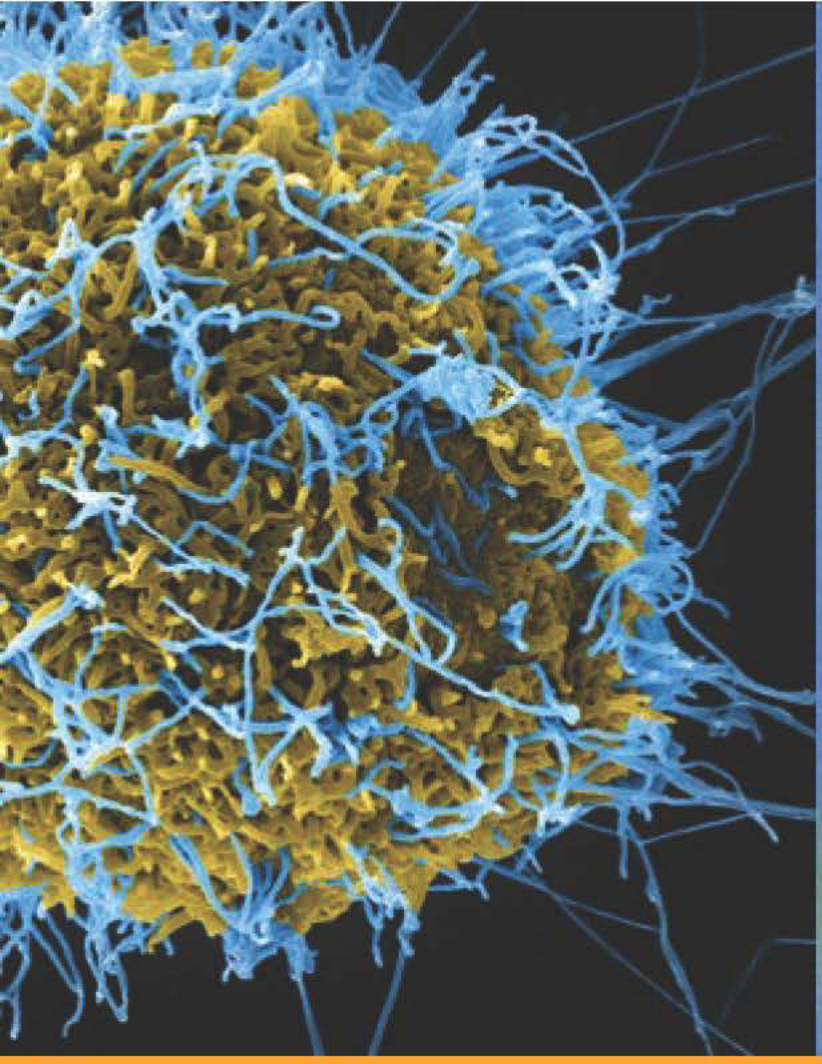
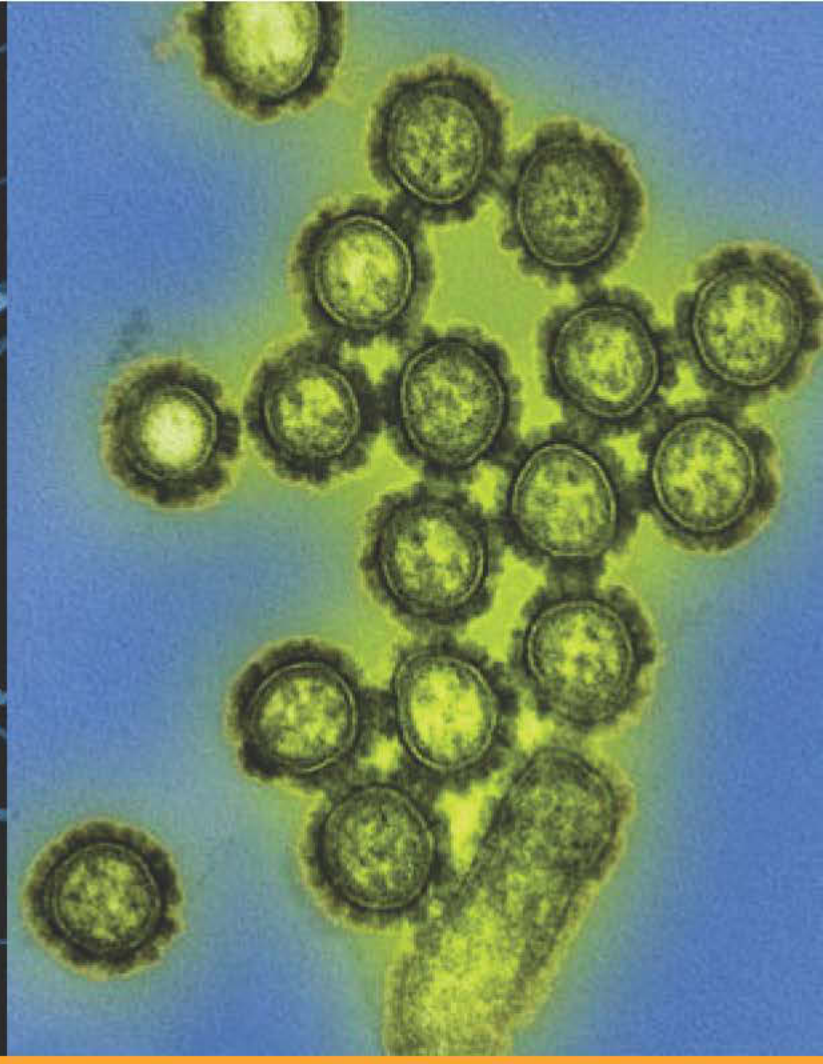
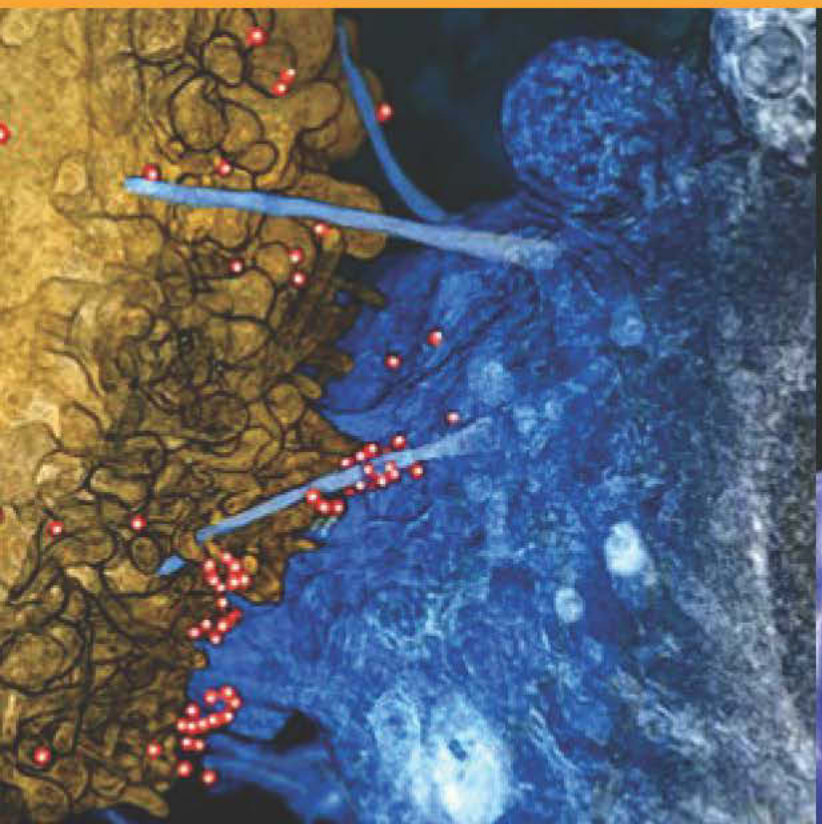




2017 VASEM Summit Report

EMERGING INFECTIONS AND PREPAREDNESS



VASEM
VIRGINIA ACADEMY OF SCIENCE, ENGINEERING, AND MEDICINE
JANUARY 2018

THE VIRGINIA ACADEMY OF SCIENCE, ENGINEERING, AND MEDICINE

The Virginia Academy of Science, Engineering, and Medicine (VASEM) is a nonprofit organization consisting of members of the National Academy of Sciences, National Academy of Engineering, and National Academy of Medicine who reside or work in Virginia. VASEM assists the Commonwealth of Virginia by serving as an intellectual resource to inform agencies and legislators on issues related to science and technology that affect decisions on policy, the economy, and quality of life. The organization also promotes research activities in Virginia and fosters connections between individuals and groups across the Commonwealth.

www.vasem.org

COVER PHOTOS of HIV, MRSA, and H1N1 flu viruses are courtesy of the National Institutes of Health (NIH).

CONTENTS

2017 SUMMIT SCHEDULE—EMERGING INFECTIONS AND PREPAREDNESS

Summit Host: Sen. Mark Warner
Summit Sponsor: Maximus®
Organizing Committee: X.J. Meng (chair), Florence Haseltine, Robert M. Carey, Patricia Dove

SUNDAY, OCTOBER 29

5:30–7:30 pm Summit Reception and Poster Session
Sponsored by MITRE Corporation

MONDAY, OCTOBER 30

7:00 am Registration and Breakfast
Sponsored by MITRE Corporation

7:45 am Welcome Remarks
X.J. Meng, MD, PhD, 2017 Summit Chair
Marcia McNutt, PhD, President of National Academy of Sciences

8:15–8:45 am *Emerging Infections, From Where Do They Come?* (see page 6)
Jennifer McQuiston, DVM, MS, Captain of U.S. Public Health Service; Deputy Director, Division of High Consequence Pathogens and Pathology, National Center for Emerging and Zoonotic Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, GA
Sponsored by the Eastern Virginia Medical School

8:45–9:15 am *Zika: History, Emergence, and Preparedness* (see page 8)
Ann Powers, PhD, Chief of Alphavirus Laboratory, Division of Vector-Borne Diseases, Centers for Disease Control and Prevention, Fort Collins, CO
Sponsored by the Virginia Tech Fralin Life Science Institute

9:15–9:45 am Coffee Break and Networking

9:45–10:15 am *Drug-Resistant Superbugs, a Major Emerging Threat* (see page 10)
Amy J. Mathers, MD, Associate Professor and Medical Director Antimicrobial Stewardship, University of Virginia School of Medicine
Sponsored by the UVA School of Medicine

10:15–10:45 am *Bioterrorism and Preparedness* (see page 12)
Rick A. Bright, PhD, Director of Biomedical Advanced Research and Development Authority (BARDA)
Sponsored by the UVA School of Medicine: Division of Infectious Diseases and International Health, Department of Pediatrics, Global Infectious Diseases Institute, and the Center for Global Health

10:45–11:15 am *Environmental Engineering to Forecast Transmission of Pathogens* (see page 14)
Linsey Marr, PhD, Professor, Virginia Tech Department of Civil and Environmental Engineering
Sponsored by Virginia Tech's Virginia-Maryland College of Veterinary Medicine, and College of Engineering

11:15–11:45 pm *Measles: Re-Emergence Of An Old Threat* (see page 16)
Diane E. Griffin, MD, PhD, University Distinguished Service Professor, Johns Hopkins University Bloomberg School of Public Health
Sponsored by MITRE Corporation

11:45–Noon Remarks *Virginia's Response to Emerging and Reemerging Infectious Disease* (see page 18)
William A. Hazel, Jr., MD, Secretary of Health and Human Resources, Commonwealth of Virginia

Noon–1:00 pm Lunch and Keynote Address
Emerging Infectious Diseases: Learning from the Past and Preparing for the Future (see page 20)
Anthony S. Fauci, MD, Director, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD
Sponsored by MITRE Corporation

1:00–1:20 pm Coffee Break and Networking

1:20–1:30 pm Remarks
Patricia M. Dove, PhD, President, Virginia Academy of Science, Engineering, and Medicine

1:30–2:00 pm *The West African Ebola Epidemics and Emergence of Other Filoviruses* (see page 22)
Jonathan S. Towner, PhD, Head of Virus Host Ecology Section, Centers for Disease Control and Prevention, Atlanta, GA
Sponsored by the Virginia Tech Biocomplexity Institute

2:00–2:30 pm *Engineering Pathogen-Resistant Mosquitoes* (see page 24)
Zach Adelman, PhD, Associate Professor, Department of Entomology, Texas A&M University
Sponsored by the Virginia Tech Carilion Research Institute

2:30–3:00 pm *HIV/AIDS: Will There Ever Be a Vaccine?* (see page 26)
John R. Mascola, MD, Director, Vaccine Research Center, National Institutes of Health, Bethesda, MD
Sponsored by the UVA School of Engineering

3:00–3:30 pm *Novel Biosensor Technologies to Detect Emerging and Bioterrorism Pathogens* (see page 28)
Andrew Flannery, PhD, Director of New Products, PathSensors Inc.
Sponsored by the UVA School of Engineering

3:30 pm Summit Ends

MESSAGE FROM SENATOR MARK WARNER

Dear Friends,

More than five years ago, I brought together the Commonwealth's universities and the Virginia-based members of the National Academy of Sciences, National Academy of Engineering, and National Academy of Medicine to engage in cross-disciplinary discussions on the issues of the day and to provide advice to policymakers in the state capital.

This year's summit on Emerging Infections and Preparedness and its published report highlight the research on an issue critical to both the Commonwealth and the nation. Dr. X.J. Meng is a world-renowned virologist whose breakthrough research has changed the way the world reacts to emerging infections and diseases. He and the programming committee ensured that the summit highlighted the research done here in Virginia as well as efforts being undertaken by officials across the nation and around the world.

