Current Topics

Global Tracking Ill Animals in the Wild—Not So Simple

“Unless you have a lot of dead animals, you’re not going to find it,” says Pierre Rollin of the Centers for Disease Control and Prevention (CDC) in Atlanta, Ga., referring specifically to a recent outbreak of Ebola virus among African gorillas but, more generally, to outbreaks of infectious diseases among wild animals. His statement epitomizes the challenge facing those who seek to track emerging diseases of zoonotic origin for various purposes, including to protect human and animal health, food sources, and ecological equilibriums. He spoke during a workshop on “Sustainable Global Capacity for Surveillance and Response to Emerging Zoonoses,” the first in a series on this topic that was convened by the Institute of Medicine in Washington, D.C., last June.

Within the United States, the U.S. Geological Survey (USGS) is the only federal agency with a program that is dedicated to monitoring diseases in wild animals and, even so, has “no mandate to report diseases and deaths among wildlife,” says Scott Wright of the USGS National Wildlife Health Center in Madison, Wisc. “We don’t pretend to know what’s going on every day with wildlife disease.” Thus, most such information is “anecdotal.”

Fortunately, however, worldwide surveillance of animals in the wild for infectious diseases does not rely entirely on anecdotes and body counts. In 2006, for example, the Rome-based Food and Agriculture Organization (FAO) of the United Nations established the Global Early Warning System (GLEWS) to track major animal diseases, including zoonoses, and part of its mandate is to “track rumors” about outbreaks, according to workshop participant Stephane de La Rocque of FAO. Recently, for instance, GLEWS helped to forecast an outbreak of Rift Valley fever in Africa, he says. However, and perhaps because of its high profile, infectious disease experts who work within this program tend to find themselves also acting as diplomats, forging delicate alliances with local ministries of health because, to do otherwise, is to court “disaster,” he says.

A major goal of those alliances is to convince health officials in countries to report animal disease outbreaks while adhering to standards and practices set by several international bodies, including the World Organization for Animal Health (OIE) in Paris, France. Those reporting standards, which are updated annually, are “legally binding,” and countries that uphold them “can’t be challenged,” says Alejandro Thiermann of OIE. However, in reality, health ministries in many countries tend to flout those reporting requirements if, for instance, they threaten local farmers and other businesses with economic adversity. Securing compliance, he points out, requires both “political will” and “technical ability.”

Economic pressures can undermine that will, according to William Karesh of the Wildlife Conservation Society.
in Bronx, N.Y. For instance, poachers capture, kill, and illegally trade “billions of kilograms of bush meat per year,” sometimes accelerating the diffusion of zoonotic diseases under the guise of meeting food needs, he says. Further, a “lot of problems arise from mixing of species in markets, particularly in China, Vietnam, and Cambodia, [but] it’s pretty common everywhere,” he adds. “The demand for protein is not going away.” Such practices, along with “habitat destruction,” help to drive the spread of zoonotic diseases.

Working in the reverse, zoonoses can threaten food security, points out Ilaria Capua of Instituto Zooprofilattico Sperimentale delle Venezie in Padova, Italy, and a representative of OIE. In particular, the H5N1 avian influenza virus, which infects about 50 bird and about 10 mammalian species, and is endemic on three continents, is proving challenging to monitor and difficult to manage, she says. When that virus arrived in Africa in 2006, it was a “catastrophe [because] there was no early warning system.” By now, the bare bones of a surveillance network is present in 20 African nations, but it “would help if we get support and set up collaborations so we don’t lose what we’ve invested in,” she says.

Such support along with local acceptance is utterly critical if global health surveillance programs are to succeed, according to Jeremy Farrar of the Oxford University Clinical Research Unit in Ho Chi Minh City, Vietnam. “The center of gravity has to move to where the problems lie,” he argues. “And if you want to deliver global health, the best way is through small institutions that are embedded locally.” Furthermore, he adds, public and clinical health activities “should be brought back together” with those involving animal health.

At the U.S.-domestic level, there are some ingenious efforts that appear to be moving in that direction. One example involves monitoring animal diseases of species having intimate contact with humans, according to Larry Glickman of Purdue University in West Lafayette, Ind. Specifically, a reporting network is under development that takes advantage of the Banfield Pet Hospital chain, which has facilities in 44 states and is headquartered in Portland, Ore. Data that are collected through this network and then analyzed provide a view into disease trends affecting 170 million or so companion animals—mainly dogs and cats—in the United States, most of which not only are in close contact with their owners but also are exposed to insects or other animals in the wild that sometimes carry zoonotic diseases.

