Current Topics

Promising Antiviral Strategies on the Rise

Although the human immunodeficiency virus (HIV) attracts much attention from antiviral drug developers, researchers are also making progress developing novel treatment strategies against several other viruses, including respiratory syncytial virus (RSV) and West Nile virus (WNV), according to investigators who spoke at several sessions during the 47th Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC), held in Chicago, Ill., last September.

One novel approach to developing antiviral drugs (as well as treatments for other diseases) depends on RNA interference (RNAi), an approach to shutting off expression of specific genes, according to John Devincenzo of the University of Tennessee in Memphis, Tenn., who was a coconvener and speaker during an ICAAC session, "Respiratory Viruses." Of the two known RNAi pathways in mammalian cells, one involves steps in the cell nucleus and the other does not, and it is this latter pathway on which he and his collaborators are focused as a means for blocking viruses, including RSV.

The small interfering RNA (siRNA) pathway, which is widely conserved, is a mechanism involving double-stranded RNA oligonucleotides that bind to messenger RNA (mRNA) in the cytoplasm of cells, according to Devincenzo. One promising antiviral approach is to synthesize siRNA molecules that can "down-modulate any mRNA sequence you choose," he says. In the case of a virus infecting a cell, that synthetic siRNA can be designed to interfere with an mRNA being expressed by a specific viral gene without disrupting mRNA molecules that are involved in expressing cellular genes.

That antiviral scheme sounds promising enough, but "how do we get siRNA into a cell?" Devincenzo asks. Circumstances make RSV a good, perhaps ideal, target for testing this strategy. Thus, for example, epithelial cells along the respiratory tract "naturally take up siRNA, and only the surface cells there are infected by RSV," he says. In experiments with animals, "we can follow [siRNA] uptake and modulation in an organ-specific way."

Of course, cell biology is never so simple, and it is also important to tailor siRNA molecules in such a way that they do not trigger inflammatory responses through parallel but "independent" immunostimulatory pathways, he says. Such a candidate siRNA drug that targets RSV and is being developed by Alnylam Pharmaceuticals of Cambridge, Mass., is being evaluated in phase 1 clinical trials in Europe and the United States and so far appears to be safe, he points out.

Another antiviral approach depends on tailoring monoclonal antibodies (mAbs) to bind and interfere with specific viral proteins—for example, those from WNV, according to Michael Diamond of Washington University in St. Louis, Mo., who spoke during another ICAAC session, "Viral Pathogenesis." One such mAb, E16, proves to be particularly potent against WNV, he says.

That monoclonal "can cure mice and hamsters of WNV infections, even after the virus has spread to the brain," Diamond says. E16 binds to the viral envelope protein, which is exposed along the outer surface of WNV, and "can disrupt neuronal spread of the virus, and inhibits spinal cord impairment and mitigates paralysis" that WNV can cause. One reason E16 is so potent is that it apparently blocks a key WNV "fusion site," preventing its escape from cells in which it replicates, he says. This mAb, which. is being developed commercially by MacroGenics of Rockville, Md., is expected soon to enter phase 1 clinical trials.

Jeffrey L. Fox
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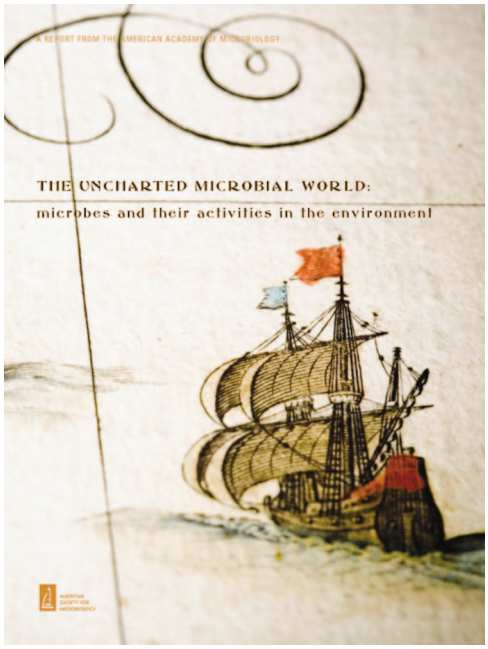
AAM Report Urges New Approaches for Analyzing Uncultivated Microbes