Our challenge moving forward is to use our established researchers to help usher forward the next generation of thought leaders in these fields. Policymakers at all levels of government need to appreciate the wide range of threats detailed by emerging researchers and the resources available in Virginia's institutions to address those threats. This summit provided a wonderful venue to begin this conversation.

Thank you again for joining us and for your involvement with VASEM.

Sincerely,

Mark R. Warner
United States Senator

MESSAGE FROM MARCIA MCNUTT

Dear Friends,

It was a pleasure to welcome the Virginia Academy of Science, Engineering, and Medicine (VASEM) to the National Academy of Sciences Building, a most fitting place to have held your 2017 summit on emerging infections and preparedness. It was wonderful to see so many brilliant scholars who are working on this important problem.

Emerging infections and preparedness is an issue that we at the National Academies take very seriously. In the last two years, we have published the proceedings of our 2016 workshop on the Zika virus, produced two consensus studies on Ebola, a report on global health with a section on emerging disease threats, and a series of studies on methods to protect the healthcare workforce in the face of pandemics. The National Academies are committed to disseminating the best information on these topics.

I would also like to say how thrilled I am by the growing movement to establish state academies, a movement that VASEM embodies. Personally, VASEM is an organization that has special meaning as my own residence is a stone's throw across the Potomac in Virginia. We have seen states step up on topics of international importance, including infectious diseases as VASEM has done, energy policy, climate change, and education. You are demonstrating that the states can fill a leadership role in using science to address the most pressing issues of our time.

Best,

Marcia McNutt, PhD
President, National Academy of Sciences

JENNIFER MCQUISTON

EMERGING INFECTIONS: FROM WHERE DO THEY COME?

As Jennifer McQuiston noted at the beginning of her presentation, the question of where infectious diseases come from is related to another question: where they are taking us? This last question, McQuiston observed, is one she has spent most of her career addressing. McQuiston is deputy director of the Division of High Consequence Pathogens and Pathology at the Centers for Disease Control and Prevention (CDC).

McQuiston is also a veterinarian by training—and reminded the audience that when thinking of infectious diseases, it is critical not to separate human beings from domestic animals, livestock, and wildlife. "We have similar immunity, similar susceptibility with our mammalian and reptilian cousins," she explained. "What makes us unique is that we make choices in our daily lives that affect the lives of all these other populations of animals."

AN OUTBREAK OF MONKEYPOX

Emerging and reemerging infectious disease is a global problem caused in large measure by these choices. To illustrate this point, McQuiston reviewed an outbreak of monkeypox in the United States. In 2003, health officials received more than 70 reports about a smallpox-like illness. This raised alarms at the CDC, which was concerned about smallpox being used as a bio-weapon. Consequently, she noted, it was a relief when it was discovered that the disease was monkeypox, which is not as lethal nor as transmissible person-to-person as its relative, smallpox.

The cases were investigated by CDC's new Emergency Operations Center. The origins of the outbreak were ultimately traced to a large shipment of rodents from Ghana, including Gambian pouched rats, dormice, and squirrels. These rodents mixed with North American prairie dogs at a pet distribution facility. The prairie dog turned out to be the perfect vector of monkeypox. The CDC worked with states to prevent humans from being exposed to infected prairie dogs and enacted bans on importing African rodents, but despite large-scale efforts over many years, no one is sure which wild African rodent first infected the others or how this persistent virus maintains its hold in nature.

The monkeypox outbreak, McQuiston said, highlights a number of challenges in dealing with emerging and reemerging infectious diseases. One is vulnerability. The smallpox vaccine protects against monkeypox, but because vaccination was discontinued when smallpox was wiped out, large populations of people are now vulnerable to orthopoxviruses like monkeypox. Currently, monkeypox appears to occur sporadically in people who have animal contact, and there have only been a few generations of person-to-person spread. "In the event that it mutates and becomes more transmissible and more lethal," she said, "we may face an epidemic." As a result, the CDC and partner agencies continue to monitor the incidence of the disease in Africa and search for its wildlife reservoir.

AN INCREASE IN THE FREQUENCY AND VARIETY OF ANIMAL-TO-ANIMAL INTERACTIONS

McQuiston noted that zoonotic diseases like monkeypox account for approximately 62 percent of emerging infectious diseases. They span the range of infectious pathogens—bacteria, rickettsia, viruses, prions, and protozoa—and they have been growing in frequency at an alarming rate. As McQuiston explained, an important reason is pathogen change. "Every living organism has a characteristic rate of mutation," she said. "Small mutations can sometimes lead to increased virulence or can enable pathogens to adapt to new species or cell types."

The consequences of these changes are magnified by animal-to-animal interactions. These include the trapping and handling of bush meat, industrial-scale agricultural practices that concentrate thousands of animals in a single facility, and vastly more efficient transportation networks that convey infected people and organisms to new locations. These are augmented by such natural phenomena as migratory bird flyways, which have been implicated in the spread of tick-borne diseases.

In the case of wildlife- and vector-borne pathogens, McQuiston said, it is difficult to track these diseases before they become a public health emergency. They often emerge along the equatorial belt, an area of rich biodiversity for both animals and pathogens. Unfortunately, these are also areas where there are few surveillance systems. In response, the CDC has adopted a One Health approach, working with physicians, veterinarians, ecologists, and many others to monitor and control public health threats and to learn how diseases spread among people, animals, and the environment.

CULTURAL CHANGES CAN PROMOTE EMERGENCE

McQuiston underscored the necessity for a comprehensive approach to understanding how infectious organisms

CENTERS FOR DISEASE CONTROL AND PREVENTION

spread—and the difficulties of acting on this knowledge once it is understood. She cited the case of a Rocky Mountain Spotted Fever (RMSF) outbreak among Native American communities in Arizona, where the hot, dry climate is not typically tolerated by wood ticks that carry the disease in the West. In the Southwest, the brown dog tick had become an unexpected and emerging vector. Contributing to the spread of RMSF in this area, CDC researchers found generational changes in Native American attitudes toward dogs. "Tribal elders reported that dogs used to be treated as outdoor animals," she said. "Now younger people are embracing the concept of dogs as pets and bringing them inside their homes. When those dogs carry ticks, the consequences can be catastrophic."

Working closely with the tribal government, the CDC helped mount an intense intervention at the San Carlos Reservation, reducing the percentage of dogs with ticks to just 1 percent, but the tick numbers tended to creep up—as did human fatalities from RMSF—when tick prevention methods lapsed. The socio-economic challenges within the community make it difficult to create sustained control solutions. The result: an emerging disease becomes entrenched.

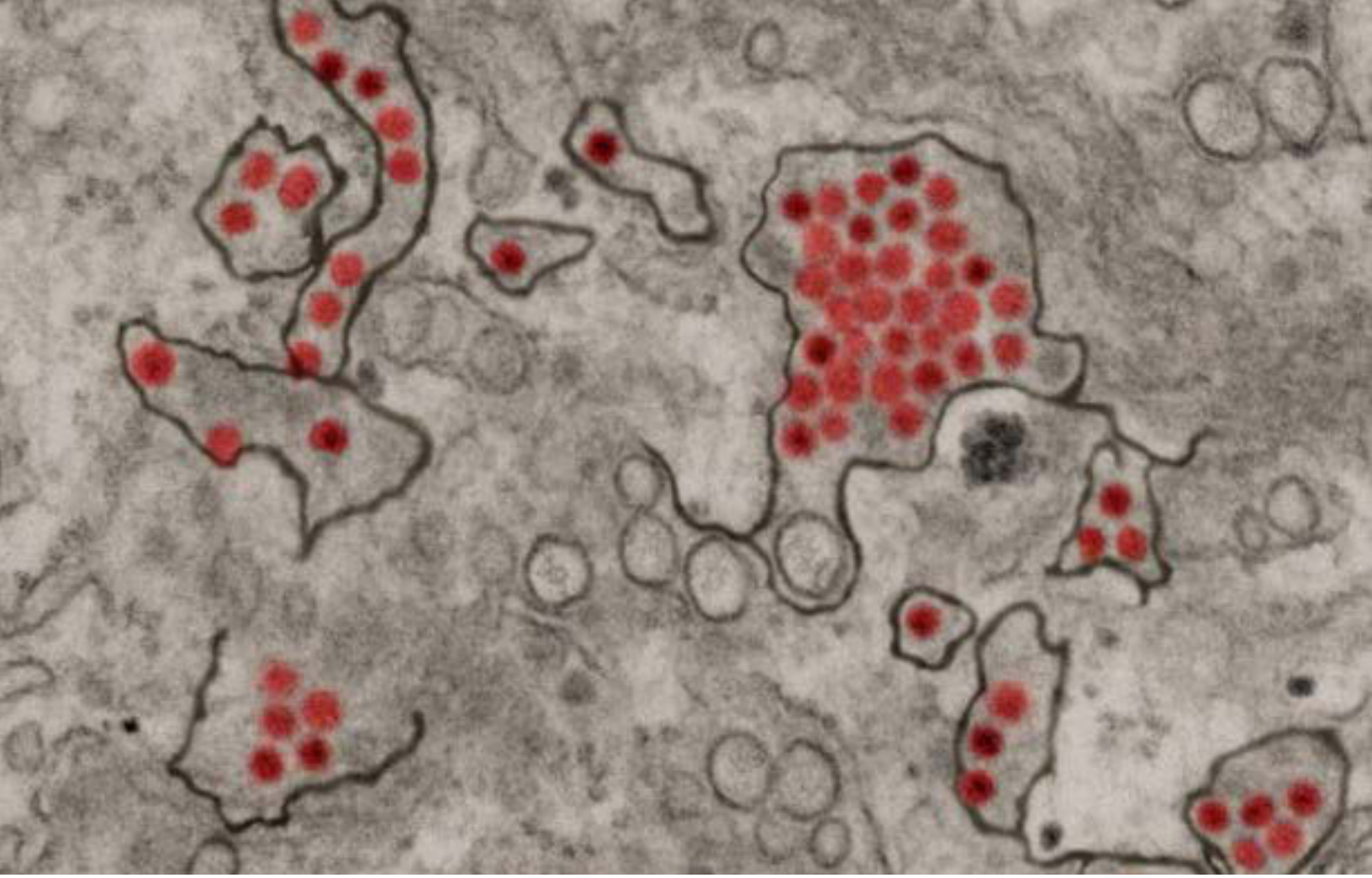
Social attitudes, McQuiston said, also contributed to the intensity of the West Africa Ebola epidemic. It became entrenched and spread quickly, thanks to years of civil conflict that led to distrust of government and lack of reporting. The unrest also weakened the public health system, so that it was more easily overwhelmed by the epidemic. These weaknesses were compounded by burial practices that inadvertently contributed to its spread.

The harmful intersection of social attitudes and infectious disease is not confined to less developed areas of the world, McQuiston pointed out. People who drink raw milk thinking it a healthy choice are placing themselves at risk of developing brucellosis, an inadvertent consequence of the live virus vaccine for *Brucella* used to prevent infertility and abortions in cattle.

McQuiston noted that these are just some of the many zoonotic infections that are emerging and reemerging. At the time of the talk, the CDC was dealing with an outbreak of anthrax in hippos in Namibia, Marburg in Uganda, leptospirosis in Puerto Rico following the hurricanes there, and pneumonic plague in Madagascar, among other outbreaks.

To underline her theme, McQuiston ended with a quote from Rick Riordan, the author of the Percy Jackson & the Olympian series of novels for teen readers: "Strange things conspire when one tries to cheat fate." Increasingly, she said, human beings are trying to cheat fate by changing things without considering the consequences. Emerging infections are one of those consequences. ■

Captain Jennifer McQuiston, DVM, MS, serves as the deputy director of the Division of High Consequence Pathogens and Pathology Science in the Centers for Disease Control and Prevention.



ANN POWERS

ZIKA: HISTORY, EMERGENCE, AND PREPAREDNESS

For the average person, emerging infectious diseases can seem to come out of nowhere. Ann Powers, chief of the Alphavirus Laboratory in the Centers for Disease Control's Division of Vector-Borne Diseases, began her presentation by observing that few people in the room had probably heard of the Zika virus a year ago. One of the goals of her presentation was to describe how Zika suddenly became part of our everyday conversation.

Powers noted that the Zika virus is the latest in a series of arboviruses (viruses transmitted by arthropod vectors) to cause epidemics in the Americas. Yellow fever and dengue fever first appeared in the 17th century, but in recent years there have been successive waves of outbreaks—West Nile virus in 1999, chikungunya virus in 2013, and Zika in 2015. These diseases are spread by mosquitos, principally *Aedes aegypti*. Powers noted that tick-borne viruses are also on the rise. These include Heartland virus and Bourbon virus. Together, these outbreaks represent an accelerating emergence of vector-borne diseases that have become or will likely become endemic in the Americas, at a time when there is decreasing national, state, and local capacity to respond.

Zika created global alarm because it is the first time since the rubella virus more than 50 years ago that an infectious pathogen has caused birth defects—and the first time such a disease was spread by a mosquito. Compounding this alarm was the fact that, until a dozen years ago, Zika was practically unknown. "We were in uncharted territory when the Zika outbreak began in the Americas," Powers said.

A GRADUALLY EMERGING THREAT

Powers recounted the history of Zika up to that point. The Zika virus was first isolated in 1947 from a rhesus monkey by scientists at the Rockefeller Foundation-funded Yellow Fever Research Institute (now the Uganda Virus Research Institute) at its Zika Forest research site. A year later, it was found in mosquitos trapped in the forest. Surveys at the time

ZIKA VIRUS: NATIONAL INSTITUTES OF HEALTH

found that 6.1 percent of Ugandans had antibodies to Zika, but it did not appear to cause disease in humans. In 1950, researchers discovered virus antibodies in Nigeria, but there was no evidence that the virus caused disease there until 1968, when it was isolated from three sick children. There were a handful of cases in ensuing decades, both from Africa and Southeast Asia, but in every case the disease was mild.