This electronic disease-tracking system collects data from more than one million visits of pets to U.S.-based veterinary facilities, extracting surveillance information that is encoded by neighborhood, Glickman says. The surveillance is so incisive that officials from the chain of veterinary hospitals now use a consent form to assure pet owners of anonymity when using data for public health purposes.

Such close and timely monitoring can be used to detect “vector-borne pathogens” to “alert vaccine producers” of anticipated product needs, and to forecast outbreaks, Glickman continues. For example, by monitoring ticks on dogs in parts of North Carolina, investigators using this system predicted by one month a surge in Lyme disease cases among humans in that area.

Jeffrey L. Fox
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If Polio Were Eradicated, Study Suggests Need for Vaccinations

After Switzerland switched from inoculating children with the live-attenuated oral polio vaccine (OPV or Sabin) to the inactivated polio vaccine (IPV or Salk), analysis proved that samples from Swiss wastewater treatment plants tested positive for at least 3 years for Sabin-like poliovirus strains, including virulent revertants, according to Alfred Metzler of the University of Zurich, Switzerland. These findings reinforce the notion that polio vaccination programs should continue for many years, even in countries where the disease itself seldom, if ever, occurs. Details of the research appear in the September Applied and Environmental Microbiology (74:5608–5614).

The findings are “timely and important” because they address issues related to the ongoing global campaign to eradicate poliomyelitis, according to Vadim Agol of the Russian Academy of Medical Sciences in Moscow, Russia. “The World Health Organization (WHO) had planned to discontinue OPV use shortly after the last known isolation of a wild poliovirus, thereby leaving many human populations, especially in developing countries, fully susceptible to poliovirus,” he says. “Since OPV strains can persist in populations for years and . . . acquire pathogenic properties, the existence of such nonimmune populations would be ‘rich soil’ for the resurrection of pathogenic viruses.”

As part of that eradication campaign, which launched in 1988, many countries now use the Salk, or IPV, instead of the Sabin vaccine, to avoid the risk of vaccine-associated poliomyelitis. Metzler as well as others calls the Salk vaccine “completely safe.” However, he points out that it is “less effective” than the Sabin vaccine because the latter “induces gut mucosal immunity, a prerequisite to prevent re-infection, fecal virus shedding, and transmission to susceptible contacts.”

Further, the Sabin vaccine can lead to persistent infections in individuals with humoral immunodeficiencies, and they can lead to emergence of strains of virulent viruses, known as immunodeficient, vaccine-derived polioviruses. If
vaccine coverage is suboptimal, these viruses may cause polio outbreaks. Put another way, exclusive worldwide use of the Salk vaccine could lead to emergence of vaccine-derived polioviruses (VDPV). Indeed, Metzler says, “VDPVs reported so far have emerged in [Sabin vaccine] settings with vaccination gaps.” Monitoring these outbreaks and trends is possible because the Sabin-type and wild-type polioviruses are “antigenically distinct,” he points out. His findings appear to confirm “silent circulation of Sabin-type viruses and emergence of VDPVs were more likely to occur in a [Salk type vaccine] setting.”

Between 2004 and 2006, and continuing into 2008, Metzler and his collaborators found 20 of 174 samples to be positive for 62 Sabin-like poliovirus strains from municipal wastewaters, as well as one sample containing wild-type poliovirus that appeared to originate from Chad. “The recovered Sabin-like viruses and VDPVs were most likely imported from countries using Sabin vaccine,” he says. However, “imported polioviruses, being either Sabin-like, vaccine-derived poliovirus, or wild-type poliovirus, do not circulate, or do so during a limited time at best.” Thus, the local setting is unlikely to serve as a reservoir.

“Finding Sabin-like polioviruses in 20 samples over a 3-year sampling period in Zurich is new for Switzerland, an inactivated polio vaccine-using country with a high standard of hygiene,” says Walter Dowdle, a founder of The Task Force for Child Survival and Development, who served for many years with the Centers for Disease Control and Prevention in Atlanta, Ga. Thus, maintaining high-coverage use of the vaccine is critical “not only because wild-type polioviruses may still be imported, but because transmission or transportation of OPV-derived strains revert to neurovirulence,” adds Tapani Hovi of the WHO Collaborating Centre for Poliovirus Surveillance and Enterovirus Research of the National Public Health Institute in Helsinki, Finland.

The study by Metzler and his collaborators “should alert both health authorities and society to the heavy danger of stopping immunization against polio,” Agol says. “WHO is now reconsidering their plans to stop OPV usage in the near future, and it is very important to additionally push it in the right direction.”