Although uncultivated microorganisms are the rule rather than the exception, microbiologists know relatively little about them, making it critical to continue developing new methods and approaches for studying these elusive organisms, according to a report from the American Academy of Microbiology (AAM), "The Uncharted Microbial World: Microbes and Their Activities in the Environment." The report summarizes the deliberations at a colloquium convened during February 2007, in Seattle, Washington, and lays out recommendations for characterizing the uncultivated microbial majority.

Fewer than 1% of microorganisms observed using microscopy can be cultivated, according to the AAM report. This shortfall seriously limits

the ability of researchers to study the microbial world, says colloquium participant Roberto Kolter of Harvard University in Cambridge, Mass. If an organism cannot be isolated and studied in a laboratory, then researchers may be forced to study it in its natural context, but the tools for studying complex communities of microorganisms are comparatively weak, he adds. "There is a sense of frustration that we are nowhere close to being able to study complex systems the way we are able to analyze pure cultures."

Separating microorganisms from habitats and from other species with which they might have coevolved is not always possible and can lead to misleading results, according to the AAM report. Hence, it urges researchers to move beyond studying such microorganisms in pure cultures and to develop better ways of studying them in their natural environments. Part of their efforts should be to develop methods for analyzing single cells and identifying responses of microbial communities to perturbations. According to the report, the consequences of disturbing microbial communities are poorly understood, even in microbial communities whose activities can impinge on or improve human health.



The topic of uncultivated microorganisms is newsworthy, particularly because science is bringing to light the wider significance of microbes in the biosphere, according to Kolter. "It's only recently that we are really getting a true sense of [microbial] diversity," he says. "It's also a special moment in the history of microbiology since other disciplines are recognizing the importance of studying microbial systems."

"There has been a lot more effort to define the bacteria that are intimately associated with humans, and trying to figure out what causes disease," says Nancy Freitag of the University of Illinois at Chicago Medical School, who was a member of the AAM colloquium steering committee. "The report brings into focus how little we know, and what a potentially rich reservoir of information there is in terms of new genes and new pathways" that could prove useful for combating diseases or for understanding industrial processes," Freitag continues.

Scientists outside the field of microbiology are an important audience for the report, according to Kolter. Because uncultivated microorganisms dominate in the environment, microbiology is wide open for discoveries and there is a great need for cross-disciplinary research. The report should "inspire scientists [in other disciplines] to go out and seek new collaborations" with microbiologists, he says. Moreover, the report is also important for those involved in science education because it outlines recommendations for training and curriculum planning.

The AAM report is available electronically at http://www.asm.org/Academy/index.asp?bid=2093 and also can be ordered via e-mail from colloquia@asmusa.org.

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White House Proposes Plan for Improving Safety of U.S. Imports

A White House-appointed, interagency federal group put forth a plan last November aimed at better protecting consumers and enhancing the safety of imported goods entering the United States, now amounting to nearly $2 trillion of goods through more than 825,000 importers each year. The new action plan proposes a strategy that focuses on a risk-based prevention with a verification model that allocates import safety resources based on risk. Among its specific recommendations, it calls for the Food and Drug Administration (FDA) being authorized to pursue mandatory recalls of food products and also supports ongoing collaborations between FDA and other federal agencies with specific roles in food safety, including the Centers for Disease Control and Prevention and the U.S. Department of Agriculture's Food Safety and Inspection Service. The plan also recommends that FDA have authority to require producers of high-risk foods in other countries certify that their products meet FDA standards and that U.S. agencies develop cooperative agreements with foreign governments, among other reasons, to increase training for foreign inspection agencies to build their capacity for assuring safety of exported products.

