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MICROBES BETWEEN US: HOW PATHOGENS MOVE BETWEEN SPECIES AND ENVIRONMENTS

AMERICAN SOCIETY FOR MICROBIOLOGY

Contents

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From the Editor

BY STANLEY MALOY, PH.D.
EDITOR-IN-CHIEF

Microbes and One Health

Although it is not a new concept, many people were not familiar with "One Health" before the SARS CoV-2 pandemic. The evidence that SARS CoV-2 came from bats, coupled with the search for other intermediate hosts between bats and humans, emphasized the importance of animal-to-human transmission as a source of emerging infectious diseases in humans. [I know, humans ARE animals! However, from a public health, agriculture and companion animal perspective, it is useful to distinguish humans from other animals.]

The One Health concept includes the environment as well as animal and human health. Environmental health also plays important roles in the transmission of infectious diseases to humans and animals. Some infectious diseases are transmitted to animals and humans directly from the environment. In other cases, disruption of the environment increases interactions between humans and wild animals, increasing the risk of exposure. [For a nice example, check out the short trailer at the end of the movie Contagion, which portrays an outbreak of Nipah virus.] Environmental health is also crucial for agriculture, including the health of livestock, fish and plants. Changes in the environment can promote the transmission of animal and plant diseases, limiting the availability of food supplies required for human health. In contrast to the natural cycles of variation that influence the environment, humans have disrupted the environment in ways that result in long-term impacts, including unnecessary use of antibiotics, pesticides and herbicides; clearing forests for agriculture or cities; and climate change associated with the burning of fossil fuels.

Microbes are core to this interplay among environmental health, animal health and human health, from the rhizobiome that influences plant growth, to healthy microbiomes in animals and humans, to infectious disease. Since microbes don't care about borders, One Health relies on international cooperation and collaboration.

A nice example of international collaboration is described by Kate O'Rourke in her article about how the mysterious disease that was killing chimpanzees in Sierra Leone was identified. Several additional examples of the teamwork required to understand microbial mysteries are described in the Hot Topics section.

The impact of One Health approaches to protect crops, livestock and fish that are essential for the global food supply is described in the article by Stephen Ornes. Rather than previous approaches that were focused on agricultural impacts by culling infected farm animals or clearing fields with plant diseases or the extensive use of antimicrobials to reduce disease, One Health approaches are focused on surveillance to allow rapid responses and enhancing biodiversity to reduce disease transmission.

We have known for a long time that most infectious diseases in humans are acquired from animals. However, we often forget that animals acquire infectious diseases from humans as well, including SARS CoV-2. The article "We Are All Connected" by Stephen Ornes describes numerous examples of animals acquiring infectious diseases from humans. Understanding the transmission of these diseases requires that physicians, veterinarians and scientists share knowledge and data. This is nicely emphasized with the story of how Tracey McNamara, a veterinary pathologist, uncovered the cause of West Nile Virus. In response to our understanding of One Health, ASM developed training programs in Africa and Asia and continues to work on enhancing One Health training around the world.

In "Hugs, Kisses and Microbes," Ashley Robbins describes the transmission of infections between people and our pets. Although pet turtles and chicks get a bad rap for the transmission of Salmonella, this article gives some insights into our microbial relationships with pet dogs. And although bats are often demonized because they transmit a variety of pathogens to humans, they play important roles in the ecosystem by pollinating plants, dispersing seeds and consuming insect pests. In the article "Are Bats Developing Resistance to White-Nose Syndrome?," Ashley Hagen describes a pandemic of a fungal pathogen that is killing bats in the U.S. and Canada. The summary from ASM experts on zoonotic diseases ties together many of these examples.

Finally, Ashley Hagen and I describe some recent publications that we found particularly interesting – a list that spans a range of topics from issue to issue that reflects our eclectic fascination with microbes.

Microbiologists propagated the concept of One Health over a century ago – notable figures in our discipline like Louis Pasteur, Robert Koch and others whose early discoveries were influenced by the ubiquitous presence of microbes in humans, animals and the environment. Likewise, because we teach about how microbes affect the health and disease of soil, water, plants, animals and humans in introductory microbiology courses, I think microbiologists are predisposed to understand the importance of One Health. We have made strides in integrating this concept into a variety of applications, but there is still tremendous opportunity and need to apply One Health approaches to our thinking and response to global health.

Stanley Maloy, Ph.D.
Microcosm Editor-in-Chief

We Are All Connected: Looking at the Big Picture with One Health

BY STEPHEN ORNES

Early in the summer of 1999, county health departments across New York began to report an alarming and widespread uptick in the number of dead birds found on the ground. Between May and November, there would be more than 15,000 dead birds reported.

At the time, Tracey McNamara, D.V.M., D.A.C.V.P., was a veterinary pathologist at the Bronx Zoo — one of only a handful of zoos with a pathologist on staff. Dead crows had shown up on the city sidewalks outside the zoo, and by August, Dr. McNamara was finding dead birds in animal exhibits as well. "We were worried that whatever they had would spill over into our captive collection," she recalled. And it did. Within a few weeks, three flamingoes and a few other birds, including a bald eagle, had died.

Dr. McNamara practices what she calls a Zen approach to comparative pathology: Keep an open mind, and don't rule anything out. "Every case you see may be something new," she said. So it went with the birds. At the same time as the mysterious bird die-off, the city was facing another growing menace, a surge in the number of human deaths from a kind of encephalitis. Dr. McNamara suspected the two were connected. She called the Centers for Disease Control and Prevention but says the agency repeatedly ignored her concerns because the CDC studies people, not animals. "They said, 'We don't do flamingoes.'"

Her suspicion would prove correct, however. After exhaustive tests, researchers found identical genomic sequences in the infected birds and humans. The culprit was West Nile Virus, which before then had never been seen in North America. There's no evidence that birds pass the virus to people or vice-versa; rather, it is transmitted by infected mosquitoes. But if vets and human health researchers had been listening to each other, Dr. McNamara said, they might have caught the encephalitis outbreak two or three months sooner, and saved lives.

The emergence of West Nile virus in North America illuminated a stark divide between veterinary and human medicine, even though, as Dr. McNamara says, "the link between them is so obvious." West Nile isn't a freak outlier. Many dangerous and infectious pathogens have close ties to animals and the environment.