It was not until 2007 that health officials on Yap, a member of the Federated States of Micronesia, reported an outbreak of illness characterized by a rash, conjunctivitis, and arthralgia. Testing by the CDC determined that the disease was caused by Zika virus. Once again, the symptoms were mild, but the number of cases—1,000 out of a total population of 7,000 over a four-month period—and an infection rate of 73 percent marked a significant change. A typhoon striking the island and killing the mosquitos helped to end the epidemic.

Zika returned to the South Pacific, however, in late 2013. In a matter of months, there were 19,000 cases. This outbreak was accompanied by reports of serious disease, including patients who presented with Guillain–Barre syndrome. But even this dramatic expansion of cases did not prepare health officials when, two years later, Zika arrived in Brazil. As of February 2017, there were an estimated 1.5 million cases, including incidences of microcephaly and other severe fetal birth defects in addition to Guillain–Barre. There were also cases of Zika being spread through sexual transmission.

MOBILIZING THE INTERNATIONAL COMMUNITY

In the face of this outbreak, there was what Powers characterizes as "a very rapid and extensive public health response," starting in May 2015 when the Pan American Health Organization (PAHO) issued an alert on the first confirmed Zika virus infections in Brazil. In January 2016, the CDC activated its Emergency Operations Center. Eleven divisions of the CDC eventually joined the response to this outbreak.

> "In the United States over the last two years, there were 5,000 travel-related cases as well as some local transmission in Florida and Texas."

A month later, the World Health Organization (WHO) declared a public health emergency of international concern (PHEIC), and two months later the CDC determined that there was enough data to state definitively that Zika was causing microcephaly. In September 2016, President Obama signed a continuing resolution that provided $1.1 billion in emergency funding for the Zika response. By November, WHO declared the end of the PHEIC, although it asserted that Zika virus and its associated consequences remained a significant enduring public health challenge requiring concerted action.

Even with this extensive, broad-based response, Powers said, there are 61 countries and territories worldwide, including 50 in the Americas, now reporting active Zika virus transmission. "In the United States over the last two years, there were 5,000 travel-related cases as well as some local transmission in Florida and Texas," Powers noted. "There was a huge spike in U.S. Zika cases in summer 2016, after which it dropped off precipitously but still occurs."

BUILDING A RESERVE OF RELEVANT INFORMATION

Although there are insufficient resources to study all 500 arboviruses individually, the world was not totally unprepared for Zika. In 2000, an assay was developed to detect the presence of *flavivirus*, a virus genus that includes arboviruses like Zika as well as dengue and West Nile. Unfortunately, it did not distinguish one *flavivirus* from another. This was followed by the publication of the first full-length genome sequence of Zika, which allowed scientists to develop a molecular detection test that was used in the Yap outbreak. The Yap outbreak itself gave scientists an opportunity to develop tools necessary to respond to the successive Zika virus outbreaks, including compiling a clinical description of the disease that included its high rate of asymptomatic patients.

Experience with the chikungunya virus also provided preparation. Chikungunya has a similar clinical syndrome, it is transmitted by the same mosquitos, and it is capable of explosive transmission through a naïve population.

Even with this degree of preparedness, Powers said, Zika presented a number of significant challenges when it arrived in the United States. They included developing novel diagnostic methods and approaches to distinguish closely related *flaviviruses*; creating animal models to study pathogenesis of birth defects and implementing a birth-defects registry; finding ways to assess the risk of sexual transmission; and devising new ways of suppressing the vectors that cause transmission.

Powers concluded by noting that the response to Zika has generated information that will help health officials be better prepared to address the next outbreak quickly. This will be essential, she implied, noting that with modern air transportation, an emerging pathogen can travel anywhere in the world in just four days. ■

Ann Powers, PhD, is a research microbiologist and chief of the Alphavirus Laboratory in the Centers for Disease Control's Division of Vector-Borne Diseases. She has authored over 140 scientific publications on alphaviruses and other mosquito-borne viruses.

AMY J. MATHERS

DRUG RESISTANT SUPERBUGS: A MAJOR EMERGING THREAT

In her presentation, Amy Mathers provided new perspectives on the genetic transmission of drug resistance in clinical settings and described how these insights can inform the way health systems can minimize the emergence of drug-resistant bacteria. Mathers, an associate professor of medicine and pathology at the University of Virginia (UVA), began by highlighting the magnitude of the danger that drug resistance represents. She cited statistics from the Wellcome Trust estimating that 700,000 deaths can be attributed to antibiotic drug resistance annually. By 2050, drug resistance could outstrip cancer as a cause of mortality.

Among the most severe of these threats are carbapenem-resistant Enterobacteriaceae (CRE). "This is not just a species of bacteria, but an entire family," she said. "To make matters worse, Enterobacteriacea are ubiquitous in the environment, both in human and animal populations." Enterobacteriacea include such pathogens as Salmonella, Escherichia coli, Yersinia pestis, Klebsiella, and Shigella and have genes of drug resistance that can easily be exchanged.

CRE is so worrisome, Mathers stressed, because carbapenem antibiotics are considered a last line agent for effective treatment of infections with Enterobacteriaceae. Fifteen years ago, CRE had been reported in just a handful of states. In 2017, it was present in all but two. Mortality in cases of serious infections with carbapenem-resistant Enterobacteriaceae is almost double that of infections with carbapenem-susceptible Enterobacteriaceae. Although new agents are coming to market, it is very difficult to determine which patients carry a carbapenem-resistant organism and could benefit from a newer agent..

A SET OF RUSSIAN NESTING DOLLS

Mathers has focused her research on a gene of drug resistance responsible for the majority of CRE in the United States, *Klebsiella pneumoniae carbapenemase* (KPC). It is approximately 1,000 base pairs long and encodes for a protein that can hydrolyze a number of critical antibiotics: penicillins, extended-spectrum cephalosporins, aztreonam, and carbapenems. This gene is housed in a 10-kilobase transposon, which is freely mobile, can self-replicate, and move to different spots in a genome.

In 2007, the UVA Health System reported its first case of CRE to the CDC, the first in Virginia. A 45-year old liver-transplant patient transferred from a Philadelphia hospital had *K. pneumoniae* and *K. oxytoca* infections that showed evidence of carbapenem resistance. An infectious diseases fellow at UVA at the time, Mathers was interested in determining how the *KPC* gene, housed in the transposon, moved on plasmids between the two species of bacteria. Since 2009, when UVA began perirectal surveillance, the *KPC* gene has been found in 21 different bacterial species. "We have one of the most robust surveillance programs in the country," Mathers said. "This enabled us to see the extent of gene movement."

Mathers emphasized that what they were seeing was very different from outbreaks of gene resistance caused by clonal expansion. "In our case, the transposon seemed to be jumping into different plasmids and then moving from species to species." Mathers received confirmation of this theory when she spent a sabbatical at the University of Oxford, conducting whole genome sequencing of the UVA sample collection.

Mathers and her colleagues likened the process they observed to a set of Russian nesting dolls. The *KPC* gene nested in a transposon, which nested in another transposon, which nested in a plasmid housed within different bacteria. The transposons can move into different plasmids within the cell, increasing variation. They can move by conjugation between different bacterial strains and species.

TRANSMISSION THROUGH WASTEWATER

"We wanted to see if we could take this information, use it to understand how transmission was occurring, and better protect our patients," Mathers said. In a typical hospital outbreak, patients are the reservoir, and pathogens are transmitted from one patient to another by healthcare workers failing to follow good hygiene practice. UVA discovered that 70 percent of its transmissions could not be explained by these patient-to-patient interactions.

Mathers's group began looking at wastewater plumbing, focusing on the line between the sink drain and the P-trap water (standing water in a U-bend that prevents sewer off-gassing). There had been reports of outbreaks originating in P-traps, but Mathers hypothesized that the drain area might be the source of outbreaks. There is an abundance of water and nutrients, large amounts of antibiotics that could promote resistant species, and a build-up of biofilm on surfaces that is difficult to remove. The increasing number of reports of sink- or drain-related outbreaks—21 in calendar year 2017—reinforced this view.

When Mathers and her colleagues began looking at their own ICUs, the majority had *KPC*-producing organisms in their drains. The team developed two lines of attack: it avoided using the sink countertop for patient care items and attempted to eliminate or reduce *KPC*-producing bacteria in P-traps. It did so by removing drains, P-traps, and overflow and applied bleach, hydrogen peroxide, or ozone to keep it from coming back.


ALARMY

"We wanted to see if we could take this information, use it to understand how transmission was occurring, and better protect our patients."

Within 10 days, however, a third of the sinks were once again positive for *KPC*-producing organisms despite the interventions and removal of drains. To make matters worse, the new *KPC*-producing *Serratia marcescens* bacteria were highly clonal and resistant to collstin, one the last effective line agents. "Essentially," Mathers said, "we had inadvertently created a high-risk clone through our own interventions."

Mathers then took a step back, and UVA created a set of sink labs to understand the behavior of multiple drug resistant organisms in traps and drains, conferring with both the CDC and Public Health England. With the help of Shireen Kotay, an environmental microbiologist with experience in civil engineering, her group found that bacteria live in biofilms lining the pipe from the P-trap to the drain as well as in the P-trap itself and that they splashed out of the sink when water was run. They can also pass through plumbing connections to neighboring sinks.

Mathers and her group also looked for other reservoirs and identified hoppers, a toilet-like device used in ICUs for solid waste, as another source. When it sampled its hoppers, 75 percent had *KPC*-producing bacteria. Producing this result required Mathers and her colleagues to create a comprehensive data warehouse that combined patient and hospital data with genomic data from next-generation sequencing. Their ultimate response was to put lids on the hoppers and purchase sink trap heaters that vibrate and prevent biofilm formation.

After a decade of nonstop *KPC* acquisition, Mathers said, UVA was able to cut its patient infection rate in half, from 33 during the preintervention period to 13 afterwards. This took an extended effort and funds. "This work highlights just how complicated trapping potential drug resistance is going to be," Mathers said. "At the very least, it will make us think differently about plumbing design." ■

Amy Mathers, MD, ABMM, is an associate professor of medicine and pathology at the University of Virginia. She is the clinical director of the Adult Antimicrobial Stewardship Program and associate director of clinical microbiology.

RICK A. BRIGHT

BIOTERRORISM AND PREPAREDNESS

Rick Bright began his presentation by outlining the chemical, biological, radiological, and nuclear (CBRN) threats the U.S. faces and the mission of the Biomedical Advanced Research and Development Authority (BARDA), the government agency he directs. Bright made the point that these threats have both a natural and a human origin. Infectious diseases, for instance, can emerge naturally or be used as bioterrorism weapons. BARDA was created over 10 years ago to help address threats from both these sources.

"We understand that to protect America and our way of life, it is imperative that we have medical countermeasures available to detect and protect against diseases and threats around the globe," Bright said. BARDA pursues this mission by providing an integrated, systematic approach to the development, production, and purchase of vaccines, drugs, diagnostics, personal protective equipment, and anything that might protect people or treat them when they have been exposed to one of these threats. It does this by forming innovative public-private partnerships with biotechnology and pharmaceutical industry partners to accelerate the development of these medical countermeasures.

"Commitment from the U.S. government is essential because, outside of emergencies, there is no market for these countermeasures, no incentive for development, and no entity willing to purchase these countermeasures after they are created," he said.

BARDA also encourages the development of manufacturing capacity, specifically in the United States, and will also consider support to entities outside the United States so that there are companies ready to make the medical countermeasures when they are needed. Recognizing that partners in the private sector might lack specific technologies needed to create or manufacture these measures, BARDA offers a core set of services and competencies, including animal studies, clinical studies, regulatory and quality affairs, quantitative modeling, drug formulation, and fill-finishing, to advance these efforts.

ADVANCED RESEARCH & DEVELOPMENT/PROJECT BIOSHIELD

BARDA's Advanced Research & Development program focuses on countermeasures that have successfully completed Phase 1 trials, but are stalled in "the valley of death," where promising technologies languish for lack of funding and resources. BARDA has achieved a number of significant successes. With the collaboration of the NIH, CDC, and DOD on the early stages of some of the new products, BARDA has supported 34 medical countermeasure approvals,

licensures, or clearances through FDA in the last 10 years.

Once approved by the FDA, the medical countermeasures may be added to the Strategic National Stockpile. However, funding to procure these countermeasures and to ensure there are manufacturers available to continue producing them has not kept pace with the need. Through BARDA's Project BioShield program, the agency is working to make sure the United States has the capacity to respond to threats when they occur.

Overall, Bright noted, BARDA has invested over $1 billion with its industry, academic, and government partners in each of the last three fiscal years to be able to support its Advanced Research & Development and Project BioShield Programs.

CBRN THREATS

Bright then talked specifically about the agency's effort to promote CBRN preparedness. BARDA focuses on pathogens and agents determined by the Department of Homeland Security to pose a material threat to our national security, economic security, and freedom. These include threats that range from nerve agents to anthrax to radiation. BARDA's goal is to have at least one stockpiled countermeasure by 2023 for 80 percent of material CBRN threats and to have made significant advances in all threat areas to the point where the law of diminishing returns applies. BARDA also plans to address operational gaps in its medical countermeasure response, such as the ability to lower costs, treat special populations, and improve the response timeline.

In terms of its Advanced Research & Development priorities, this means concentrating on addressing chemical and viral hemorrhagic fever threats, repurposing existing products to treat injuries from radiological and nuclear threats, sustaining investment in new antibacterial agents and vaccines, and continuing to reevaluate its activities to achieve more effective, sustainable solutions.

Bright also reported success in Project BioShield. This includes 27 products supported, 14 added to the Strategic National Stockpile, and six that have achieved FDA approval. These products include new vaccines and treatments for smallpox, anthrax, botulism, and chemical and radiological/nuclear threats.