Freeloading Mutants Arise in *Pseudomonas aeruginosa* Colonies

Some bacterial communities support freeloaders. Specifically, colonies of *Pseudomonas aeruginosa* from infected patients with cystic fibrosis contain variants that carry a mutation in the *lasR* gene that controls quorum sensing. Quorum sensing coordinates production of virulence factors, including those that control the secretion of extracellular enzymes. Some of the *lasR* mutants stop metabolizing nutrients and, instead, live off nutrients produced by their neighbors, according to microbiologist Martin Schuster and his collaborators at Oregon State University (OSU), Corvallis, who call this type of nutritional exploitation "social cheating."

"Social cheating is a mechanism for an individual to save energy and avoid the costs of cooperation," says Schuster. This particular form of social cheating occurs during quorum sensing and confers on the cheaters, at least temporarily, a growth advantage over their more productive colony mates, he and his collaborators report in the October 2, 2007 *Proceedings of the National Academy of Sciences*.

Cheaters are not more virulent—just the opposite, according to Schuster, who says that virulence is a population trait that requires a large number of cells acting together. Although an individual cheater saves energy by not making enzymes to break down food, this individual advantage goes only so far. If too many cheaters accumulate, the virulence of the entire bacterial community is threatened, and some cheaters revert to being productive community members.

When *P. aeruginosa* cells grow in media containing casein, quorum sensing triggers the cells to produce extracellular proteases that sustain growth and contribute to virulence. In laboratory experiments, Schuster showed that after about 100 generations under such conditions, *lasR* mutants emerged that did not degrade casein, yet flourished and showed a growth advantage over wild-type *P. aeruginosa* within the same community. "The cheaters rely on cells around them that secrete extracellular enzymes," Schuster explains, and because the "cheaters are not investing in metabolic energy to make extracellular enzymes, they gain a growth advantage."

When the population of social cheaters grows larger, however, it slows growth of the overall bacterial community. Thus, for example, when the researchers added increasing amounts of *lasR* mutants to cultures of *P. aeruginosa*, colony growth rates fell in a dose-dependent fashion. In some cases, social cheaters restored protease activity and reverted back to being cooperative members of the community. The signals involved in this switch are poorly understood and need to be investigated.

When the first *lasR* mutants were detected in patients with either acute or chronic *P. aeruginosa* infections, some experts speculated that quorum sensing was not important for virulence and, thus, a poor therapeutic target. However, these new findings from Schuster's team at OSU "show that quorum sensing as a whole is very important for virulence, and you have the evolution of cheaters that can survive when quorum sensing is high," says Marvin Whiteley, a molecular geneticist at the University of Texas, Austin, who was a co-organizer of the ASM Cell-Cell Communication in Bacteria meeting, held this past October in Austin. During that meeting, Stephen Diggle of the University of Nottingham, United Kingdom, presented data similar to Schuster's using the same strain of *P. aeruginosa* (since published in *Nature*, **450:**411–414). "So the data are corroborated by an independent investigation," Whiteley says.

Finding therapeutic agents to control quorum sensing could have broad applications. Acyl-homoserine lactones coordinate the virulence of not only *P. aeruginosa* but also more than

Unusual Facts about Unusual Pathogens

Here is some unusual information recently uncovered about a variety of pathogens:

- The genome of *Malassezia globosa*, a fungus responsible for dandruff and other skin conditions in humans, contains many genes encoding hydrolases, particularly lipases, according to researchers at P&G Beauty in Cincinnati, Ohio, whose findings are published in the November 13, 2007 *Proceedings of the National Academy of Sciences (PNAS)*.
- Adenovirus serotype 14, which causes severe and sometimes deadly respiratory infections, is becoming more common in the United States, according to the November 15 *Morbidity and Mortality Weekly Report*.
- Predatory species of dinoflagellates alter their swimming patterns in characteristic ways once bacterial prey become available, adjusting from random swims into helical trajectories that may lead to build-ups of toxins that help to account for fish kills, according to Joseph Katz of Johns Hopkins University in Baltimore, Md., and his collaborators, whose findings appear in the October 22, 2007 *PNAS*.