In 2009 and 2010, hundreds of thousands of people died worldwide from being infected with the H1N1 virus, or "swine flu," which was first identified in pigs in the United States 20 years earlier. The Zika virus, which caused an epidemic in 2007, was first identified in monkeys in 1947. A growing number of domesticated, wild and captive animals have tested positive for SARS-CoV-2, including pumas, wild mink, gorillas, snow leopards, and dogs and cats. The virus likely jumped from humans to the animals, where it could mutate and return to infect people in months, years or decades.

Of the more than 1,400 infectious diseases known to infect people, pathogens that infect animal species cause more than 60% of them. These diseases show that the health of humans is inexorably tied to that of animals and the environment. And those connections could be exploited, say experts, to identify infectious diseases in nature before they threaten human health. Many emerging pathogens come from the environment or are already present in animals; the challenge, then, is to be able to see them and recognize a troublesome signal, said Dr. McNamara.

That interconnectedness drives an idea called One Health, an approach to addressing and solving health issues by thinking about how these three domains influence each other, and how knowledge in one area can inform new advances in the others. Its potential impact reaches beyond infectious diseases. Antimicrobial resistance (AMR) is another area that demands a One Health approach: The use of antimicrobials in animals — like cows or chickens — can contribute to the emergence of antimicrobial-resistant bacteria, which in turn can end up in humans and undermine the effectiveness of medical treatments for infections. The World Health Organization has identified a One Health approach to AMR as a challenge that will shape global health.

The idea of One Health likely seems intuitive to researchers in the microbiology community, said microbiologist Stanley Maloy, Ph.D., at San Diego State University, who has long been involved with One Health projects at the American Society for Microbiology (ASM). The One Health concept, he says, is like a three-legged stool, with microorganisms playing key roles in processes that bridge the three domains, he says.

> *"There's a lot of exchange between the environment, animals, and humans, and that comes through microbes," Dr. Maloy said.*

Many ongoing global One Health projects focus on sharing knowledge and resources with lower and middle-income countries, said microbiologist and immunologist Martin Evans, Ph.D., who consults for the ASM on international AMR surveillance projects sponsored by the CDC.

"What a One Health approach to AMR does is promote public health decision-making in all sectors based on facts and data," he said. "The ASM joins a global effort to provide technical guidance and expertise in making this happen."

TURNING OLD KNOWLEDGE INTO MEANINGFUL ACTION

An awareness of the inexorable connections between the health of people and the health and well-being of animals and the environment isn't new; it's as old as medicine itself. About 2,400 years ago, the Greek physician Hippocrates speculated that human diseases weren't just a result of an imbalance in the life forces known as "humours," they also arose from animal or environmental sources. Over a century ago, the German physician and pathologist Rudolf Virchow — who gave us the term zoonosis — argued for the necessity of a comprehensive approach to health.

> *"Between animal and human medicine there are no dividing lines — nor should there be," Virchow wrote. "The object is different, but the experience obtained constitutes the basis of all medicine."*

Although a series of physicians and researchers rearticulated Virchow's idea during the 20th century, it wasn't until recently that disparate fields began to collaborate on an overarching plan to apply a One Health approach in disease prevention and AMR. The ASM became involved with global One Health efforts about 15 years ago, after the American Veterinary Medical Association highlighted the importance of bridging the disciplines and proposed an interdisciplinary global consortium of experts in the three fields.

"That really got the ASM thinking about all the things we do in this One Health way already," said Dr. Maloy. Researchers within the ASM community have long studied the roles of microbes in the environment and in human health, for example, and shared that knowledge not only with other scientists but with Ministries of Health and public health departments around the world. The 2008 collaboration suggested a new way to move forward. "That led to a lot of thinking about future pandemics and disease," says Dr. Maloy. "These were all topics within ASM before that, but One Health gave us a context for thinking about them in a valuable way."

Throughout Africa and Asia, in projects in Cameroon, Kenya, Tanzania and elsewhere, ASM has been establishing laboratories and training scientists to promote evidence-based public health programs. The goal is to provide these countries with the resources and training to get started — and then ultimately to run them on their own, said Koss Mensah, M.P.H., who works on ASM's global public health programs. "We are supporting them right now, but the key is to ensure sustainability," Mensah said. "We work with them to understand the problem, and then they can carry their own weight."

Those international programs must be based on robust surveillance, said Dr. Evans. "A major part of One Health, whether in humans or animals or the environment, is AMR surveillance," he said. "Indeed, surveillance is the key component." Only by tracking the emergence and evolution of AMR can researchers determine the best antimicrobial treatment for people and animals, influence policy decisions, and measure the benefit of interventions.

Photo above: Preparing EQA samples for distribution in Tanzania.

In Tanzania, for example, ASM has been involved in AMR efforts for more than a decade. In 2019, bolstered with financial support from the Fleming Fund and in collaboration with the Tanzanian government, ASM launched a national program to build local and sustainable resources for AMR surveillance in humans and animals.

The effort includes renovating and supplying laboratories in Dar es Salaam and beyond — not only labs for human health, but also for animal health. The program goal is to ensure that each laboratory implements and follows the same guidelines. That means that samples must be collected, prepared, transported and analyzed according to best practices, and that data are managed, verified and reported within the national AMR surveillance network in uniform ways. It also means that animal and human health experts, communities and policymakers near need clear paths of communication to be able to share findings related to AMR surveillance status and any public health responses, including risk.

As a result of these efforts, said clinical microbiologist and immunologist Mtebe Majigo, M.D., of the Muhimbili University of Health and Allied Sciences, "the data from animal and human health will come together." When the program began, said Dr. Majigo, the animal health program lagged behind, lacking facilities and infrastructure. ASM and its partners procured supplies, and "we were able to train lab staff to implement all of our surveillance activity," Dr. Majigo said.

In Cameroon, working with the One Health Institute at the University of California, Davis, ASM is helping develop a One Health curriculum for a post-graduate diploma. It will be used to train local scientists to develop expertise and help promote the sustainability of a One Health approach to AMR. "We have experience in AMR that they don't have yet," said Dr. Evans. "The ASM includes a really diverse group of scientists, with significant expertise and specialists in human or animal or environmental science."

Photo above: Three of the technologists at Mnazi Mmoja Laboratory, the first public laboratory to obtain ISO accreditation in Tanzania.

FUTURE CHALLENGES

Like many countries, Cameroon currently lacks a trained workforce with a skillset that could successfully implement a robust One Health program. "If you're going to do this well, you need expertise in every area," said Dr. Evans. "That's why educational environments are a really good idea. This is the first time I've seen One Health develop into an academic program in Africa."