Bright noted that BARDA's focus on new antibiotics and drug-resistant bacteria reflects the realization that exposure to most CBRN threats, over the long term, can lead to opportunistic and persistent infection. To address this issue, BARDA and a group of academic, nonprofit, and government institutions last year launched CARB-X, the Combating Antibiotic Resistant Bacteria Biopharmaceutical Accelerator. This is now the world's largest public-private partnership specifically focused on identifying promising new antibiotics or other approaches to reduce the threat of drug-resistant bacteria.

CDC, STRATEGIC NATIONAL STOCKPILE STAFF

Stockpiled supplies enabled Federal Medical Stations to be set up in five hours during Hurricane Harvey.

THE THREAT OF PANDEMIC INFLUENZA

In addition to CBRN threats, BARDA is deeply involved in preparing for and responding to pandemic influenza, the cause of 500,000 deaths worldwide each year. Over the last 10 years, BARDA has invested billions of dollars to collaborate with industry and federal partners to identify new ways of producing influenza vaccines, diversifying vaccine substrates, shortening the time to development, and boosting our capacity to produce up to 600 million doses of influenza vaccine within six months from emergence. All this work, Bright said, lays a foundation for mastering the ultimate challenge, producing a universal influenza vaccine.

BARDA is also working with its partners to develop faster, more easily administered diagnostic tests so those with influenza can be informed and treated quickly and the path of the pandemic can be identified more readily. It is also investing heavily in new therapeutics. Bright reported that breakthroughs recently have opened the door to new classes of antiviral drugs for influenza, drugs that are now in Phase 2 and Phase 3 trials. Finally, it is also investing in reusable respiratory devices that can be used to reduce influenza transmission.

THE THREAT OF EMERGING INFECTIOUS DISEASES

The last area of BARDA focus is emerging infectious diseases. "We have to identify new systems and platforms for diagnostics, vaccines, and therapeutics that are flexible enough to respond more quickly to emerging outbreaks," Bright said. Through its National Medical Countermeasure Response Infrastructure, BARDA is working to shift from a pathogen-based emergency preparedness posture to a capability-based emergency response posture. This effort has entailed leveraging established FDA-approved production processes and the launch of three Centers for Innovation and Advanced Development in Manufacturing to develop an immediate response using existing technologies in the interim before vaccines are scaled up.

Bright concluded his presentation by emphasizing the overarching challenges that BARDA faces. These include sustaining the enterprise to ensure that a vibrant pipeline exists upstream and a firm marketplace commitment exists downstream; maintaining an industry base by providing the best incentives to bring/keep partners to the space; improving coordination across the U.S. government to leverage expertise and resources to be more effective; and operationalizing a rapid response coordinated at all levels to effectively utilize medical countermeasures and production capabilities to respond to any threat. ■

Rick A. Bright, PhD, is the deputy assistant secretary for preparedness and response and the director of the Biomedical Advanced Research and Development Authority in the U.S. Department of Health and Human Services.

CDC, JAMES GATHANY

LINSEY MARR

ENVIRONMENTAL ENGINEERING TO FORECAST TRANSMISSION OF PATHOGENS

Not all researchers studying infectious diseases are scientists or clinicians, a point Linsey Marr made when introducing her presentation. "While many researchers study the interaction between a pathogen and a disease, our group focuses on the relationship between a pathogen and the environment" she said, "This is something environmental engineers are well qualified to do."

A professor in Virginia Tech's Department of Civil and Environmental Engineering, Marr applies engineering tools—the same they might use to study pollution—to investigate the dynamics of pathogens in the atmosphere. This is particularly relevant because, as Marr noted, two of the four generally defined modes of disease transmission between people—large droplet transmission and aerosol transmission—occur through the atmosphere. When people cough or sneeze, they emit large droplets containing pathogens, which can land on the mucus membrane of another individual. They can also produce smaller droplets—aerosols—that can stay suspended in air for long periods of time. When others inhale them, they can become ill.

The engineering approach that Marr uses to study transmission via droplet or aerosol is the same one she uses for traditional pollutants such as carbon dioxide, nitrogen oxide, and particulates. She tracks the transport of the pollutant from its source, whether it is a power plant or a person, into the atmosphere, where it can undergo physical and chemical transformation. "We know, for instance, that ultraviolet radiation can degrade certain pathogens," she said. She then tracks its transport and ultimate deposition, whether in the environment or in a person.

In real estate, Marr noted, location is everything. With airborne particles, size is the characteristic that matters most. In the case of airborne pathogens, size differences are caused by the amount of respiratory fluid that surrounds the pathogen. An influenza virus is 0.1 micron in diameter, but it is emitted in particles with diameters of between 0.2 and 100 microns. Size is important because it determines the pathogen's lifetime in the atmosphere. Anything much larger than 25 or 50 microns, Marr said, will settle in a minute. Smaller droplets can remain suspended for hours if not days. Size is also important because it determines where in the respiratory system a pathogen will be deposited if it is inhaled.

IS AIRBORNE FLU VIRUS A HAZARD?

Marr illustrated the value of engineering methods to study the airborne transmission of pathogens by applying it to four separate questions. The first: Is flu virus present in small enough droplets to remain airborne?

In response, Marr and her colleagues went to a series of sites—a healthcare center, a daycare center, and an airplane interior—with a tool that is able to separate particles by their size. "We found virus in all particle sizes from less than 0.25 microns in diameter to greater than 2.5 microns," she said. "Sixty-four percent of the total virus that we measured was associated with aerosols, those particles smaller than 2.5 microns," she said. "This means that influenza can remain suspended for long periods of time and be inhaled."

CAN EBOLA BE AEROSOLIZED FROM WASTEWATER?

Whether the Ebola virus can be aerosolized from wastewater was the second question that Marr used to illustrate the value of engineering methods. "Here we took our tools to infrastructure," Marr said. "We looked at toilets, aeration basins at wastewater treatment plants, and converging sewer pipes."

Ebola patients expel copious amounts of diarrhea, which can contain virus concentrations as high as 107 per milliliter. Although toilets emit approximately 2 million aerosols per flush, Marr's group found, using Ebola surrogates, that the aerosol virus emission was slight, an estimated one virus every 100 flushes. Marr's group built models that simulated aeration basins at wastewater treatment plants and the convergence of sewer pipes and found that aerosol production of these continuous processes was up to four virus particles per minute. "We concluded that there is potential for aerosolization of the Ebola virus," Marr said. "It is not huge, but it should be monitored."

RELATIVE HUMIDITY AND FLU SEASON

How humidity affects airborne transmission of the flu was the third question that Marr gave as an example. The relative humidity in the respiratory tract is 100 percent. If the humidity in the surrounding air is also 100 percent, a one micron droplet will remain unchanged. If the relative humidity is less than 40 percent, as might be found in offices in the winter, the droplet will shrink to 0.4 microns. This difference affects transport. In addition, when the droplet's diameter decreases by half, its volume shrinks by a factor of eight, which raises the concentration of any dissolved solutes such as salt. This drastically changes the microenvironment of a pathogen and can have a substantial effect on its viability.

Marr and her colleagues devised a set of laboratory models to track the response of aerosols of various sizes, with different salt and protein concentrations, when exposed to different levels of relative humidity. They placed droplets with media representing pathogens in a chamber with controlled humidity for one to three hours. They also performed a similar experiment with suspended aerosols in a custom-built rotating drum, which enabled them to increase the time the aerosols are suspended.

> "We looked at toilets, aeration basins at wastewater treatment plants, and converging sewer pipes."

They found that at 100 percent humidity, there was no loss of pathogen viability, but viability dropped as humidity approached its mid-range and partial evaporation led to higher salt concentrations. When relative humidity is very low, the respiratory fluid evaporates and the salt crystallizes, making it harmless to bacteria. In other words, viruses survive in both very humid and very dry conditions. These results might account for the seasonality we see in influenza transmission in temperate regions.

LONG-DISTANCE VIRUS TRANSPORT

The final question Marr posed is whether diseases can be spread through long-distance transmission through the atmosphere. She currently is trying to determine if virus transport from mainland China to Taiwan is feasible at relevant concentrations. In doing so, she is building on a previous study of influenza transport in outdoor air that was conducted in Taiwan in 2006. The researchers collected samples at the northern tip of Taiwan and measured their virus concentrations. Using meteorological records to model back trajectories, Marr correlated high virus concentrations to airflow coming from China and low concentrations to airflow from the Sea of Japan.

To better determine the mechanism of transmission, Marr is currently embarked on an experiment in which she will release simulated viruses from Shanghai based on a realistic estimate of how much virus people with influenza might introduce into the air. She will run a concentration model and compare estimated concentrations in Taiwan with actual measurements.

"In all these circumstances," Marr noted, "engineering tools can shed light on transmission of infectious diseases, helping public health officials better understand and prevent their transmission." ■

Linsey Marr, PhD, is a professor of Civil and Environmental Engineering at Virginia Tech. Her research group studies the emission, transformation, transport, and fate of air pollutants. She is especially interested in emerging or nontraditional aerosols like viral pathogens.

DIANE E. GRIFFIN

MEASLES: REEMERGENCE OF AN OLD THREAT

Unlike many of the other viruses discussed during the VASEM Summit, there is an effective and dependable vaccine for measles. Nonetheless, the disease has proven to be stubbornly resistant to control and remains one of the most important causes of childhood deaths. In her presentation, Diane Griffin, a professor of molecular microbiology and immunology at Johns Hopkins, shed light on the reasons for this situation.

Griffin noted that much of the epidemiology about measles was established in 1846 during an outbreak after a 60-year lull in the North Sea's Faroe Islands. Peter Panem, a Danish physician on the islands, made a number of fundamental observations about the disease: measles is contagious; there is a 14-day incubation period; the attack rate for those who are susceptible is 100 percent; and exposure to the disease confers lifelong immunity. Those who were present on the island during the outbreak 60 years before remained healthy.

THE STATE OF MEASLES KNOWLEDGE

Since that time, Griffin pointed out, a great deal has been learned about the pathogenesis of RNA virus infections like measles. Some are persistent in human populations in the absence of treatment—HIV is a good example. This group of RNA viruses has accordingly not evolved a very efficient method of transmission. There are other viruses like herpes that can cause an acute transmissible disease, only to become latent for long periods of time punctuated by periods of reactivation and transmission. Acute viruses like measles are highly transmissible because they seem to be cleared from the body relatively quickly.

A key immunologic issue in combatting measles was determining the size of the susceptible population required to sustain its transmission. From data collected in the United Kingdom before the measles vaccine was available, scientists determined that a population of between 100,000 and

WORLD HEALTH ORGANIZATION

> "The primary cause of death is usually bacterial pneumonia, not measles pneumonia."

500,000 was enough to maintain the virus.

Scientists have also determined that complications arising from a measles infection—primarily other infectious diseases—are the main reason it is so much more lethal than other rash diseases. "The primary cause of death," Griffin noted, "is usually bacterial pneumonia, not measles pneumonia." In one in a thousand cases, patients develop encephalomyelitis, an autoimmune disease that produces mental retardation, seizures, and paralysis. As long as 10 years after exposure, a very few patients develop subacute sclerosing panencephalitis, a fatal condition that suggests that measles, normally thought of as an acute virus, can persist for long periods of time.

Despite progress, there are still many unanswered questions about the disease. Measles is asymptomatic during the initial 10-to-14-day period after exposure, which increases the chances of the virus being transmitted. "From a biological point of view, we want to know how the virus shuts down the host response to infection so that there is no fever or other symptoms during this period," Griffin said.

Griffin herself is very interested in the persistence of the virus. She and her colleagues found that children in Zambia had detectable measles virus RNA either in their respiratory tract, urine, or blood for months after they had apparently recovered. They noted a similar pattern in rhesus macaque models, finding viral RNA in their lymph nodes a year after infection. Epidemiological studies of European children who had measles showed an increased susceptibility lasting as long as two or three years to other infections, especially respiratory disease. In essence, Griffin concluded, although it has a low mortality rate in developed countries, even there it can have serious long-term consequence for children who have had measles.

THE CHALLENGES OF VACCINATION

The persistence of measles adds to the reasons that vaccination is critical. The vaccine in use today—an attenuated live virus developed by John Enders—is safe and efficacious. It is given at nine months in developing countries, where, because of ongoing measles transmission, children are exposed at a young age. In the United States, Canada, and Europe children are vaccinated at 12 to 15 months, partly to avoid the potential of maternal antibodies to neutralize the vaccine.

The vaccine had a dramatic effect on the incidence of measles in the United States when introduced in 1963—and public health officials came to the conclusion that measles could be eradicated globally. In 1997, the World Health Organization set a target date for measles eradication between 2005 and 2010. By 2008, WHO and its partners had made significant progress, only to face large outbreaks in Europe and a resurgence in Asia and Africa.

A fundamental cause of these outbreaks is a failure to vaccinate a sufficient portion of the population. Because measles is so infectious, high levels of immunity—between 92 and 95 percent—are required to interrupt transmission. Raising the bar even higher, approximately five percent of the vaccinations do not induce immunity. This means that if 95 percent of children are vaccinated, immunity levels are just 90 percent. In the United States, the response was to require a second vaccination before children enter school.

Griffin cited a number of reasons why officials around the world have not been able to achieve the necessary vaccination levels. The first is donor fatigue caused by the prolonged, resource-intensive efforts that measles eradication requires. The second is the difficulty of delivering the vaccine, especially in developing countries where it can be challenging to sustain the cold chain needed to protect the live vaccine.

The result is that immunization rates remain low in Africa and India, as well as in parts of Europe. A strategy that has worked well in these circumstances is to routinely vaccinate everyone under the age of 15, followed up every three-to-five years by comprehensive vaccination of all children under five. This approach was pioneered successfully in South America by Pan American Health Organization, but it requires both a massive and sustained effort.