70 other gram-negative species, including many pathogens. "There's still a lot to understand about quorum sensing as a therapeutic target," says Whiteley, but Schuster and Diggle's results support this approach for developing novel agents to use in treating chronic infections associated with diseases such as cystic fibrosis.

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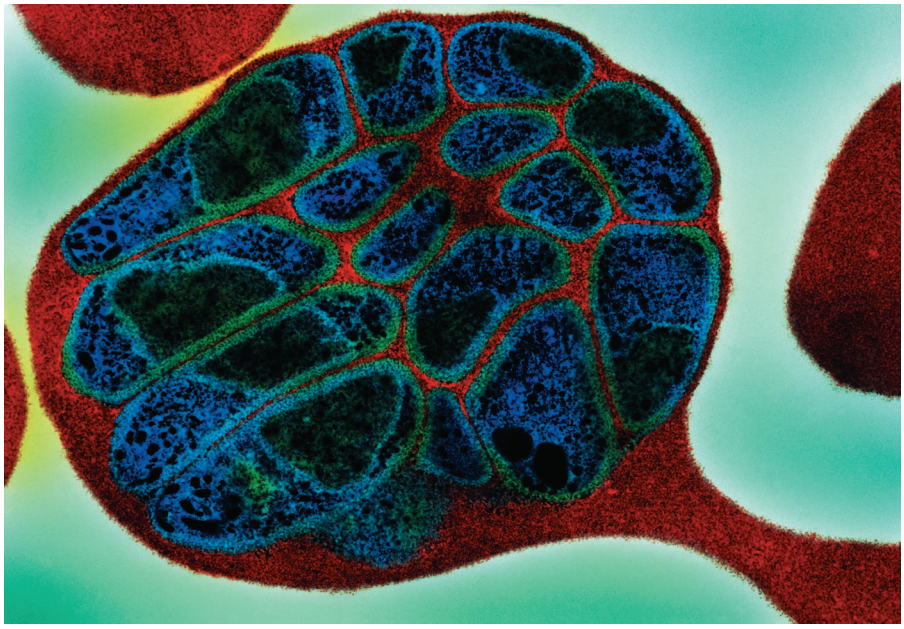
Money, Technology, and Fresh Ideas Converge on Malaria



Colored transmission electron micrograph (TEM) of a human red blood cell (red) infected with the malarial parasite *Plasmodium* sp. (blue). This deadly parasite continues to be the focus eradication and vaccination efforts. (Magnification, ~×22,000. © Moredun Scientific Ltd/Photo Researchers, Inc.)

What will it take to eradicate malaria, an ancient disease that continues to kill more than a million people each year, many of them babies and most of them poor? The answer is a coordinated, multifaceted attack, according to participants at a symposium, sponsored by the Malaria Research Institute at the Johns Hopkins Bloomberg School of Public Health (JHBSPH) and held at the New York Academy of Sciences last October.

Big donors, including the Global Fund, the Bill and Melinda Gates Foundation, the World Bank, and the U.S. President's Malaria Initiative, are fueling the current campaign, and technical breakthroughs, especially genomic sequencing of the *Plasmodium falciparum* parasite and the *Anopheles gambiae* mosquito, are enabling development of innovative scientific weapons against this deadly protozoan.

Effective vaccines, key to the antimalaria campaign, continue to prove difficult to develop. "*P. falciparum* has about 5,300 different antigens and a very complicated lifecycle, spent mostly in hepatocytes and erythrocytes," says Christian Loucq who directs the PATH Malaria Vaccine Initiative (MVI) in Bethesda, Md. Moreover, there are four distinct plasmodia that infect humans—*P. vivax*, *P. malariae*, and *P. ovale* in addition to *P. falciparum*, the deadliest of this group.

Several vaccine candidates in the MVI portfolio of eight are considered promising, Loucq says. One of them—RTS,S, developed by Joe Cohen at GlaxoSmithKline—has recently proved clinically safe, partially effective, and immunogenic in infants, *P. falciparum*'s most vulnerable population. This is good news in a research area more used to disappointment than success (http://www.malariavaccine.org/ab-current_projects.htm).