> *Programs that take a One Health approach have the potential to identify trouble in the environment or in animals before it wreaks havocs on humanity. "It could be in plants, it could be animal diseases," said Dr. Maloy. "If you have some big outbreak that influences plants, then you could impact food security. There's this clear interrelationship among these things."*

Predicting new threats, he said, is the core issue behind One Health, "that idea of changing the way we respond to infectious diseases." Instead of responding to outbreaks with treatments, he said, One Health offers a way to focus on early identification: "Potentially, you could develop an upstream block." That ability will become critical to human health, he said, as climate change will likely impact the way that diseases and pathogens move through plant, animal and human populations.

COVID-19 offers a grim reminder of the consequences, said Dr. Maloy. "One thing that COVID has made us realize is that pandemics will be an integral part of our life," he said. "We need a better way of predicting outbreaks before they happen."

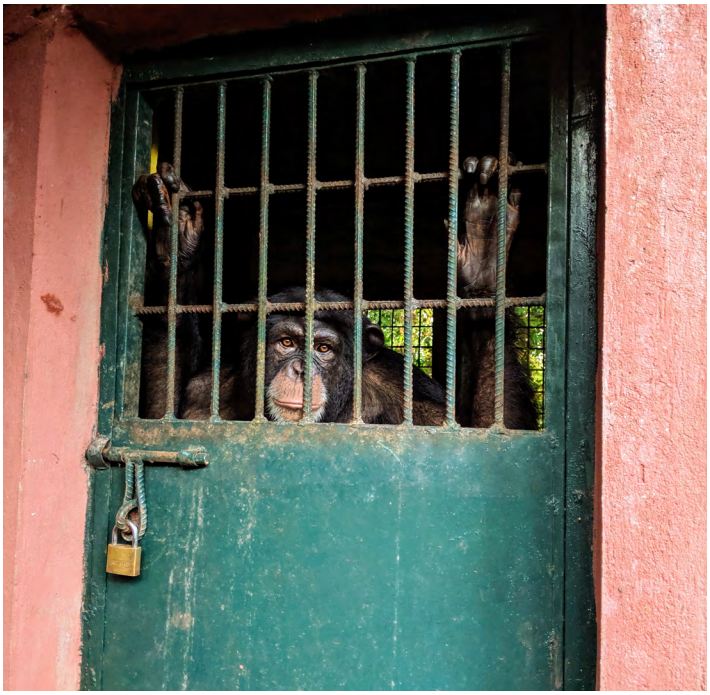
Investigating a Mysterious Disease of Sanctuary Chimpanzees

BY KATE O'ROURKE

In 2005, chimpanzees at the Tacugama Chimpanzee Sanctuary in Freetown, Sierra Leone, started dying from a mysterious illness. Characteristic signs were neurologic, such as weakness, ataxia and seizures, and gastrointestinal, such as abdominal distension, anorexia and vomiting. The illness came on quickly, with some chimps being found dead before caretakers even knew they were sick. Even after staff veterinarians aggressively treated the chimps, the fatality rate was 100%.

The mystery disease continued to plague chimpanzees year after year, mostly affecting animals during March and April. Histopathology investigations were unsuccessful at identifying a culprit. Researchers couldn't find a parasite, toxic plant or virus that was responsible for the disease. Testing of stomach contents and water quality didn't turn up anything. Years went by, and chimpanzees kept dying.

A social group of chimpanzees at the edge of a forested enclosure at Tacugama Sanctuary.



An adult chimpanzee in an indoor enclosure at Tacugama Sanctuary.

Ismail (Izzy) Hirji, D.V.M., was the resident veterinarian at Tacugama Sanctuary from 2016-17 and now serves as a consultant veterinarian for the sanctuary. He said the sanctuary instituted several management changes that decreased the prevalence of the disease. "Because the deaths were happening in the afternoon, the hottest part of the dry season, we kept the chimps indoors for the afternoon. We also added additional water stations to make sure they were adequately hydrated. Those two things made a difference in mortality, but we don't know why," said Dr. Hirji.

In 2016, Tony Goldberg, Ph.D., D.V.M., professor of epidemiology in the School of Veterinary Medicine at the University of Wisconsin-Madison, received an email pleading for help from Gregg Tully, CEO of the Pan African Sanctuary Alliance, an umbrella organization for primate sanctuaries across Africa. "Greg knew through connections that I do work on epidemiology and microbiology of primate diseases, and he told me about this terrible problem that they had been having for a decade at Tacugama. He said they were at the end of their rope and they didn't know what to do," said Dr. Goldberg.

Dr. Goldberg agreed to help. "My lab has developed techniques for pathogen-hunting, where we can take any type of biological sample and process it to identify all the microbes in it using a specialized set of lab and bioinformatic methods," he said. "It involves massively parallel deep sequencing and metagenomics. We have used these methods to crack other mystery diseases in primates in the past."

Getting the chimpanzee samples out of Sierra Leone during a West African Ebola outbreak was the hardest part of the investigation. Chimpanzees have the most highly protected status of any animal or plant by the Convention on the International Trade of Endangered Species (CITES).

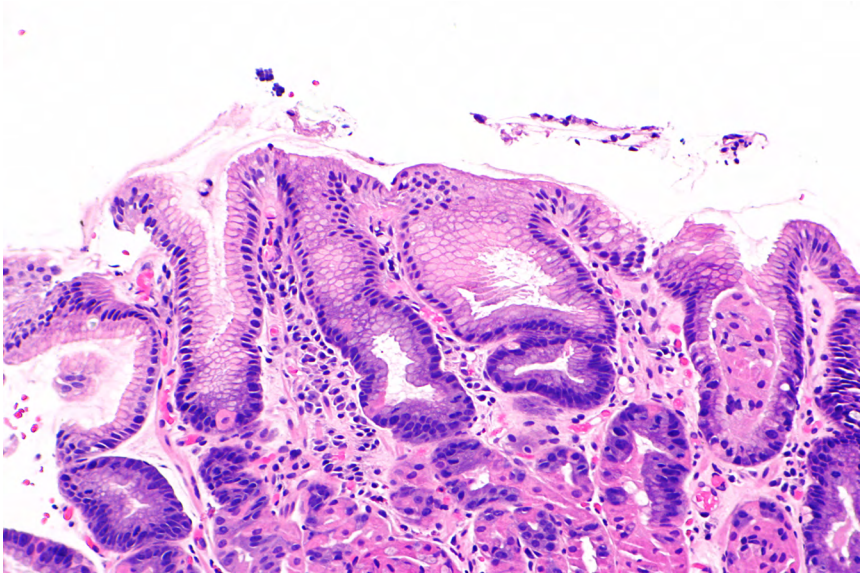
"We had to get CITES import permits from the United States and export permits from Sierra Leone, and we had to have them match," Dr. Goldberg said. "We had to coordinate everything so that the samples, which were being stored in a freezer at a local hospital, could somehow be shipped here frozen." The permitting process was a real challenge, and even more frustrating due to the fact that chimps were dying during the struggle to get the permits. From 2005-18, 56 chimpanzees died at the sanctuary.