David Holzman
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MicroRNAs Appear To Govern Latency of Herpesviruses

The RNA molecule known as the latency-associated transcript (LAT) of herpes simplex virus 1 (HSV-1) is hydrolyzed into microRNAs that suppress active replication of this virus, which lies dormant within trigeminal nerves of the face between cold sore flare-ups. This finding helps to explain the “mystery of what LAT RNA does,” says Bryan Cullen of Duke University Medical Center in Durham, North Carolina.

Noting that several other herpes viruses encode microRNAs, Cullen suspected that HSV-1 also does so. LAT seemed a potential source for micro-RNAs because it is transcribed at substantial levels within infected neurons, is highly unstable, and does not encode proteins, he says. For years, he and other scientists wondered why the virus makes this RNA molecule that does not produce a protein.

Cullen, collaborating with Donald Coen and others at Harvard Medical School in Boston, Mass., isolated RNA molecules from the trigeminal ganglia of mice that were latently infected with HSV-1; they then sequenced cDNA molecules derived from those RNA species. LAT, they found, is the source of four micro-RNAs, designated miR-H2, miR-H3, miR-H4, and miR-H5. A fifth HSV-1 microRNA, miR-H6, does not derive from LAT, and its precursor remains unidentified.
Among this set of microRNAs, miR-H2 is transcribed in an antisense orientation to ICP0, an HSV-1 protein that helps to reactivate latent HSV-1. Specifically, miR-H2 reduces ICP0 protein expression, but does not significantly affect ICP0 messenger RNA levels, suggesting translational regulation of viral gene expression. MiR-H6 is complementary to, and inhibits expression of, viral RNAs that encode ICP4, a transcription factor required for viral replication and infection. Additional details appear in the August 7, 2008 Nature (454:780–783).

The functions of the other microRNAs are not known. Cullen suspects that miR-H3 and miR-H4 down-regulate ICP34.5, a key virulence factor for HSV. They are transcribed in an antisense direction to the mRNA that encodes this protein.

In a related development, Philip R. Krause of the Food and Drug Administration in Rockville, Md., and his collaborators coincidentally described a similar, LAT-related microRNA, designated miR-I, in ganglia of guinea pigs infected with HSV-2, the herpesvirus that infects genital tissue. The miR-I from HSV-2 reduces expression of ICP34.5, according to their report in the August 5, 2008 Proceedings of the National Academy of Science.

“It was a surprise to read about the other group’s work that parallels ours,” Cullen says. HSV-2’s miR-I is about 70% similar in sequence to miR-H3 from HSV-1. “These two microRNAs likely perform the same function in these two related viruses,” Cullen says.

Cullen is collaborating with researchers at Regulus Therapeutics in Carlsbad, Calif., to test new drugs designed to bind microRNAs that keep HSV-1 dormant. In the overall scheme, such drugs should activate the virus, making it vulnerable to killing with drugs such as acyclovir that inhibit replicating viruses. “In principle, you could activate and kill all of the virus in a patient and completely cure a person,” he says. If successful, the same approach could be followed for treating genital herpes or shingles, which is caused by Varicella zoster. It is distantly related to the other two herpesviruses, and sometimes remains dormant for decades after infecting children and causing chickenpox.

However, microbiologist Patricia Spear of Northwestern University Medical School in Chicago, Ill., raises doubts about such plans to inactivate microRNAs as a step toward flushing such latent viruses from their tissue reservoirs. “Latent viruses reside in neurons,” she says, and “if viral reactivation was induced in all latently infected neurons, there may be considerable damage to these neurons.” Despite this reservation, she calls Cullen’s work “high quality” and “an important discovery,” noting it may provide insights about how HSV replicates or remains latent.

Carol Potera
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Noteworthy Federal-Level Developments on Food Safety

Recent noteworthy developments pertaining to food safety at the federal level include:

- Officials at the Food and Drug Administration (FDA) said in August that food producers will be permitted to irradiate iceberg lettuce and spinach up to a maximum absorbed dose of 4.0 kilograys as a way of controlling food-borne pathogens and extending shelf lives of these fresh products.

- Officials at the U.S. Department of Agriculture (USDA) said in August that they are amending meat inspection regulations to ban the slaughter of cattle that become nonambulatory, widely known as “downer” cattle, to “maintain consumer confidence in the food supply, eliminate further misunderstanding of the rule, and to make a positive impact on the humane handling of cattle,” says USDA Secretary Ed Schafer.

- The U.S. Court of Appeals in August reversed a lower-court ruling and stated that USDA has the authority to restrict the use of a rapid diagnostic test for bovine spongiform encephalopathy, thus blocking efforts by Creekstone Farms Premium Beef of Arkansas City, Kansas, to test cattle before exporting them to Japan and Korea.