Vaccine developers need the right antigens, and Susan Kraemer of the Johns Hopkins Malaria Research Institute (JHMRI) in Baltimore, Md., is investigating a protein family called PfEMP1 (*P. falciparum* erythrocyte membrane protein 1), considered a prime protective immunity target. "In addition to proving useful in vaccine design, these proteins can help us understand how the parasite causes severe disease and how it evades the immune response," she says.

One of Kraemer's colleagues at Johns Hopkins, Marcelo Jacobs-Lorena, is focusing his research on the mosquito rather than the parasite. "Transmission of *Plasmodium*," he says, "depends on the successful differentiation of *P. falciparum* in its mosquito vector." Transgenic malaria-resistant mosquitoes are already thriving in Jacobs-Lorena's lab and, importantly, proving more fit than the *Plasmodium*-infected controls. Jacobs-Lorena's next step is to introduce resistance genes into wild mosquito populations to evaluate their effect on malaria control.

"As endemic countries scale up interventions, fast, accurate, and noninvasive parasite detection is necessary," says Sungano Mharakurwa, scientific director of JHMRI's field research site in Zambia. "Current definitive malaria testing requires drawing blood, which is difficult in children, the de facto sentinel malaria survey group, and problematic in communities with blood taboos." Mharakurwa uses polymerase chain reaction (PCR) technology to detect *P. falciparum*

DNA fragments in saliva and urine. "After some technical refinements, these sharp- and needle-free detection methods are expected to be ready for drug and vaccine trials, efficacy-monitoring programs, and wide-reaching epidemiological surveys," he says.

Another important technical achievement is the simplified, home-use screening test—a dipstick that detects a parasite-specific protein in urine samples—developed by David Sullivan, also at JHMRI. "Home testing will allow much greater access to malaria diagnosis and enable earlier treatment," according to Sullivan.

Recognizing the need for their scientists to work in a malaria-endemic setting, JHBSPH established a research and training station in rural Zambia in 2003.The Malaria Institute at Macha (MIAM), a collaborative effort with the Zambian government, the Macha Mission Hospital and the Macha Malaria Research Institute, provides the facilities and infrastructure for multifaceted research.

"Here we study malaria with all the confounding factors of climate, movement of peoples, and cultural perceptions," says Douglas Norris, who studies mosquito biology, behavior, and genetics at MIAM. "Macha, where people live in scattered clusters of huts connected by dirt roads and *Anopheles* mosquitoes breed in small pools filled by periodic wet-season rains, is our best hope of conquering malaria. It's here in this living laboratory that we gain a true appreciation and understanding how people and insects interact, and it's here that we'll be able to evaluate the tools and ideas needed to eradicate the disease."

Marcia Stone
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Look Carefully for Viruses, and Novel Examples Can Be Found

Humans harbor a richer and more diverse array of viruses, one that has experts marveling over what they missed until recently, according to virologists who spoke during the 47th Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC), held in Chicago, Ill., last September. Although many of those viruses cause diseases, determining which of them instead are mere bystanders sometimes proves a challenging task, say several researchers who reviewed recent findings during the ICAAC symposium, "New Viruses: Old Diseases?"

There is an "explosion" of new viruses associated with the human respiratory tract, according to Kenneth McIntosh of Harvard University Medical School in Boston, Mass. One of the more recently recognized among them, called the bocavirus, "doesn't resemble anything we've seen before," he says. "It's been found every time we've looked for it, among children all over with world with respiratory diseases."

First identified in samples from children in Sweden, the DNA-based bocavirus is part of a family that includes the bovine parvovirus and canine minute virus, both of which cause diarrhea in their respective hosts, according to McIntosh. Although the human bocavirus can be detected in stool samples, its presence there appears to "be incidental," he says. Instead, the human bocavirus causes infections of the lower respiratory tract in children, often coinfecting with the winter respiratory virus.