In 2017, the frozen samples of tissue, blood and stomach contents from affected chimpanzees and healthy chimpanzees from the sanctuary finally arrived in the United States. Dr. Goldberg and colleagues, including Leah Owens, a D.V.M./Ph.D. candidate working in Dr. Goldberg's laboratory, got to work conducting a case-control study.

While some parasitology had been done on site at Tacugama, an additional parasitology examination confirmed that no parasites were case-associated, said Owens. Virological examinations also did not identify a smoking gun. The researchers found viruses in all of the samples, but they found no different viruses in the cases than in the controls.

The researchers then turned their efforts toward examining bacteria of the samples through microbiome analysis. They found a bacterium that did not match any known species in 13 of 19 cases of what was now being called epizootic neurologic and gastroenteric syndrome (ENGS), but not in any controls. That bacterium turned out to be a member of a genus called Sarcina.

"Almost all of the cases had sequences of a bacterium of a genus that I had never heard of before, Sarcina, and none of the controls had it," said Dr. Goldberg. "In the cases, we found it not just in the stool samples, but we found it in the heart, the brain, the kidney, the liver and the deep organs of some of these sick and dead chimps."



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Sarcinae belong to the family *Clostridiaceae*, which contains known pathogens like *Clostridium difficile* and *Clostridium botulinum*, which are bacteria known to produce toxins and cause severe gastrointestinal disease. The researchers first thought the bacteria might be *Sarcina ventriculi*, which was discovered in 1840 in the vomit of a patient suffering from chronic gastrointestinal disease. Further sequencing, however, determined that they had found a new species of Sarcina, which they named *Sarcina troglodytae*. "We did a little bit more sequencing and discovered it was a separate species of Sarcina that was not one of the two known strains, *Sarcina ventriculi* or *Sarcina maxima*," said Dr. Goldberg.

All *sarcinae* share a distinct and unusual morphology: Upon dividing, the cells stick together and create tetrads, or square packets of cells. "We had seen the evidence in the sequencing data, but it was when we started looking in the tissues and saw the unmistakable cubes of cells that we knew for certain this was a definitely a Sarcina," said Owens.

Like other Sarcina species, *S. troglodytae* did not contain any toxin genes, but it did have pyruvate decarboxylase (PDC), an unusual gene that allows *sarcinae* to produce large amounts of ethanol and gas in acidic environments like the stomach. Unlike any other known *sarcinae*, the researchers found evidence of a functional urea degradation pathway, which enables pathogens to invade host tissues. "These two biochemical pathways in our bacterium together allow it to invade tissues very easily and, as a byproduct of its unique metabolism, to make ethanol. In other words, it can leave the gastrointestinal tract and travel to other organs, like the brain, and produce ethanol there, which is highly toxic," said Owens.

In addition to neurological signs, the researchers say this newly identified bacterium causes gastric dilation and a very serious gastrointestinal disease caused emphysematous gastritis, owing to its ability to produce copious amounts of carbon dioxide. "Emphysematous gastritis is basically gas bubbles in the walls of your gastrointestinal tract. It is deadly," said Dr. Goldberg. "If your stomach tissues and intestinal tissues are full of gas pockets, you will die very quickly of shock, which is exactly what we have seen in some of the chimpanzee ENGS cases."

> *"I am confident that this bacterium plays a significant role in this disease, but we are equally confident that it is not the whole story," said Dr. Goldberg.*

A member of the Tacugama staff disinfecting and preparing fruit to feed to the chimpanzees.

Based on their new findings, veterinarians have made several changes at Tacugama. First, all chimpanzees get probiotics to encourage the growth of healthy bacteria to discourage the uncontrolled growth of *Sarcina troglodytae*. Second, affected chimpanzees receive antacids to take down the acidity level that the bacteria thrives on. Third, because the bacteria also thrives on carbohydrates, the chimpanzees' diet changed from a carbohydrate-rich diet to a fiber-rich diet. And fourth, impacted chimpanzees are given antibiotics that are known to be effective against *Sarcina* in humans.

Kirsten Gilardi, D.V.M., executive director of Gorilla Doctors, a program that provides veterinary care to eastern gorillas in East Central Africa, and director of the Karen C. Drayer Wildlife Health Center at the School of Veterinary Medicine at the University of California, Davis, says she is very impressed with the investigation, which was laid out in a study published in *Nature Communications* (2021;12(1):763). "What really struck me about this paper was the elegance with which the investigation rolled out," said Dr. Gilardi. "I

remember hearing about this mystery disease that was causing mortality at the sanctuary, and lots of people around the world were scratching their heads about what it could be. They were ruling out potential obvious causes early on and kept coming up with no etiology. So then to follow in this paper, the solving of this mystery ... the investigation was really well done. I was really impressed with how they really brought all the tools to bear to diagnose this fatal disease in these animals."

Sharon Deem, D.V.M., Ph.D., Dipl. A.C.Z.M., director of the Saint Louis Zoo Institute for Conservation Medicine and a wildlife veterinarian and epidemiologist, also said she was impressed with the investigation. "I think the investigation is incredibly strong in the various diagnostics that they did and the epidemiological tools they used," said Dr. Deem. "The sequencing was really nice science."

Dr. Goldberg said they are applying for funding to work on such unanswered questions as where the bacteria comes from, why it impacts chimpanzees in a seasonal pattern, why it does not impact humans, and why it seems to only impact animals at the Tacugama Sanctuary.

Are Bats Developing Resistance to White-Nose Syndrome?

BY ASHLEY HAGEN, M.S.