Finally, there is vaccination refusal, especially in the United States and Europe. In 2001 measles was declared eradicated in the United States. However, in 2014, there was a large outbreak among visitors to Disneyland and among Amish populations due to vaccination refusal. Griffin notes that each state has its own criteria for allowing children to go unvaccinated. They include medical, religious, and personal belief exemptions.

"Unlike influenza, measles does not change immunologically and the vaccine remains highly effective," Griffin said. "Whether a state will recognize religious or philosophical objections to a vaccination policy greatly influences the number of children who remain unvaccinated and the likelihood of more outbreaks occurring." ■

Diane E. Griffin, MD, PhD, is the University Distinguished Service Professor and former chair of the W. Harry Feinstone Department of Molecular Microbiology and Immunology at Johns Hopkins Bloomberg School of Public Health. She is also vice president of the National Academy of Sciences.

VIRGINIA DEPARTMENT OF HEALTH

WILLIAM A. HAZEL

VIRGINIA'S RESPONSE TO EMERGING AND REEMERGING INFECTIOUS DISEASE

Virginia Secretary of Health and Human Resources William Hazel began his presentation by commending members of the audience for their work. "The agencies of the Secretariat have the obligation to promote opportunities for individuals to have healthy, prosperous lives," he said. "You can't do that when you are sick. Your expertise in public health, virology, bacteriology, and other fields aids us immensely."

ACCOUNTING FOR PUBLIC PERCEPTION

Hazel connected the topics of many of the day's presentations on infectious disease and preparedness to his responsibilities. For instance, he noted that the Virginia Department of Health (VDH) continued to address the issue of vaccination refusal and the reemergence of diseases like measles that had been controlled when he was a medical student. "If you want a passionate, heated policy discussion, come to our meetings about mandatory vaccination for schools and listen to the debate between people resisting and those advocating vaccination," he said. Vaccination is critical for suppressing or eliminating deadly disease, but he acknowledged that health officials have not been as effective as they could have been in making the case to the public.

One of the lessons learned from the Disneyland outbreak in California, Hazel said, is that transparent, freely available data available can help build public trust in vaccinations. He noted that California published data on the outbreak and vaccination rates on its state websites, allowing people to evaluate the information for themselves. Hazel noted that scientists like those assembled at the VASEM Summit could, by speaking out, help reinforce the case made by public health officials that vaccination is beneficial.

PLANNING FOR EMERGENCIES

Hazel noted that disaster medicine and preparedness, the topic of another presentation, also falls under the legal authorities of the governor of Virginia. The governor has the ability to declare an emergency, create emergency regulations, and secure funding to address it, including petitioning the federal government for support. As part

of this authority, the VDH participates in a number of multi-agency task forces on federal, state, and local levels that prepare for health emergencies. This includes a wide variety of responders, including law enforcement as well as hospitals and nursing homes. "We have a very broad, comprehensive response capability," he said. "This has proven to be invaluable given significant health emergencies during my tenure."

These emergencies have included the H1N1 influenza pandemic of 2009–10, preparation for Ebola virus in the United States in 2015, and the Zika virus in 2016–17, as well as the opioid addiction crisis the U.S. is currently experiencing. "I learned during the Ebola outbreak, as with measles vaccination, that we have to deal with the public perception as well as the reality of the disease," he said.

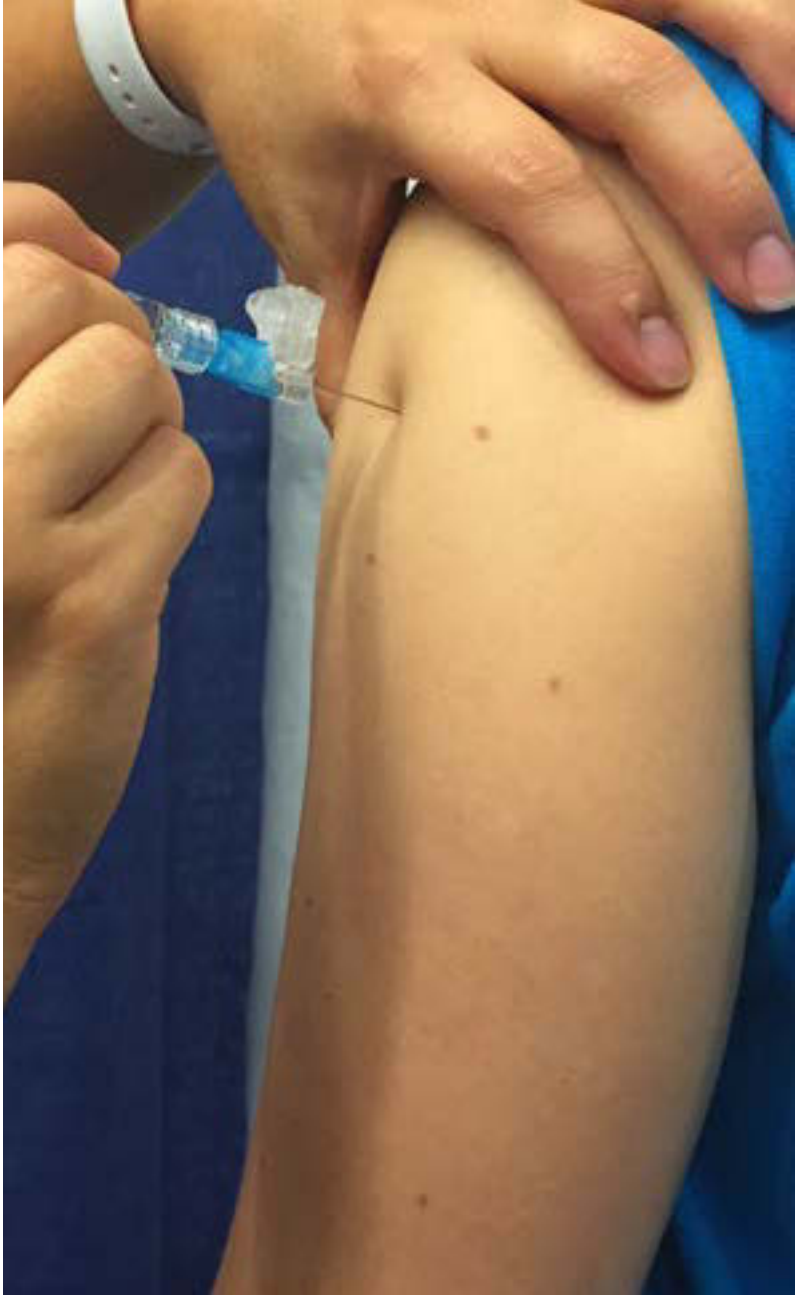
BUILDING CAPACITY FOR SURVEILLANCE, RAPID ANALYSIS, AND COMPREHENSIVE RESPONSE

The first line of defense for public health, Hazel noted, is surveillance, backed by robust information technology capacities. "We see surveillance on a daily basis," Hazel said. "We are always on the watch to see if there is something that we need to be aware of to protect the public." When the VDH discovers an emerging health threat such as an increase in HIV, hepatitis C, and endocarditis due to needle sharing, it is able to take the lead in addressing the problem with local departments of health and even advocating for changes in legislation based on best practices.

An important surveillance tool used by VDH is dashboards, which are available to staff members on a daily basis. Hazel noted that the department is considering making these dashboards public, though there is the ongoing issue of informing public perception.

One of the issues that the VDH monitors, Hazel said, is emerging infections. Because Virginia has large poultry and swine businesses, it tracks the incidence of animal as well as human illnesses. Vector-borne illnesses are another concern, especially the post-acute phases of Lyme disease, as is the emergence of new strains of organisms due to drug resistance. "In the case of drug resistance, we have the opportunity to use our authority over the medical system—through Medicaid and the state employee health plan—to drive changes in the antibiotic prescribing patterns of physicians." In addition, the VDH is always on the alert for new illnesses introduced by overseas travelers arriving at Virginia's ports and airports.

Addressing these issues—and others—requires a massive effort. Hazel cited several examples to provide a sense of the scale of its monitoring efforts. During an influenza outbreak, this can include travel monitoring, tracking avian flu outbreaks, analyzing influenza strains from patients at state labs, looking for unusual cases, clusters, and outbreaks, and following those with high-risk exposures.



NATIONAL INSTITUTES OF HEALTH

During the Ebola and Zika crises, these campaigns had to scale up on very short notice. In the case of Ebola, Virginia eventually monitored more than 2,200 travelers, only one of whom was considered high risk. VDH mounted a similar campaign for Zika.

Hazel concluded by again urging academy members in the room to lend their voices to the discussions about public health. There are issues like vaccination, he reiterated, where their advice and counsel could affect the public discussion. "We need to speak the language that the public uses," he said. "Every one of us has some responsibility to speak up." ■

William A. Hazel, MD, an orthopedic surgeon, served as Virginia's secretary of health and human resources between 2011 and 2017. As secretary, he oversaw 11 state agencies with over 16,000 employees.

From left: Anthony Fauci, HHS Secretary Sylvia Matthews Burwell, President Barack Obama, and Nancy Sullivan, chief of NIAID's Biodefense Research Section.

ANTHONY S. FAUCI

EMERGING INFECTIOUS DISEASES: LEARNING FROM THE PAST AND PREPARING FOR THE FUTURE

The VASEM Summit's keynote address was delivered by Anthony S. Fauci, Director of the National Institute of Allergy and Infectious Diseases (NIAID) at the U.S. National Institutes of Health. He began his talk by recounting a recent incident:

In the interim between the election of President Trump in November 2016 and his inauguration in January 2017, Denis McDonough, President Obama's chief of staff, invited future members of the Trump Cabinet to the White House to brief them on unexpected emergencies they may experience.

Fauci joined then-Secretary of Health and Human Services Sylvia Mathews Burwell and former Centers for Disease Control and Prevention (CDC) Director Tom Frieden to talk about pandemic response. While each pandemic has unique features, Fauci was clear on one point: administrations should expect to face infectious disease crises.

Fauci had faced them throughout his career. When he first testified before Congress in the mid-1980s, he presented a map of the world displaying emerging and reemerging infectious diseases. At that first hearing, he put a single virus on the map: HIV. The last time he testified, in spring 2017, the map was crowded with pathogen names, the cumulative effect of decades of outbreaks.

"Each presidential administration in which I have served has faced multiple crises caused by infectious diseases, usually at least one in its first year," he said. Fauci devoted his presentation to reviewing this record.

THE RONALD REAGAN ADMINISTRATION

Five months after President Reagan was inaugurated in 1981, CDC's Mortality and Morbidity Weekly Report (MMWR) first reported five gay men presenting with an unusual form of pneumonia. Similar cases that soon began appearing in the MMWR included Kaposi sarcoma and other opportunistic infections. Fauci made the decision to refocus his career on this syndrome, later shown to be caused by the human immunodeficiency virus (HIV). The Reagan administration initially treated this emerging infectious disease—one that would ultimately affect 76 million people and cause 35 million deaths—as a limited event in a discrete population. "One lesson I stressed during the cabinet presentation," he said, "was to take infectious diseases seriously because they can surprise you."

NATIONAL INSTITUTES OF HEALTH

THE GEORGE H.W. BUSH ADMINISTRATION

President George H.W. Bush took a different approach, Fauci noted. Bush had realized as Vice President that the challenge of HIV/AIDS would continue. During his administration, he increased the budget for HIV research exponentially. "If you make investments in biomedical research and recruit very smart and eager people, the advances in science are nothing short of breathtaking," Fauci said. Thanks to this funding, there are now more than 30 approved anti-HIV drugs, and some regimens are combined in a single daily pill.

"We started with low-tech things like condom use, needle exchange, and behavior modification," Fauci said. "Today we have the tools that if we implement them properly, we likely could put an end to the epidemic."

THE BILL CLINTON ADMINISTRATION

During the two terms that President Bill Clinton was in office, the HIV/AIDS pandemic continued to grow. President Clinton was interested in the science, and at Fauci's suggestion, he established the Vaccine Research Center (VRC) at NIAID, initially focusing on HIV and later on a variety of infectious diseases including chikungunya, Ebola, and Zika.

During the Clinton Administration, the United States experienced outbreaks of several reemerging infections, including West Nile virus. Although the VRC completed early development of a DNA vaccine candidate for West Nile, it was unsuccessful in finding a pharmaceutical partner to complete development and produce it. "This was another lesson I conveyed to the Trump Cabinet," Fauci said. "We have to find a way to engage the pharmaceutical industry in becoming involved in areas of public health that are not necessarily big money-makers."

THE GEORGE W. BUSH ADMINISTRATION

President George W. Bush faced both terrorism and bioterrorism, when letters tainted with anthrax spores were sent to members of Congress and other luminaries through the mail. Collaborating with counterparts across the federal government and industry, NIAID helped establish a mechanism to accelerate countermeasures development for bioterror threats.

When asked by President Bush what scared him most about bioterrorism, Fauci stated that he was in fact most afraid of naturally occurring outbreaks of infectious diseases. Soon afterward, a series of outbreaks, including H5N1 influenza (bird flu) threatened to, but fortunately did not, emerge as a pandemic. President Bush responded in 2005 by announcing an international partnership on avian and pandemic influenza. Fauci helped develop the National Strategy for Pandemic Influenza.

Severe Acute Respiratory Syndrome or SARS, a newly emerging infectious disease, was yet another infectious disease crisis during the Bush Administration. As part of its response, NIAID used a vaccine platform, the DNA vaccine, to rapidly create a vaccine candidate to prevent SARS infection. The time between sequencing this coronavirus and the initiation of phase 1 vaccine trials was just 20 months. Fortunately, classic public health measures alone put an end to the epidemic. The lesson for Fauci: "In this age of technology, don't abandon low-tech methods like identification, isolation, contact tracing, and quarantine."