The main features of mamavirus closely resemble those seen in other mimiviruses. For instance, it forms complex virus-producing factories, and the particle is surrounded by a characteristic, fibril-covered, multilayered membrane. By contrast, Sputnik is rich in novelty and its genes appear to derive from several possible sources, including an unknown family of small viruses, an archaeal virus or plasmid, and a mimivirus. “One of Sputnik’s most remarkable characteristics is its apparent chimeric origin,” La Scola says. “[It] seems to be one of the most convincing cases so far for gene mixing and matching within the virus world.”

La Scola and his collaborators spotted the smaller viruses reproducing inside mamaviruses, and transmission electron microscopy reveals that mamavirus and Sputnik particles are produced simultaneously but at different locations and rates. Importantly, Sputnik reproduction interferes with that of APMV, resulting in deformed mamavirus progeny. In most cases, several capsid layers accumulate asymmetrically at only one pole of the mamavirus particles, while fibril distribution becomes erratic. Further, when mamavirus and Sputnik are coinoculated into amoebas, the yield of infective mamavirus particles decreases by roughly 70%. Sputniks, it seems, sicken the mamavirus.

“The finding that Sputnik can pirate the factory of another virus to propagate at the expense of its host makes it a ‘virophage,’ an infecting agent functionally analogous to bacteriophage,” La Scola says. He urges a search for additional virophages “to shed more light on the unique modes of viral interactions.” For example, virophage might serve as vehicles for lateral gene transfer between giant viruses, which constitute an important part of the DNA virus population in diverse marine environments, he points out. “Indeed, the presence of three APMV genes in Sputnik suggests that gene transfer between a virophage and a giant virus is not only possible, it’s probably central to viral evolution.”

Jean-Michel Claverie, codiscoverer of the first mimivirus and director of the Mediterranean Institute of Microbiology in Marseille, considers the discovery of Sputnik a pivotal one and says “the fact that a virus can get sick makes it more alive.” According to Claverie, “discovery of a virophage infecting a mamavirus factory lends credit to the radical new view known as ‘Girus,’ which defines the factory, not the infecting particle, as the viral organism.” It also holds that viruses are alive, even though they cannot reproduce entirely on their own. Thus, Claverie and others in this camp see no difference between the cell-like virus factory and intracellular bacteria such as Rickettsia and Chlamydia, rendering viruses as bona fide “living” microorganisms.

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Blocking Bacterial Sensor Protein Protects Infected Mice

Molecules that disrupt the outer-membrane sensor protein QseC, which is widely distributed among gram-negative bacterial pathogens, also can interfere with their capacity to produce damaging and sometimes lethal bacterial toxins, according to Vanessa Sperandio of the University of Texas Southwestern Medical Center in Dallas and her collaborators. Thus, targeting QseC provides a novel and potentially powerful means for

Noteworthy Activities Affecting Companies Developing Vaccines, Antimicrobials

Recent noteworthy developments affecting companies that are developing vaccines, antimicrobials, or related products include:

- **Emergent BioSolutions of Rockville, Md., in September received a four-year, $24.3 million contract from the Department of Health and Human Services to develop its monoclonal antibody-based anthrax drug AVP-21D9, including for clinical testing and to increase production capacity.**

- **Maxygen of Redwood City, Calif., was awarded $3.4 million from the U.S. Department of Defense to work on ParallelaVax, a technology for accelerating production of experimental vaccines, and to test them in animal models.**

- **Replidyne of Louisville, Colo., in August announced plans to cut staff and to try to merge or sell programs for developing DNA-replication inhibitors such as faropenem, which targets gram-positive pathogens such as Clostridium difficile.**

- **XOMA of Berkeley, Calif., in September received a $65-million contract from the National Institute of Allergy and Infectious Diseases to continue developing and to begin clinical safety and animal efficacy testing of monoclonal antibody-based products for protecting against botulinum toxins.**
protecting hosts against the havoc caused by a diverse set of pathogens, including *Salmonella enterica* serovar typhimurium, *Escherichia coli*, and *Francisella tularensis*, that infect mammals as well as other bacteria that infect plants, without directly blocking bacterial growth, they report in the August 22 issue of *Science* (321: 1078–1080).

At least 25 pathogens depend on QseC to respond to chemical signals in the host—for instance, to hormones such as epinephrine and norepinephrine—by producing virulence factors including toxins, according to Sperandio. She and her collaborators subjected enterohemorrhagic *E. coli* (EHEC) to a set of 50,000 organic molecules, determining which of them blocked expression of bacterial virulence factors without being toxic for other reasons, such as by inhibiting transcription.