Although the origin of SARS-CoV-2 has been hotly debated and remains under scientific review, it is hard to argue against the notion that the COVID-19 pandemic has cast a shadow over the bat name. Bats are proven reservoirs of many different microbes that can cause severe disease in humans, including, but not limited to, *Bartonella spp.*, *Leptospira sp.*, *Salmonella spp.*, *Escherichia coli*, Rabies virus, Ebola virus, Influenza virus and several coronavirus species. However, they are also key contributors to global and environmental health. Bats are principal pollinators and seed dispersers. Without them, plants like bananas, mangoes, cashews, avocadoes, peaches and cloves would cease to exist, leaving animals at the bottom of the food chain vulnerable to starvation and lack of cover, and entire ecosystems would eventually deteriorate. Furthermore, bats feed on insects and help with the population control of mosquitoes, beetles, months and leafhoppers, thus limiting the spread of mosquito-borne diseases and lessening the need for chemical pesticides in agriculture.

Unfortunately, these winged mammals are currently in the throes of a pandemic of their own, one that has killed more than 6 million North American bats in a little less than two decades. White-nose syndrome (WNS) is an especially lethal fungal disease caused by the ascomycete *Pseudogymnoascus destructans*. Data collected from 200 sites across 27 U.S. states and two Canadian provinces from 1995-2018 indicate that three bat species have been particularly devastated by the disease. Ninety percent decreases in population size have been observed in the decade since WNS emerged for the northern long-eared bat, little brown bat and tri-colored bat. All three species were listed as endangered in Canada under the Species at Risk Act in 2014. But studies are now suggesting that one of these endangered species, the little brown bat, may be developing resistance to the deadly fungus, and as it turns out, temperature regulation and metabolism during hibernation may be both the cause of and the solution to the problem.

How does white-nose syndrome kill bats?

P. destructans is an invasive, psychrophilic (cold-loving) fungus that grows optimally at temperatures ranging from 12.5-15.8°C and cannot grow in temperatures over 20°C. Active bats maintain body temperatures of 37-41°C and are therefore protected from infection. But hibernating bats reduce body temperatures in the winter to control metabolic rates and conserve energy, and that's when *P. destructans* strikes. Bats and human spelunkers carry the fungus from cave to cave, and it can survive without any hosts present for 10+ years, but when the temperature of an unsuspecting bat drops to levels that make it a hospitable host, *P. destructans* invades tissues of the ears, muzzles and wing membranes. The characteristic white fuzz that develops on the bat's bare skin is what gives WNS its name.

Infection causes skin irritation, tissue damage and dehydration, as well as behavioral activity suggestive of fever response, and these trigger WNS-infected bats to wake from hibernation prematurely when food sources are scarce. Combatting infection and repeated waking demand energy and deplete the bat's limited fat reserves, causing many to exhibit unusual behavior such as flying outside in the middle of winter in search of food and water. With little to no food available, victims of the disease eventually starve or freeze to death.

What's different about white-nose syndrome survivors?

Bats in Eurasia, where the pathogen is thought to have originated, do not appear to be as severely affected by *P. destructans* as North American bats. In fact, scientists have demonstrated that Asian bats carry lower fungal loads than bats in the United States. The reason for this is not fully understood, but coevolution and ecological factors are likely both at play. For example, in the winter, less fungus has been found growing in Eurasian caves compared to North American ones. As a result, scientists have hypothesized that local bats are becoming infected less often, or later in the season, and becoming less sick from infection.

Now, a growing body of research indicates that North American little brown bat populations may be rebounding as well. In fact, population increases between 5-30% from previous pandemic lows have been observed at certain hibernation sites in New York. In an effort to determine whether survivors are developing resistance, scientists collected wing punch samples from survivors and victims of a WNS-induced mass mortality event in two little brown bat populations that are showing signs of recovery. Whole genome sequencing from 132 bats identified 63 unique single nucleotide polymorphisms (SNPs) that are more common in WNS survivors than in bats who died with the fungus. Scientists mapped these SNPs to reference genomes of bats and other animals, such as ground squirrels and mice, and identified one loci in a gene associated with the immune system. The remaining SNPs were located within genes linked to host response to WNS and changes in metabolism during hibernation. The results are supported by a 2019 study conducted in Michigan that concluded that survivors of WNS are better at fat storage than those that succumb to the disease. However, a 2020 study that pooled genetic information, as opposed to sequencing individual bat genomes, reported no population-wide signs of selection, leading some to hypothesize that different bat populations may be adapting differently.

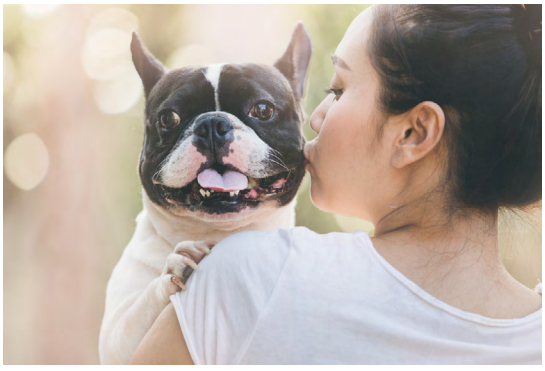
What can be done to mitigate further ecological losses of bat populations?

While bats do the heavy lifting, their human counterparts are continuing to investigate ways to help. Conservation strategies aim to slow human transport of *P. destructans* by closing hibernation caves to the public. Additional mitigation strategies include vaccine development, fungicide treatment, habitat improvement and food-supply increases prior to hibernation. But all of these methods are expensive, and none of them are particularly effective. Further research is needed to understand how the genotypic, phenotypic and behavioral changes of WNS survivors are related, but recent findings provide hope that little brown bats will have a future.

Pet Pathogens: What Dog Owners Need to Know

BY ASHLEY MAYRIANNE ROBBINS, M.E.L.P.

It's no secret that humans love their pets. The American Veterinary Medical Association (AVMA) estimates 57% of U.S. households own at least one companion animal, with nearly 150 million dogs and cats in the United States as of 2016. Pet ownership has steadily increased by 20% over the last 2 decades, rising again amid the pandemic. Dogs lead the pack as the most popular traditional pet (sorry, cats), with 38% of U.S. households owning at least 1 dog. While fewer households own cats (25%), those that do are more likely to include multiple. Exotic pet ownership has also grown 25% in the last decade, with more people than ever caring for fish, ferrets, reptiles, hamsters, rabbits, birds and amphibians and the like.