The human metapneumovirus is another recently identified and also widely distributed viral pathogen that infects the human respiratory tract, affecting individuals of all ages but more commonly children, McIntosh continues. "After rhinovirus and RSV [respiratory syncytial virus], human metapneumovirus is the most common. It's very similar to RSV, exacerbates asthma, and often is a coinfecting virus." Rhinoviruses, meanwhile, are the most frequently found viruses

Hepatitis A Vaccine Good News, HIV Vaccine Bad News

The hepatitis A vaccine proves effective when administered to individuals after they are exposed to the hepatitis A virus, making it at least as effective as immune globulins for postexposure prevention of such infections, according to Beth Bell of the Centers for Disease Control and Prevention in Atlanta, Ga., and collaborators, whose findings appear in the October 18, 2007 *New England Journal of Medicine*. Meanwhile, findings from international studies of a vaccine meant to protect against HIV continue to prove puzzling. Specifically, volunteers with immunity to the adeno-associated virus used as a carrier for synthetic HIV genes in the Merck & Co. investigational HIV vaccine, called V520, developed more infections with HIV than did those volunteers who received a placebo. Furthermore, the V520 vaccine was not effective at either preventing HIV infection or at reducing viral loads in those volunteers who became infected with HIV during the trial. The "reasons for this result are still being studied," according to officials from Merck and investigators associated with the U.S. HIV Vaccine Trials Network.

in humans of all ages who have acute infections of the respiratory tract. The rhinoviruses also can coinfect with other viruses targeting the respiratory tract, and can exacerbate asthma among children or lead to bronchitis in adults.

Yet another novel virus is found in the human respiratory tract and is associated with, if not the outright cause of, infections, according to David Wang of Washington University School of Medicine in St. Louis, Mo. This recently identified virus, designated WU, is a polyoma virus that he and his collaborators uncovered while screening respiratory secretions for nucleotide sequences with virus-like signatures. Separately, Tobias Allander, Bjorn Andersson, and their collaborators at the Karolinska Institute in Sweden, early in 2007 reported finding a similar polyoma virus, designated KI, also in human respiratory secretions. That virus is "related to, but divergent from WU," and those two viruses are still more divergent from two other frankly pathogenic polyoma viruses that were isolated from immunocompromised individuals several decades ago, according to Wang. Although the pathogenicity of WU is "unknown," it is found in "patients with respiratory disease," he says.

Uncertainties about health effects are not peculiar to newly recognized viruses found in respiratory secretions. Human herpesvirus-6 (HHV-6), which is one of eight known herpesviruses, is the only one of this group that integrates into the genome of its human host, according to Kate Ward of University College in London, United Kingdom. Once there, the virus "persists for life" and "often is silent," she says. Whether it is merely a passenger of its hosts or can become an active pathogen remains uncertain. During its unintegrated phase, HHV-6 typically infects children who are two or younger, leading to a rash and fever, or sometimes to a fever and fits that occasionally are "prolonged," Ward says. Also, among some adults who become immunocompromised, the virus "causes a nasty encephalitis that is about 50% lethal." In such cases, it "almost certainly comes from inside the person, but there is no evidence it comes from the integrated [version of the virus]. There's so much we don't know."

Jeffrey L. Fox

Plenty of Novelties To See in Green and Red Alga Genomes

The genome of the single-celled green alga *Chlamydomonas reinhardtii* contains 15,000-plus genes, including hundreds that are associated with carbon dioxide capture and generation of biomass, according to the team of about 100 scientists from the U.S. Department of Energy Joint Genome Institute (DOE JGI), the University of California, Los Angeles, and the Carnegie Institution, whose findings appear in the October 12, 2007 issue of *Science*. Among other points of interest, this species "possesses the largest known array of enzymes that manufacture the signaling molecules cyclic AMP and cyclic GMP," says Simon Prochnik of DOE JGI. "These cyclic nucleotides play key roles in shuttling nutrients into the cell, controlling motility of the organism via flagellar function, and determining sexual development." Meanwhile, the compact genome of the much smaller red alga, *Cyanidioschyzon merolae*, which contains only 30 transfer RNA (tRNA) genes, includes another 11 tRNA genes that are each divided into two segments, according to Yasuhiko Sekine and collaborators at Rikkyo University in Tokyo and several other institutions in Japan, whose findings appear in the October 19 *Science*. Although appropriate tRNA molecules are produced, they arise through processing of an unusual circular RNA intermediate, the researchers note.