THE BARACK OBAMA ADMINISTRATION

Within months of President Barack Obama's inauguration, the H1N1 pandemic influenza epidemic expanded rapidly across the world. While the U.S. government worked with industry to manufacture and test a vaccine, disease incidence peaked before vaccine supplies were widely available. This experience demonstrated the need to improve our approach to influenza vaccine development.

Several years later, Ebola emerged in West Africa. At the invitation of the government of Liberia, NIAID and Liberian investigators created the Partnership for Research on Ebola Virus in Liberia (PREVAIL). PREVAIL launched a series of vaccine, treatment and survivor trials, demonstrating the feasibility of rigorous research in outbreak settings. An important lesson of Ebola was that public health responses must address both the disease itself and the fear it creates. This lesson was also relevant to the Zika virus outbreak, which followed closely on the heels of Ebola. The response to the Zika virus once again highlighted the benefits of the development vaccine platform technology utilized by the VRC and NIAID. A DNA vaccine for Zika took less than four months to go from sequence identification to phase 1 clinical trial.

A PERSISTENT CHALLENGE

Fauci concluded by noting three decades of pandemic threats have shown that every administration is likely to experience at least one infectious disease crisis and that new administrations should heed lessons from previous pandemics: the need for global surveillance; transparency and communication; infrastructure and capacity building; coordinated and collaborative basic and clinical research; adaptable platform technologies for vaccines, diagnostics, and therapeutics; and a stable funding mechanism. ■

Anthony S. Fauci, MD, was appointed Director of the National Institute of Allergy and Infectious Diseases at the U.S. National Institutes of Health in 1984. He is also chief of the NIAID Laboratory of Immunoregulation, where he has made numerous important discoveries related to HIV/AIDS and is one of the most-cited scientists in the field.

JONATHAN S. TOWNER

THE WEST AFRICAN EBOLA EPIDEMICS AND EMERGENCE OF OTHER FILOVIRUSES


CENTERS FOR DISEASE CONTROL AND PREVENTION

To begin his presentation, Jonathan Towner, head of the Virus Host Ecology Section at the Centers for Disease Control and Prevention (CDC), oriented the audience by placing the genus *Ebolavirus* in the context of the larger filovirus family, which also consists of the genus *Marburgvirus* and the genus *Cuevavirus*. Most filoviruses, he noted, are found in Africa, and all species of filovirus, with the exception of the *Reston ebolavirus*, cause disease in humans. Among those viruses affecting humans, there is quite a bit of genetic diversity.

Most filovirus outbreaks in Africa over the last forty years, Towner said, have been geographically constrained, presumably by the distribution of their natural reservoir. Ebola virus, species *Zaire ebolavirus*, has been historically limited to the central Congo Basin, until it emerged in West Africa in 2014. Similarly, Marburg virus outbreaks have been mostly confined to East Africa, though there was an outbreak in Angola in 2005. "I spend a lot of time thinking about where the natural reservoir is," Towner said. "Their distribution is an important clue."

A DEPARTURE FROM THE NORM

The recent large outbreak of Ebola in West Africa took the world by surprise, particularly because of its scope. The virus eventually infected more than 28,000 people. Towner noted that the next largest outbreak, caused by Sudan virus (*Sudan ebolavirus*), generated just 425 cases. The West Africa outbreak also took a massive toll on healthcare workers. Almost 900 healthcare workers throughout West Africa, Europe, and the United States were infected, and of those, more than half died.

The CDC mounted a comprehensive response. In the end, it deployed more than 1,000 staff members to West Africa, helping establish with international partners 22 diagnostic labs in rural and urban locations. The CDC lab (in Bo, Sierra Leone) alone processed more than 27,000 specimens over the 15-month period between March 2014 and July 2015. CDC worked in the United States to implement enhanced screening at airports and improve hospital readiness.

"Our experience in West Africa confirmed much of what we knew about Ebola, but also expanded our knowledge," Towner said. Person-to-person transmission is the dominant way filoviruses spread. Risk factors include contact with patients, especially in the late stages of illness; contact with bodily fluids and stools; touching cadavers as part of burial practices; and sexual contact. Researchers discovered Ebola could be persistent. They discovered RNA in the semen of a few patients more than 500 days after the onset of the disease.

TRACKING THE VIRUS FROM THE CONGO BASIN

"Our group was interested in how the spillover from the Congo Basin to West Africa occurred," Towner said. Historically, he noted, Ebola virus (in Democratic Republic of the Congo (DRC), Gabon, and Republic of Congo) had been associated with consumption of nonhuman primates, but they are not the reservoir. In 2005, a search for the Ebola reservoir in Zaire found genetic material in three species of fruit bat. Interestingly, their range extends into West Africa. "This put these bats on the map as a likely source," Towner said.

There had been reports that the index case in Guinea, a two-year old boy, had been playing in a hollow tree, which was infested by bats of a different species. Because this tree, and the bats, were later burned by the inhabitants of a nearby town, a bat connection could not be verified.

APPLYING LESSONS FROM MARBURG

To shed light on the Ebola virus reservoir, Towner and his team focused on a related pathogen, Marburg virus. In 1967, Marburg virus was the first filovirus discovered, and it is responsible for outbreaks of Marburg hemorrhagic fever in sub-Saharan Africa. This disease is similar to that caused by Ebola virus, with easy person-to-person spread and high case-to-fatality ratios of between 23 percent and 85 percent.

Between 1998 and 2000, there was a large Marburg hemorrhagic fever outbreak in the DRC. Over 80 percent of cases were found among miners working in an illegal subterranean gold mine or their direct contacts. "What was further compelling was that when we looked at the sequences of the virus isolates from the miners, there were a minimum of nine different genetic lineages, implying that the miners were coming into repeated contact with the natural reservoir," Towner said. However, before any further conclusions could be drawn, the mine flooded, and all transmission stopped.

In 2007, a smaller outbreak occurred in southwest Uganda, in the Kitaka lead and gold mine. Towner and his colleagues investigated and found the mine to be infested

with the Egyptian rousette fruit bat. They collected samples from this species and another that was living in the mine. Twenty-two of 23 Marburg-positive bats were Egyptian rousettes. Furthermore, infectious Marburg virus isolates were secured from four of them. "We finally had proof," Towner said, "that these bats carried infectious Marburg virus." The researchers determined that the virus sequences in the bats were identical or nearly identical to those found in the infected miners. Furthermore, the diversity of these sequences suggested a long association with a reservoir host. There was another outbreak of Marburg hemorrhagic fever a year later among visitors to a cave in Uganda, about 50 kilometers from the Kitaka Mine, that also had Egyptian rousette bats, a further indication that this species of bat is a natural source of Marburg virus.

NARROWING IN ON THE EGYPTIAN ROUSETTE BAT

Given these connections, Towner and his colleagues concluded that understanding Marburg transmission requires knowledge of the natural history of the Egyptian rousette bat. Its geographical distribution encompasses the locations of all known Marburg outbreaks, and its reproductive capacity, combined with colony sizes of greater than 100,000 individuals, suggests it exists in large metapopulations. The Egyptian rousette bat reproduces twice a year, giving birth in February and August. Long-term (longitudinal) studies have shown that the timing of more than 80 percent of known spillovers to humans have occurred during these birthing seasons, giving the sense that there are seasons of increased human risk. Since that study was published, the last three known outbreaks have all started during these birthing seasons.

To determine how Marburg is transmitted from this reservoir, a number of research teams experimentally infected captive bats. Towner's team conducted a nine-month experiment with infected bats housed with naïve contract bats in various cage arrangements to test different modes of transmission. They found that virus is shed primarily in saliva and that the bats could transmit the virus to each other over time and in the absence of any other things found in a natural cave like ticks, mosquitos, or bats of another species. They also found that a minority of the infected bat population was responsible for a disproportionately large percentage of viral shedding.

Uncovering this mystery has become more pressing as filoviruses continue to emerge in different species and locations. Lloviu virus was recently found in dead bats in Europe, and Reston virus, of *Hot Zone* fame, was found in pigs in the Philippines and in China. Recently, a new filovirus was detected in an Asian fruit bat. "If filoviruses follow a one-host, one-virus principle, we have a lot of work to do," Towners said. "There are 1,200 species of bats." ■

Jonathan S. Towner, PhD, is head of the Virus Host Ecology Section of the National Center for Emerging and Zoonotic Infectious Diseases at the Centers for Disease Control and Prevention.

AEDES AEGYPTI: NATIONAL INSTITUTES OF HEALTH

ZACH ADELMAN

ENGINEERING PATHOGEN-RESISTANT MOSQUITOS

Dengue, chikungunya, yellow fever, West Nile, and Zika are only the tip of the iceberg when it comes to viruses transmitted by mosquitoes. As Zach Adelman observed, there are scores of other viruses capable of being vectored by mosquitoes that may eventually cause disease. Adelman, an associate professor of entomology at Texas A&M University, made this point to highlight the challenge of engineering pathogen-resistant mosquitoes.

THE NATURAL HISTORY OF *AEDES AEGYPTI*

Appreciating the advantages of the engineering approach that Adelman and his colleagues have developed, he said, requires an understanding of how the *Aedes aegypti*, the mosquito most commonly implicated in vector-borne diseases, transmits pathogens. The female *Aedes aegypti* requires a blood meal to acquire the protein she requires to produce eggs, which she lays around water that collects in small containers. If she feeds on someone who carries a virus, she will ingest that virus along with the blood. Viruses like dengue have evolved methods to survive in the *Aedes aegypti* digestive tract and find their way back to its salivary glands. The virus is then transmitted to the next person she bites.

To succeed in transmitting a pathogen, the mosquito has to both find and survive encounters with two human hosts. In addition to finding accessible capillary beds, it has to cope with an immune response and avoid detection. Once it has taken on its blood meal, it must also survive so it can digest it, produce eggs, and deposit them.

"Breaking this chain of events at any point will stop transmission," Adelman said.

Aedes aegypti evolved to live in and around human dwellings about 5,000 years ago. In tests, approximately 99 percent of blood found in this species is human blood. Originally found in Africa, it was brought to the rest of the world thanks to colonialism and the slave trade.

POPULATION CONVERSION

To interrupt transmission, Adelman is pursuing genetic control, which entails editing the DNA of the mosquito itself so it takes on new properties or loses properties it has. He noted that there are two approaches to genetic control. The first is population conversion. Rather than disrupt an ecosystem by eliminating them, population conversion entails inserting genes that interfere with the mosquito's vector competence. These genes could be inserted using any one of several gene drive techniques, methods that ensure the allele frequencies of a desirable gene increase with every generation.

> "If you eliminate the vector from an area and keep on eliminating it, you also end transmission of all pathogens, known and unknown."

An important objection to population conversion is that there is no guarantee that the vector-interrupting gene will limit the transmission of all pathogens that the mosquito could possibly carry, known pathogens as well as emerging ones. Studies of engineered pathogen resistance have confirmed this drawback. They have been shown to be effective but are limited to a single species of virus. "To be efficient, any approach we adopt should block all viruses we know about as well as viruses that emerge in the future," Adelman said.

Laboratory experiments have also found that, although the number of mosquito progeny carrying the transgene exceeds 90 percent in a few generations thanks to the gene drive, 100 percent modification is required over a long period of time to have a meaningful effect.

Finally, Adelman noted, there is the concern among scientists and the public that there is no way to recall a transgene linked to a gene drive and no guarantee that it will not jump to another species. The National Academy of Sciences issued a report on gene drives, acknowledging their value but concluding that "there is insufficient evidence available at this time to support the release of gene-drive modified organisms into the environment."

POPULATION SUPPRESSION

For Adelman, another method of gene control—population suppression—is a more promising approach. It is open-ended. "If you eliminate the vector from an area and keep on eliminating it, you also end transmission of all pathogens, known and unknown," he said. The bar for an effective suppression strategy is high, however. Adelman noted that spraying with DDT was a suppression strategy, but because it did not kill all mosquitoes, populations bounced back when it was stopped, due in part to the evolution of resistance.

Working with Zhijan Tu, a professor of biochemistry at Virginia Tech, Adelman is developing population suppression strategies that he believes will overcome this objection, as well as objections to using gene drive. His goal is to insert a gene that biases the population toward males, which do not make eggs, do not drink blood and do not transmit disease.

Adelman noted that researchers have been pursuing the gene for sex determination in mosquitos with exactly this goal in mind since at least 1967. In 2013, researchers zeroed in on the area of the genome where this gene resides, and Tu, Adelman, and their colleagues discovered the gene itself in 2015. When injected into embryos that were to develop as females, it changed them into males. Adelman and Tu, theorize that the release of these modified male mosquitoes, which would only be capable of producing other male mosquitoes, could over several generations reduce and eventually eliminate the female population—and the mosquito species itself.

CHALLENGES TO IMPLEMENTATION

As Adelman pointed out, there are many issues (technical, social and political) that must be addressed before their technology can be deployed.

From Adelman's perspective, the effects on the environment are likely to be minimal, due to the fact that *Aedes aegypti* is an invasive species throughout most of its range. His goal is to eliminate *Aedes aegypti*; there are 3,000 other mosquito species that could take over its environmental niche. In comparison with other methods used to combat mosquito infestations, such as the use of pesticides, his approach to population suppression is benign. There are also benefits, in terms of slowing the progression of transmission, even before suppression is complete.

Adelman is hoping to develop a large-scale mosquito factory at Texas A&M University. It could be used for experimental purposes and to produce transgenic mosquitos on a scale required to begin effective suppression. Before he could release genetically altered mosquitoes into the environment, Adelman would require approval from the FDA and/or EPA. Right now, recent FDA guidance does not seem to pertain to technology that changes the sex of mosquitoes, but is ultimately unclear. "We are very excited by the possibilities," Adelman said, "but will need a clearly defined regulatory pathway before we can move forward." ■

Zach Adelman, PhD, is an associate professor in the Department of Entomology at Texas A&M University. His research has focused on the development of novel gene editing/gene replacement approaches for disease vector mosquitoes.