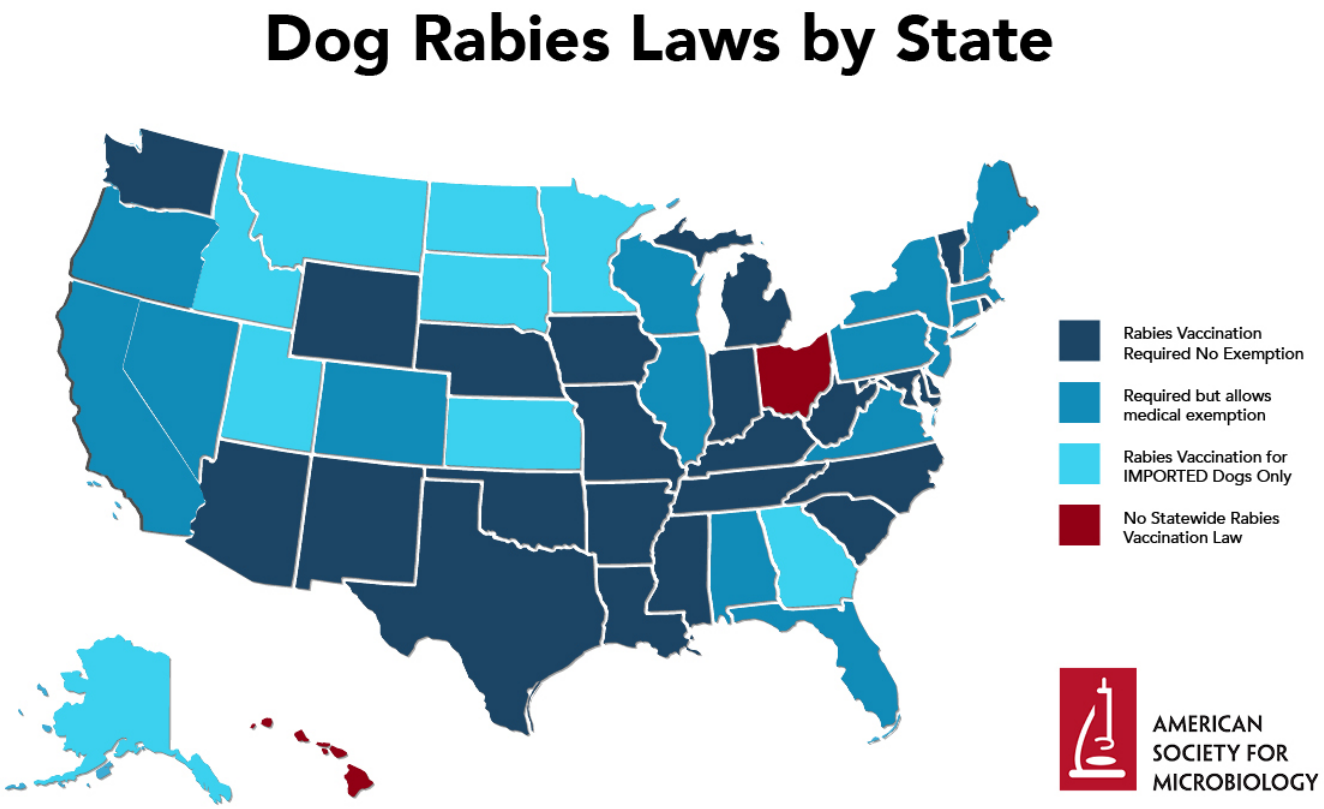
Human Care

Although the modern veterinary profession began in the 1760s with the establishment of the first school of veterinary medicine in France, animal care has been practiced for thousands of years and likely preceded human medicine. Ancient civilizations relied on healthy animals to sustain their food supply, and some cultures, like the Egyptians, revered animals on a spiritual level. Early veterinary science drew on lessons learned from human cases of tuberculosis, typhoid and cholera to prevent diseases in livestock, and has expanded in the last few decades to focus on the preventive and specialized care of companion animals.

The average pet-owning household will spend over $1,100 on their pets each year, which is not surprising, since over 90% of owners consider them to be family members. Over 37% of the $95 billion pet industry is spent on medical and veterinary care, and 40% is spent on food and diet. Some owners report spending more on their pet's medical expenses than they do on their own health care costs, and the novel-but-trending pet insurance industry is estimated to be worth more than $1 billion. Proper pet care is essential to keeping animals and human caretakers healthy, and this includes understanding the microbes that live on and within our canine companions.

Vets, Viruses and Vaccines

Much like humans, preventive care for dogs should begin from a young age and include regularly scheduled examinations. Newborn puppies derive some maternal immunity at birth and, assuming they nurse appropriately, through their mother's milk. Maternal immunity begins to wane at around six weeks, and puppies should receive their "core" DHPP (distemper, adenovirus/hepatitis, parainfluenza and parvovirus) vaccinations every few weeks up until four months of age to ensure an effective immune response. Early vaccination is crucial: Dogs are most susceptible to infection at between six weeks and six months, especially to parvo, a highly contagious viral disease that causes acute gastrointestinal illness and, if left untreated, has a 91% mortality rate. During the first year, veterinarians may recommend other common optional vaccinations for canine influenza, leptospirosis (caused by a bacteria found in urine) and bordatella ("kennel cough"). These vaccinations may be suggested for dogs that are in close contact with other dogs in kennels, and they may be required for boarding and grooming.



One vaccine is not optional: Dogs must be vaccinated against rabies in many states. *Rabies lyssavirus* is a mammal-specific disease that results in brain inflammation, neurological symptoms, behavioral changes and sometimes death. Although occurrences of rabies in the U.S. have decreased dramatically since the development of a vaccine, the inflammatory brain disease affects hundreds of thousands of animals and kills an estimated 50,000 people in developing nations each year. Ninety percent of rabid animal cases in the U.S. occur in wildlife, marking a dramatic change since 1960, when the majority of cases were in domestic animal species, primarily dogs. All but nine states have some kind of regulation or requirement regarding rabies vaccination, though some allow medical exceptions.

Despite the importance of vaccination, there are gaps, and the Humane Society estimates that 69% of the 19 million pets living in underserved communities have never seen a veterinarian. To combat this, many organizations offer free rabies clinics and low-cost veterinary services. Meanwhile, there is a reported increased rate in vaccine hesitancy among pet owners: Britain's People's Dispensary for Sick Animals (PDSA) found that in 2018, about 25% of dogs — 2.2 million of them — had not had their necessary vaccinations as puppies, primarily because their owners deemed it "not necessary."

Diagnosing and Treating Infections

Fortunately, for vaccinated animals, diseases are relatively benign. The main reasons for a vet visit include skin conditions, stomach issues, urinary tract infections and ear infections, according to Healthy Paws Pet Insurance's third annual Cost of Pet Health Care 2018 report. Common infectious agents include staphylococcus and *Escherichia coli*, often following a superficial injury like a bite or scratch. Dogs, with their curiosity for garbage scraps and others' feces, can also pick up infections while exploring unhygienic places. Largely, these are cases of bacteria "in the wrong place at the wrong time," says University of Pennsylvania Assistant Professor and Veterinarian Dr. Stephen Cole.