Yellow Fever Virus-Based West Nile Vaccine Edges Others Protecting Horses

In veterinary clinical trials involving a direct viral challenge to the central nervous system of horses, three commercially available vaccines protected animals against severe brain and spinal cord disease that West Nile virus (WNV) can cause, according to Kathy Seino and her collaborators at the College of Veterinary Medicine, University of Florida (UF) in Gainesville. Moreover, the most recently licensed of those vaccines protected animals against developing even mild clinical signs of WNV infection. Details appear in the November 2007 issue of *Clinical and Vaccine Immunology* (**14:**1465–1471).

"This is the first direct comparison of all three vaccines using this highly virulent challenge model," says Aaron C. Brault of the School of Veterinary Medicine at the University of California, Davis (UCD), who was not involved in the research. "This study showed that vaccination was able to protect against virus delivered to the central nervous system, for me an unexpected result," adds Bill Reisen, also of UCD.

That challenge model, which involves injecting high titers of WNV directly into the central nervous system of horses, is considered an advance over two alternative approaches, according to Seino. Even though the better of those two other

approaches for challenging horses with WNV "could induce viremia and neutralizing antibody," she says, it failed to produce "consistent clinical signs of WNV encephalomyelitis."

In the UF study, some horses were left untreated while others were vaccinated with one of three different vaccines. The first of them, designated K-WN and marketed as West Nile-Innovator, consists of a formalin-inactivated, whole-virus vaccine that came on the market in 2001. The second, designated CP-WN and marketed since 2004 as Recombitek equine WNV vaccine, consists of an attenuated canarypox virus vector carrying genes encoding WNV membrane and envelope proteins. The third, designated WN-FV and marketed as PreveNile since 2006, consists of an attenuated yellow fever virus (a flavivirus) vector carrying WNV genes encoding membrane and envelope proteins.

"These studies along with the safety and efficacy work performed in this laboratory demonstrate that the flavivirus chimera is safe and results in at least a 12-month duration of immunity in horses," says Seino, referring to the third of those vaccines, WN-FV or PreveNile. "Vaccine development, licensing, and testing for protection against disease in horses is crucial for understanding protective immunity in humans, since these are outbred hosts and variation in immune responses can actually be accounted for."

"The study definitely confirms what we have been seeing in field situations and reported in the literature," says Tasha Epp of the University of Saskatchewan in Saskatoon, Saskatchewan, Canada. "It is wonderful to see that there are other vaccine technologies that provide the same type of protection [as the formalin-inactivated whole virus vaccine]. This has been an opportunity to study the differences in immune response, clinical presentation [from different vaccines that could lead to] creating specialized protocols that minimize bad reactions. . . I think it is important to note that the level of immune response does not predict whether a horse is protected."

Since WNV emerged in the United States in 1999, it has caused disease in nearly 25,000 horses, killing about 6,000, as well as hundreds of thousands of birds, according to Seino. One of 11 horses infected with WNV develops disease.

Meanwhile, WNV infections lead to about 4,000 reportable cases among humans in North America each year, with many thousands of asymptomatic infections going undetected. At least one-third of the confirmed cases involve the central nervous system, and there are more than 90 fatalities per year. For such reasons, a vaccine similar to WN-FV, which protects horses against both mild and severe clinical symptoms of WNV infection, is being developed for human use by Acambis, a biotechnology company with facilities in Cambridge, Mass., and Cambridge, England. ChimeriVax-West Nile is undergoing Phase 2 clinical testing, the company reports.

David Holzman
David Holzman is the *Microbe* Journal Highlights Editor.