One member of this set, a synthetic compound with the formula [N-phenyl-4-[[phenylamino]thioxomethyl]amino]-benzenesulfonamide], mercifully designated LED209, does not blunt growth of *S. enterica*, *E. coli*, and *F. tularensis*, but does bind to the QseC receptor, disrupting signaling that leads to production of toxins. It was chosen for further study based on potency, minimal toxicity to human and animal cell lines, and potential for chemical modification.

LED209 protects mice against otherwise lethal doses of *F. tularensis* strain SCHU-S4, according to Sperandio. For example, 80% of mice that received LED209 orally 3 hours after inhaling *F. tularensis* were alive after 9 days, whereas only 10% of similarly exposed control mice survived. LED209 also protected 80% of mice from *S. typhimurium* for 24 hours, with 20% being spared for a full 12 days. In the absence of LED209, all *S. typhimurium*-infected mice died within 6 days. LED209 is not toxic to such animals and does not interfere with adrenergic signaling.

“The sensors in bacteria are waiting for the right signal to initiate the expression of virulent genes,” Sperandio says. “When we [blocked the sensors with LED209], the bacterial pathogens could not effectively cause disease in the treated animals.” Because this sensor is present in such a wide variety of pathogenic bacteria, it could prove an effective broad-brush approach to combating such infections, especially since interfering with their action does not involve resistance-promoting growth inhibition.

“Because LED209 has never been used as an antibiotic, it’s a completely different type of drug,” Sperandio says. “In addition, its target, QseC, is also different from current antimicrobial drug targets. This study demonstrates that LED209 has promise in fighting at least three pathogens and likely many more.” However, she adds, LED209 “is not a magic bullet that can circumvent evolution. Over time, resistance will occur, and this is why we chose a molecule that can be meaningfully chemically modified.”

This study describes “an interesting concept,” says Brett Finlay of the University of British Columbia in Vancouver, B.C., Canada. However, he adds, “Regulation of virulence factors is incredibly complex, and involves many different regulatory circuits.” For example, complexity may help to explain the survival of some infected mice in the absence of LED209. “The study does point to an attractive alternative approach to anti-infectives, and we really need such alternatives given the significant problems with antibiotic resistance,” he says.

**Brian Hoyle**

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**Densovirus Infects Malarial Mosquitoes, Could Lead to Control Strategies**

A virus that infects larvae and adults of the mosquito *Anopheles gambiae*, the major vector for malaria, might provide novel means for controlling this parasitic disease, which kills more than 1 million people worldwide each year, according to entomologist Jason Rasgon of the Johns Hopkins Bloomberg School of Public Health Malaria Research Institute in Baltimore, Md.,
ampire disease, as reported in the January 2008 issue of the Journal of General Virology. “We found either the same virus or something very similar,” Rasgon says. Densoviruses are nonenveloped, single-stranded DNA icosahe- dral viruses that belong to the family Paroviridae and the subfamily Densovirinae, which infect arthropods.

For a decade, several researchers studied Aedes densoviruses as a potential means for interfering with the spread of mosquito-borne diseases. However, Aedes densoviruses do not infect An. gambiae, ruling out such viruses for playing a role in curtailing the spread of malaria. However, because AgDNV does not infect humans or other vertebrates, it might offer a way to attack mosquitoes, including those that spread malaria.

One part of such a scheme calls for inserting genes into AgDNV that would then be expressed once the virus infects mosquitoes. To test this possibility, Rasgon and his coworkers inserted the gene for enhanced green fluorescent protein into AgDNV, then infected mosquito larvae. The emerging adults expressed fluorescently tagged AgDNV in their midgut and ovaries. Additionally, AgDNV was passed from adult mosquitoes to subsequent generations by vertical or horizontal transmission.

Rasgon and his collaborators similarly incorporated genes for antimalarial peptides and insect-specific toxins into AgDNV and plan to test how the engineered virus affects mosquitoes. An antiplasmodium peptide, for instance, might prevent such insects from becoming infected with the parasite. Another approach would be to treat larval breeding grounds with a microbial pesticide similar to the insecticidal toxins produced by Bacillus thuringiensis. “These are ideal schemes, but we’re not there yet,” he says.

Engineered expression vectors that deliver genes to Anopheles mosquitoes “could have a role in the control of mosquito-borne diseases,” says microbiologist Jonathan Carlson of Colorado State University in Fort Collins. Similar vectors for the Aedes denso- virus developed in his laboratory shorten the lifespan of adult mosquitoes and reduce the likelihood that they will transmit dengue virus. Infected females can spread densoviruses to new sites, making viral vec- tors attractive as control agents, he adds. “However, much more work needs to be done.”

Carol Potera