Dr. Cole's work focuses on the clinical and molecular epidemiology of carbapenem-resistant *Enterobacteriaceae* and other cases of antibiotic-resistant bacteria in animals, which has been increasing in recent years. The issue can be attributed mainly to the overuse of broad-spectrum antibiotics and is particularly problematic in facilities where a large number of animals or multiple species are housed together (*i.e.*, kennels, shelters and pounds.) Because the antibiotics used to treat canine infection are the same medications used in humans, it is especially important to exercise discretion when using or prescribing antibiotics, which may require more time and resource-intensive, culture-based diagnostics.

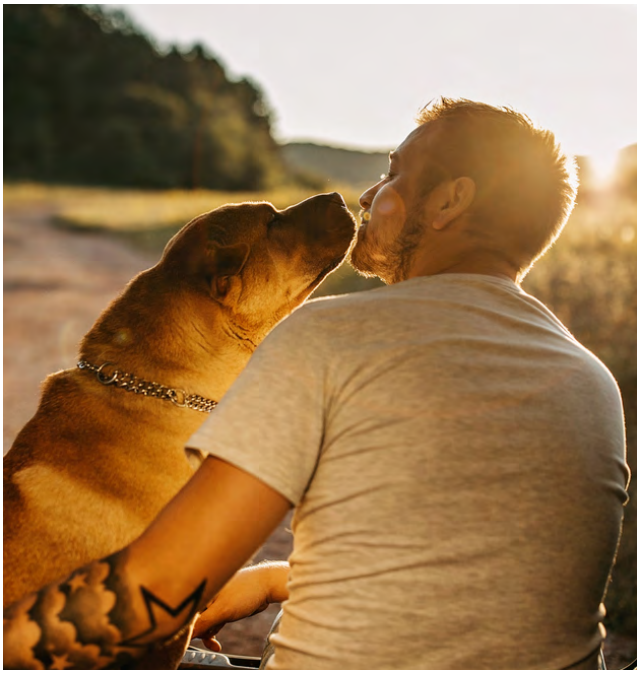
A rapid alternative to growing bacteria in culture is the use of next-generation DNA sequencing, which can help identify novel bacteria or those that do not grow well in culture, says Dr. Janina Krumbeck of private testing facility MiDog. This technique uses a fecal, blood or saliva sample to genetically identify both pathogenic and benign bacteria present in the canine microbiome by comparing the results to a database curated from previous samples from healthy and infected dogs. Dr. Krumbeck says accurate species-level identification can also reveal which antibiotics a sample may be resistant to, and thus it is a powerful tool in prescribing proper treatment. NGS is not widely used in the veterinary field yet, a key barrier being cost, but Dr. Krumbeck has high hopes that the technology may be used more widely to complement bacterial cultures, especially as MiDog extends to more host species. "Successful treatment begins in accurate identification," she said. "We need to use all the tools available."

Parasites, Pets and People

Dog owners and their pets will likely be familiar with routine parasite prevention. A number of over-the-counter methods are available to discourage parasitic insects and arthropods, including flea collars, topical ointments and shampoos that work by interfering with the parasite's nervous system. While fleas are mainly just a nuisance, ticks may jump from animal to human and are known to carry a multitude of pathogens, including viruses, protozoans, and bacteria *Borrelia ssp* (which causes Lyme disease) and *Rickettsia spp* (which causes Rocky Mountain Spotted Fever). The risk of disease transmission varies among tick species, seasonality and geography, says L. Rainer Butler, a Ph.D. student at the University of Maryland Medical School studying immune responses to tick-borne diseases. Ticks are most active in the warmer months, but they tend to prefer amphibians and reptiles as hosts in regions where the temperature is mild year-round; in fact, Lyme disease is relatively absent south of the Virginia border. The best practice is to check your pet frequently and remove ticks within 24-48 hours to prevent disease transmission, she says.

Dogs are natural hosts for heartworms (*Dirofilaria immitis*), and over a million pets in the U.S. harbor the parasite, according to the American Heartworm Society (AHS). Heartworms are transmitted by mosquitoes and reside in the dog's lungs, spreading to the heart and causing long-term damage if left untreated. Because prevention in the form of chewable tablets is easier and more affordable than months of medication, AHS recommends year-round heartworm preventatives, which veterinarians can prescribe. Many dogs will also harbor intestinal parasites — roundworms, hookworms or tapeworms — at some point in their lives, although dogs are most vulnerable in puppyhood when nursing. Dogs with parasites may lose weight, vomit or pass diarrhea; these infections are diagnosed following a fecal examination for microscopic eggs in the dog's stool. Owners can mitigate the risk of an asymptomatic dog passing on the parasite by visiting the vet for annual checkups.

A healthy diet can address many health issues, and some dog food companies are now creating probiotic and prebiotic formulas specifically suited to the canine microbiome. Studies indicate mixed results from a raw-meat diet: Although dogs fed raw meat may harbor a more diverse microbiome, they are also at higher risk for parasitic or bacterial infection. Dogs with allergies or other underlying immune conditions may benefit from a hypoallergenic diet, as inflammation is often associated with an unbalanced microbiome. More research is needed to fully understand the potential of nutrition as a treatment measure.



Pets Love Us

We love our pets, and they love us. But can this connection go too far? The relationship between dogs and their owners is intimate; they eat together, play together, even snuggle together on the couch. Over half of all pet owners report kissing their pets more often than their significant other! Yet such closeness can increase the risk of pathogen transmission between dogs and humans, and vice versa. It may be difficult to determine whether a pet is ill, especially because microbes manifest differently in dogs than in humans, and dogs have an evolutionary instinct to hide signs of illness or injury from potential threats.

Can you hug, kiss and love your pets despite the risk of zoonotic transmission? As Dr. Cole says, "It's like kissing a dumpster vs. kissing a toilet seat." Such is the importance of proper health care, not just for humans, but for the pets with whom they share their homes. This builds a safer environment for all, one in which our love of dogs can continue with minimal risk to us, or our pets. And yes, this includes hugs and kisses.

ASM Experts Answer Pressing Questions About Zoonotic Diseases

BY GEOFF HUNT, PH.D. & ASHLEY HAGEN, M.S.