JOHN MASCOLA

HIV VACCINES: CHALLENGES AND PROGRESS

John Mascola titled his presentation on HIV vaccines Challenges and Progress to convey to his audience not simply the complexity of the challenges vaccine researchers face but also the substantial accomplishments they have achieved, both in developing an active vaccine as well as providing passive immunity. He is the director of the Dale and Betty Bumpers Vaccine Research Center (VRC) of the National Institute of Allergy and Infectious Diseases.

THE LIMITATIONS OF ANTIBODY-BASED PROTECTION

To give the audience a better sense of why it has been so difficult to develop an HIV vaccine, Mascola began his talk with a brief description of how the HIV virus infects a CD4 immune cell. HIV has an envelope surface protein consisting of two protein subunits of gp160 that binds to the primary cellular receptor CD4 and then to a cellular coreceptor, the CCR5. This sequential binding triggers fusion of the viral and host cell membranes.

"A key component to developing an effective viral vaccine," Mascola said, "is coming up with a neutralizing, antibody response to the virus, an antibody that binds to the viral surface protein." Such an approach is the basis of the Hepatitis B vaccine and the development of hemagglutinin for influenza A.

The first vaccine trial was in 1987, Mascola noted, just four years after HIV was described. Mounting a vaccine trial so quickly was a remarkable achievement. Using recombinant DNA technologies, researchers developed a subunit vaccine based on genetically engineered antigens of gp160, with the goal of generating neutralizing antibodies, which were thought to be sufficient to confer protection against HIV. The results, however, were disappointing. No significant neutralizing antibodies were produced.

Efficacy trials in 2003 targeting the gp120 subunit failed to confer protection. In 2007, attempts to put internal viral genes in an antiviral vector were halted for similar reasons—and because those taking the vaccine were found to have an increased risk of developing HIV. In 2013, a vaccine based on DNA priming with an adenovirus type 5 boost was also stopped for lack of efficacy. A trial in Thailand combining two vaccines did manage to produce 31 percent efficacy. "At best, these vaccines induced weak neutralizing antibodies, with low potency and limited breadth," Mascola said. "That can be compared to measles vaccines, which produces a robust immune response."

The good news, Mascola said, is that we now understand why this should be. Comparison to the respiratory syncytial virus (RSV) and the influenza virus is illuminating. In RSV, the virus is exposed and not diverse. The influenza virus show more diversity and some glycosylation, a process that generates a glycan coating of sugars outside of the envelope protein. In HIV, there is much more glycosylation and much more diversity, making the envelop protein an extremely difficult target for antibody-based protection.

Since 2013, researchers have been able to examine the complete structure of the HIV envelope protein in detail. Most of the protein surface is covered by a glycan shield.

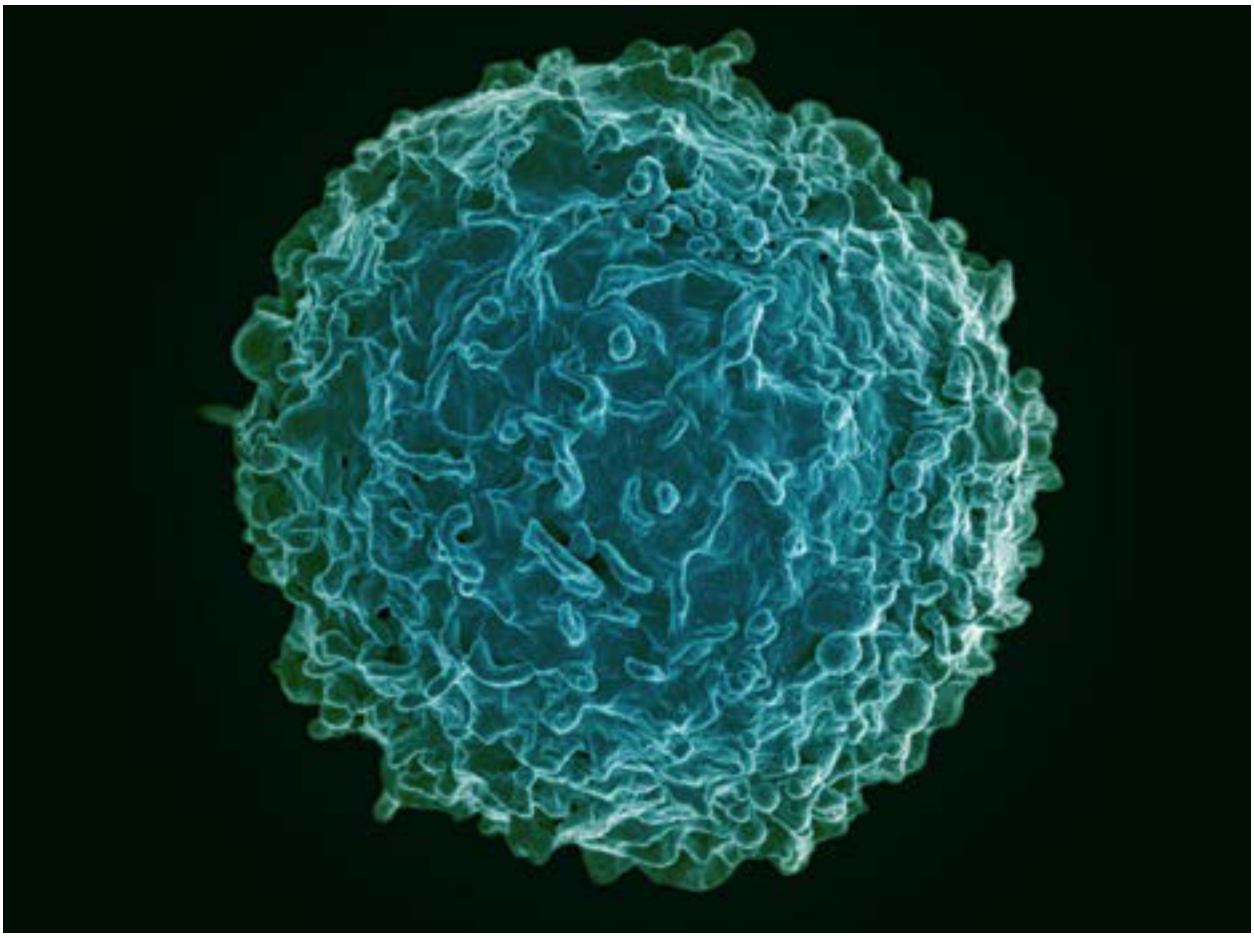
A SHIFT TO STRUCTURE-BASED VACCINE DESIGN AND B-CELL ONTOGENY

The lessons learned from these failures has led researchers to new directions. They are focusing on the native HIV-1 trimer, a protein spike on the viral envelope that mediates attachment and entry into the host cell. They are also identifying potent neutralizing antibodies that arise naturally during HIV infection to understand how these antibodies work.

As a step in this direction, researchers studied 80 relatively healthy subjects with HIV, tested their sera against HIV, and identified those who made potent, cross-reactive neutralizing antibodies against HIV. They then isolated those antibodies and determined their binding sites on the virus envelope. This knowledge is the basis of structure-based vaccine design. "These antibodies have fairly unusual characteristics," Mascola said. "They often have long binding loops that enable them to reach in past the glycan shield."

However, they also have a high level of affinity maturation, which means their affinity for the envelope protein gradually increases over time. Although, as Mascola noted, this is not an ideal quality for a vaccine, which requires an immediate and strong response, it opened up another investigative pathway. Starting with a single B-cell (the cell in the immune system that is responsible for generating antibodies), researchers tracked the evolution of antibody lineages as they evolve to reach the point of effective neutralization.

Mascola cited an experiment that traced an antibody lineage as it increases in effectiveness over time while monitoring specific changes on a molecular level. The antibody evolves in tandem with the virus and becomes increasingly cross reactive and capable of binding at multiple sites, but destruction of CD4 cells negates this progress. "The antibody is just a step behind where it needs to be to stop the infection," Mascola said. "But if you had an antibody of that potency early on, the outcome might be



B LYMPHOCYTE: NATIONAL INSTITUTES OF HEALTH

different." This approach is the basis of the lineage-based vaccine design that has just entered phase 1 clinical trials.

Looking forward, Mascola noted that there was cause for guarded optimism. Structure-based design has led to better antigens, and B-cell ontology produced more effective antibodies. Scientists have also developed nanoparticle vaccine platforms and shed light on the role of T follicular helper cells in assisting B-cells and establishing germinal center reactions, which include the production of high-affinity antibodies.

Mascola concluded, however, that an effective HIV vaccine is not imminent. Given the fact that there are 2 million new cases of HIV a year, there is a need for passive strategies that can prevent people from acquiring HIV, although they do not confer lasting immunity.

PASSIVE IMMUNITY

Using passive antibodies to provide prophylaxis protection has a long history, Mascola noted, in diseases as varied as polio, hepatitis A and B, and measles. Many studies have shown it provides protection in non-human primate models of HIV, and there are many different binding sites available.

Mascola used an antibody produced at the VRC called VRC01, which targets the CD4 binding site, to illustrate the potential of passive immunity. The Antibody Mediated Prevention (AMP) Study, based on VRC01, is now in Phase 2b clinical trials. Antibodies are being given intravenously every two months at two different infusion doses to members of two cohorts. He said that that the study was 70 percent enrolled and is being conducted at 47 sites in 11 countries.

"If the study is a success, we hope to learn two main things," Mascola said. "How much antibody is needed for protection and if viral resistance will necessitate the use of two antibodies." If the trial demonstrates efficacy, it would provide an incentive for researchers to develop next-generation antibodies. Work has already been done on antibodies that are more potent, longer acting, and provide broader coverage. Given this momentum, Mascola concluded, we will have gained the ability to use antibodies to prevent HIV infections in the next five years. "It will be part of our toolbox," he said. ■

John Mascola, MD, is the director of the Dale and Betty Bumpers Vaccine Research Center of the National Institute of Allergy and Infectious Diseases, part of the National Institutes of Health.

ANDREW FLANNERY

NOVEL BIOSENSOR TECHNOLOGIES TO DETECT EMERGING AND BIOTERRORISM PARTICLES

Andrew Flannery began his presentation by introducing his company, PathSensors, which makes diagnostics not just for bioterrorism agents but also for plant pathogens, human pathogens, and food-borne illnesses. Flannery is vice president of product development and chair of the Scientific Advisory Board at PathSensors. Rather than present a survey of new biosensor technologies, Flannery opted to redirect his talk to describe the development process for new detection tools for pathogens and toxins.

Flannery began by highlighting the impetus for bioterrorism detection. "We all know about the 2001 attack when anthrax spores were sent through the mail, leading to four deaths and treatment of 30,000 with antibiotics," he said. "But bioterrorism has been a concern at least since 1984, when member of the Rajneehsee religious sect sprinkled salmonella on salad bars at in Oregon restaurants in a bid to influence an election." The biological agents that terrorists might use are almost limitless, Flannery noted, and are extensive, including bacteria, viruses, and toxins.

THE FUNDAMENTALS OF A BIODETECTION DEVICE

Flannery declared that any biodetection technology designed to identify these threats must accomplish a series of tasks. It must be able to sample specific biothreat media, for instance, soil, water or powder. It must have a system to isolate the agent from the media, and another system to recognize it. The technology must also be capable of amplifying the signal and then detecting and interpreting it.

Whatever the technology, it will be judged on the basis of three primary characteristics Flannery enumerated. They are 1) sensitivity: the ability to detect small amounts of target within a background matrix, 2) specificity: the ability to discriminate between closely related pathogenic and nonpathogenic organisms or toxins, and 3) speed: the ability to conduct analysis rapidly with fast time-to-result.

Quite often, Flannery noted there are trade-offs among these three criteria. If you have high sensitivity but low specificity, for instance, you may get a large number of

PATHSENSORS

false positives, lessening trust in the diagnostic. It is important to be able to have a back-up test, preferably one that relies on a different method, for definitive agent identification. Flannery also pointed out that one diagnostic test does not fit all situations. For instance, a test that works well in a laboratory may be inappropriate for field work.

COMPARING COMMONLY USED DIAGNOSTIC TECHNOLOGIES

"The challenge we face is that current pathogen detection products on the market today are either fast, sensitive, or easy to use, but rarely all three," Flannery said. Culture methods, which are the gold standard for most situations, form the basis of many products. While culture methods are extremely precise, they are time-consuming to use, taking as long as seven days to produce a result depending of the threat. This is certainly not the time frame that first responders require, Flannery observed.

The second approach is the immuno-based assay. One form of immune-based assay is ELISA (enzyme-linked immunosorbent assay). In ELISA, an antibody is used to recognize the agent of interest, and that signal is then amplified. ELISA is both fast and cheap, but in most instances, it must be done in a laboratory. Another immune-based assay is the lateral flow test. It uses a piece of paper with a capture antibody bound to it. The sample is introduced, and if the agent of interest is present, the flow will reach a predetermined line. Lateral flow tests are extremely fast and can be used in the field, Flannery noted, but they have poor sensitivity and are prone to false positives.

Finally, there is the polymerase chain reaction, which allows an investigator to amplify the DNA or RNA from an agent of interest. Dyes are used to identify the target or monitor its accumulation, providing a gauge of quantity. Flannery pointed out that although PCR is highly sensitive, it is relatively slow, requiring more than 30 minutes for an assay, expensive, and requires a certain amount of technical sophistication to manage. In addition, because the background material can inhibit the reaction, it is liable to false negatives.

CANARY® TECHNOLOGY

PathSensors has turned to CANARY® technology as a way to bypass the limitations of these three approaches. It has the advantages of being sensitive, quick, easy to use, and inexpensive. CANARY, which stands for cellular analysis and notification of antigen risks and yields, was developed by MIT's Lincoln Laboratory following the 2001 anthrax attack as part of a Defense Advanced Research Projects Agency initiative.

In CANARY, an immune cell, the B-lymphocyte, is transformed into a biosensor that can respond to virtually all threats. To create a CANARY biosensor, a mouse is inoculated with an agent of interest and the resulting antibodies characterized. The variable regions of the antibodies are identified, cloned, and transferred to a B-lymphocyte containing the Aequorin luminescence gene, producing pathogen-specific receptors on the surface of the B-cell. When the receptors bind the pathogen, they activate an endogenous signaling pathway within the B-cell, producing a transduction cascade that results in the release of calcium. The calcium activates the Aequorin, which then emits light. One advantage of this process, which is extremely rapid, is that the light output reflects the concentration of the agent of interest

Flannery noted that PathSensors has developed three instrument platforms that incorporate CANARY: BioFlash for aerosol pathogen detection, and the Zephyr and Navigator systems for liquid-based pathogen detection. BioFlash is a portable system that combines CANARY detection with a proprietary aerosol collecting technology that captures particles up to 10 microns in size. It is currently being used for screening mail.

The Zephyr system for liquid-based pathogens includes a centrifuge, biosensors, and a luminometer that reads the results. It can be used in the field by operators without specialized training. It has been developed for a number of agents of interest to first responders, including anthrax and plague. It can detect anthrax in concentrations down to 1,000 spores per milliliter.

At the prompting of the U.S. Department of Agriculture, which is interested in screening imported crops for plant pathogens, PathSensors developed Navigator, a liquid-based pathogen detection system with 96 wells. PathSensors uses Navigator in concert with an assay capable of detecting *Phytophthora*, a water mold that causes crown and root rot diseases in plants. The assay has high specificity, excluding such closely related species such as Pythium and other fungi. In comparison tests, the CANARY assay significantly outperformed lateral flow assays.

Currently, PathSensors has a CANARY library of several dozen biosensors including such biothreats such as *Yersinia pestis* and *Bacillus anthracis* spores, plant pathogens that include *Ralstonia solanacearum* in addition to *Phytophthora* species, human pathogens like Ebola and dengue virus, and food-borne pathogens like *Listeria* species and *Salmonella* species. Flannery noted it is continuing to build this library. The company is developing biosensors for Zika virus, avian influenza and other human pathogens as well as a potato virus panel. ■

Andrew Flannery, PhD, is vice president of product development and chair of the Scientific Advisory Board for PathSensors, Inc., a company developing systems that provide high-speed, high-sensitivity pathogen detection.

VASEM MEMBERS

Faye Glenn Abdellah, NAM
Uniformed Services University of Health Sciences

Edward Aldridge, Jr., NAE
U.S. Department of Defense

John Anderson, NAE

Norman Anderson, NAM
American Psychological Association

**James Aylor *University of Virginia*

Bruce Baker, NAS
Howard Hughes Medical Institute

Craig Benson, NAE *University of Virginia*

Toby Berger, NAE *University of Virginia*

Peter Bethell, NAE *Cardno MM&A*

Eric Betzig, NAS
Howard Hughes Medical Institute

Enriqueta Bond, NAM
Burroughs Wellcome Fund

Richard Bonnie, NAM *University of Virginia*

Jay Boris, NAE
U.S. Naval Research Laboratory

Thomas Bostic, NAE *Intrexon Corporation*

Dushan Boroyevich, NAE *Virginia Tech*

**Barbara Boyan, NAE
Virginia Commonwealth University

L.D. Britt, NAM
Eastern Virginia Medical School

John Bryant, NAM

Sheila Burke, NAM
Howard Hughes Medical Institute

Wesley Bush, NAE
Northrop Grumman Corporation

Dennis Bushnell, NAE,
NASA Langley Research Center

**Joe Campbell, NAE *University of Virginia*

**Robert M. Carey, NAM
University of Virginia

Vinton Cerf, NAE *Google, Inc.*

Douglas Chapin, NAE *MPR Associates, Inc.*

Roger Chevalier, NAS *University of Virginia*

James Childress, NAM *University of Virginia*

Rita Chow, NAM
National Council on Aging

Robert Clagett, NAE

Henry Cox, NAE
Lockheed Martin Corporation

F. Fleming Crim, NAS
University of Wisconsin-Madison

Lance Davis, NAE

Don Detmer, NAM *University of Virginia*

John Dorning, NAE *University of Virginia*

*Patricia Dove, NAS *Virginia Tech*

James Duncan, NAE *Virginia Tech*

Lloyd Duscha, NAE

**Antonio Elias, NAE *Orbital ATK*

Robert Epstein, NAM *University of Virginia*

Stephanie Ferguson, NAM
International Council of Nurses

William Friend, NAE *Bechtel Group, Inc.*

Gerald Galloway, NAE
University of Maryland

Jacques Gansler, NAE
University of Maryland

Nicholas Garber, NAE
University of Virginia

Naomi (Lynn) Gerber, NAM
George Mason University

Thomas Giallorenzi, NAE
Naval Research Laboratory

Richard Guerrant, NAM
University of Virginia

**Florence Haseltine, NAM
Haseltine Systems

Henry Hatch, NAE

Ulrike Heberlein, NAS
Howard Hughes Medical Institute

Barry Horowitz, NAE *University of Virginia*

James Hubbard, NAE
National League for Nursing

John Hudson, NAE *University of Virginia*

Betsy Humphreys, NAM
National Institutes of Health

Peter Hutt, NAM *Covington & Burling LLP*

Ada Jacox, NAM *Athena Vineyards*

VASEM BOARD OF DIRECTORS

Antonio Elias, Robert Kahn, X.J. Meng, Patricia Dove (president), Andrew Densmore, (executive director), Florence Haseltine, Joe Campbell, Thomas Young, and James Aylor (secretary/treasurer).
Not pictured: Senator Mark Warner (honorary chair), Barbara Boyan, Robert Carey, Anita Jones, and Lester Lyles.

Elaine Jaffe, NAM *National Cancer Institute*

Stuart Jessup, NAE
Naval Surface Warfare Center

**Anita Jones, NAE *University of Virginia*

**Robert Kahn, NAE/NAS
Corporation for National Research Initiatives

Paul Kaminski, NAE *Technovation, Inc.*

Isabella Karle, NAS
Naval Research Laboratory

Kenneth Kellermann, NAS
National Radio Astronomy Observatory

Paul Kern, NAE *The Cohen Group*

Kenneth Kendler, NAM
Virginia Commonwealth University

Richard Knapp, NAM

Leonard Konikow, NAE
U.S. Geological Survey

Frederick Krambeck, NAE *ReacTech Inc.*

John Langford, NAE *Aurora Flight Sciences*

Judith Lean, NAS *Naval Research Laboratory*

Fred Lee, NAE *Virginia Tech*

Allen Lichter, NAM
American Society of Clinical Oncology

Jennifer Lippincott-Schwartz, NAM
University of Wisconsin-Madison;
University of Virginia School of Law

Andrew Lovinger, NAE
National Science Foundation

Gerald Luttrell, NAE *Virginia Tech*

**Lester Lyles, NAE
Aeronautics and Space Engineering Board

Malcolm MacKinnon, NAE *U.S.N. Retired*

David Maddox, NAE
National Academy Board on Army
Science & Technology (BAST)

Beverly Malone, NAM
National League for Nursing

Gerald Mandell, NAM *University of Virginia*

Robert McCabe, NAE
National Science Foundation

Marcia McNutt, NAS
National Academy of Sciences

Kishor Mehta, NAE
National Science Foundation

**Xiang-Jin Meng, NAS
VA-MD College of Veterinary Medicine

Richard Merrill, NAM *University of Virginia*

Richard Meserve, NAE
Carnegie Institution for Science

Martin Mikulas, Jr., NAE
University of Colorado Boulder

James Mitchell, NAS/NAE *Virginia Tech*

John Monahan, NAM
University of Virginia School of Law

Arthur Money, NAE
U.S. Department of Defense

Charles Moorman, NAE
University of Maryland

Ferid Murad, NAM/NAS
The George Washington University

Thomas Murray, NAE *Virginia Tech*

David Nash, NAE
Dave Nash and Associates, LLC

Alan R Nelson, NAM *ACP*

John Niederhuber, NAM
Inova Fairfax Hospital

James Nuckolls, NAM *Carilion Health System*

Kenneth Olden, NAM
U.S. Environmental Protection Agency

Malcolm O'Neill, NAE *U.S. Army*

Elaine Oran, NAE *University of Maryland*

Joseph Ornato, NAM
Virginia Commonwealth University

Hilliard Paige, NAE *University of Virginia*

Sorab Panday, NAE *GSI Environmental*

Arun Phadke, NAE *Virginia Tech*

Robert Phillips, NAM
American Board of Family Medicine

Dolores Piperno, NAS
Smithsonian Institution

Stephen Plog, NAS *University of Virginia*

John Edward Porter, NAM/NAS
Hogan Lovells US LLP

Arati Prabhakar, NAE
Hogan Lovells US LLP

Charles Pryor, Jr., NAE *Urenco U.S.A.*

Bhakta Rath, NAE
Naval Research Laboratory

Morton Roberts, NAS
National Radio Astronomy Observatory

Alton Romig, NAE
National Academy of Engineering

David Roop, NAE *Dominion Virginia Power*

Sara Rosenbaum, NAM
The George Washington University
School of Public Health and Health Services

Gerald Rubin, NAM/NAS
Howard Hughes Medical Institute

Barbara Rusinko, NAE *Bechtel Group, Inc.*

Robert Schafrik, NAE *GE Aviation*

Bruce Smith, NAS *Smithsonian Institution*

Edgar Starke, NAE
University of Virginia (retired)

John Stenbit, NAE

Timothy Stolzfus-Jost, NAM
Washington and Lee University

Lawrence Stone, NAE *Metron, Inc.*

Jerome Strauss, NAM
Virginia Commonwealth University

Stanley Suboleski, NAE
Evan Energy Investments

Karel Svoboda, NAS
Howard Hughes Medical Institute

David Thompson, NAE *Orbital ATK*

James Turner, Jr., NAE
General Dynamics Corporation

Harry Van Trees, NAE
George Mason University

Walter Wadlington, NAM
University of Virginia School of Law

Bailus Walker, NAM *Howard University*

George Watkins, NAS *Lehigh University*

Peter Wilhelm, NAE
Naval Research Laboratory

Donald Winter, NAE

Maurice Wood, NAM
Virginia Commonwealth University

Steven Woolf, NAM
Virginia Commonwealth University

Mary Woolley, NAM *Research! America*

Carl Wu, NAM/NAS
Howard Hughes Medical Institute

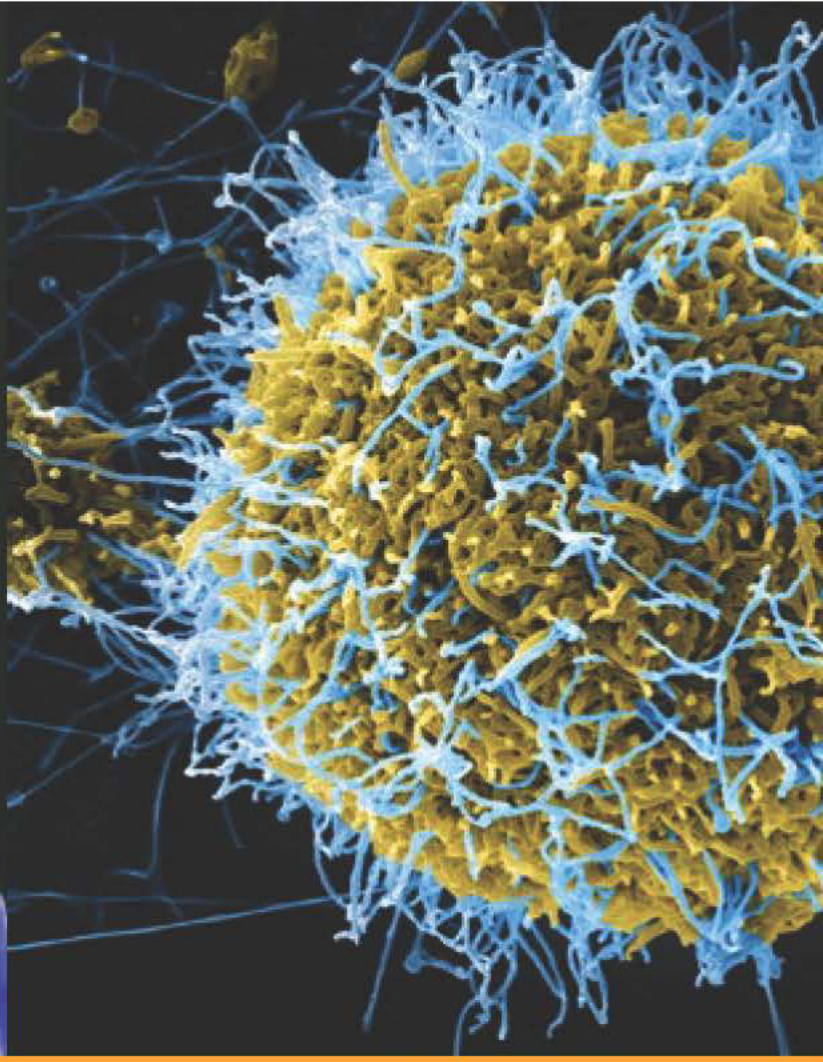
William Wulf, NAE *University of Virginia*

Roe-Hoan Yoon, NAE *Virginia Tech*

**Thomas Young, NAE

*President **Board Member

VASEM
2920 West Broad Street
Suite 120
Richmond, VA 23230
www.VASEM.org



VASEM VIRGINIA ACADEMY OF SCIENCE, ENGINEERING, AND MEDICINE