Zoonotic diseases, those transmitted between humans and animals, account for 75% of new or emerging infectious diseases. The future of public health depends on predicting and preventing spillover events, particularly as interactions with wildlife and domestic animals increase. In this article, several infectious disease experts answer pressing questions related to zoonoses.

Note: This article is adapted from a May 27, 2021, ASM-organized Reddit Ask Me Anything discussion on zoonotic diseases. The full discussion can be found here. Panelists are listed below:

DR. DAVID BLEHERT, PH.D.
Chief, Laboratory Sciences Branch, U.S. Geological Survey National Wildlife Health Center

DR. GREG GRAY, M.D., M.P.H.
Professor of Medicine and Global Health, Duke University

DR. BARBARA HAN, PH.D.
Disease Ecologist, Cary Institute of Ecosystem Studies

DR. TARA SMITH, PH.D.
Professor, Department of Epidemiology, Kent State University College of Public Health

Q: Do humans have reservoirs of viruses that don't affect us (are not pathogenic to humans) but can spread to animals and cause disease?

I think humans likely have viruses that we tolerate well but that may harm other animal species. Certainly, we know of human disease outbreaks (like measles) among non-human primates that have had relatively high mortality. - *Dr. Greg Gray, M.D., M.P.H.*

We can certainly spread viruses to other species, though perhaps we do not function as often as asymptomatic reservoirs as we know other animals, such as rodents or bats, do. We probably gave human rhinovirus C to non-human primates. Other pathogens, like MRSA, can also be spread from humans to animals, including our pets and zoo animals. - *Dr. Tara Smith, Ph.D.*

Q: How do climate change and environmental factors impact zoonotic disease type and frequency?

There are many ways that climate change can increase the risk of emerging infections and zoonotic disease in humans. Deforestation can lead to diversity loss and force populations to congregate in smaller areas, bringing new species into contact with each other and potentially with humans, which can allow for spillovers from species to species. Warming can allow disease vectors such as mosquitoes and ticks to move into new areas that were previously inhospitable to them. Climate refugees may be forced to move, potentially bringing animals with them into new areas, which again risks spillovers. Land may no longer be farmable, forcing rural individuals into cities that are denser and can lead to outbreaks. - *Dr. Tara Smith, Ph.D.*

One of the results of climate change is that temperatures are warming in northern regions of the world. As this occurs, the population ranges of various cold-sensitive animal species are expanding northward. As the population ranges of these animals expand, they bring along various microbes, including pathogens, that may be new to the regions into which these species are expanding. As the ranges for various wildlife species expand, they also begin to interact with other wildlife species and populations from which they used to be isolated. Thus, changing ecological conditions result in the movement and spread of pathogens among animal species, which could result in increased risk for outbreaks of wildlife disease among previously naïve wildlife species and populations, increased risk for spillover of novel wildlife pathogens to domestic animals, and increased risk for zoonotic transmission. - *Dr. David Blehert, Ph.D.*

Q: Large-scale animal operations are a common focus of the origin or spread of zoological diseases. What can be done to minimize the risk?

The Food Safety and Inspection Service of the U.S. Department of Agriculture (USDA), the Centers for Disease Control and Prevention (CDC) and the Food and Drug Administration all hold food-production industries accountable for zoonotic pathogen contamination in food. Food industries work hard at reducing the risk of food contamination. You really need to tour a modern plant to understand the many safety measures they employ. I think it's very feasible to work with the industry to make livestock farming safer. I also see the benefit of conducting surveillance for novel viruses among livestock workers. - *Dr. Greg Gray, M.D., M.P.H.*

It's still tough to know exactly how many spillovers we see from farming, especially "factory farming." Surveillance is notoriously difficult for a lot of reasons. It's tough to get on farms to acquire samples from animals and workers. In the U.S., many farm workers may be undocumented and in precarious positions regarding employment, so it's tough to get them to participate in studies if they don't necessarily trust researchers or worry it may "out" them to authorities. If they do get sick from something they may have acquired on-farm, they may not seek treatment for those same reasons. So we have incomplete knowledge in this area. - *Dr. Tara Smith, Ph.D.*

The USDA routinely screens poultry farms for avian influenza viruses and removes infected animals from farms to prevent further losses to the poultry industry and prevent zoonotic transmission. Another mechanism to control outbreaks of highly pathogenic avian influenza is to conduct routine surveillance for avian influenza viruses in wild birds. When wild bird surveillance indicates that transmission risks are elevated, poultry producers can be advised to increase biosecurity. - *Dr. David Blehert, Ph.D.*

Q: What are the arguments for why we will likely never get rid of influenza?

The historic low in influenza cases last year was brought about because of global dampening in human-to-human transmission. Continuing to reduce human-to-human transmission will keep spillover events from turning into epidemics, but it will not eradicate the spillover events from animals from occurring. - *Dr. Barbara Han, Ph.D.*

I don't see eradication of influenza viruses in the near future. I would argue that avian species, swine, humans and cattle are all reservoirs for influenza A, B, C and D viruses, but swine influenza viruses concern me the most. - *Dr. Greg Gray, M.D., M.P.H.*

Q: What are the primary drivers of zoonotic disease?

Zoonotic diseases, or diseases of animals that can also infect humans, can be transmitted between animals and humans by a number of mechanisms, one of which is human interaction with infected domestic animal(s). - *Dr. David Blehert, Ph.D.*

There are a lot of other ways that diseases can be transmitted from animals to humans. We know they can also come from farmed animals, and diseases such as Ebola, HIV and the original SARS can come from animals hunted/butchered/consumed as wild game. - *Dr. Tara Smith, Ph.D.*

Spillover events, where pathogens are transmitted from an animal host into a human, occur frequently, but the vast majority of these don't cause any problems in humans. Each spillover event is not a complete roll of the dice; there is consensus about certain groups of pathogens that are more likely to cause severe or highly transmissible disease in humans. - *Dr. Barbara Han, Ph.D.*

Visit our website to learn more about zoonotic disease and access an interview with Dr. Rima Khabbaz, director of the CDC's National Center for Emerging and Zoonotic Infectious Diseases.

MICROBIOLOGY ON THE FARM:

Protecting Crops, Livestock and Fish from Diseases

BY GEOFFREY HUNT, PH.D.

Infectious disease outbreaks are always a major health concern. Pathogens that affect humans naturally get most of the headlines, but perhaps as important are the ones that affect and threaten the global food supply. Diseases that impact plant crops are estimated to cost $220 billion annually worldwide, while the U.S. Department of Agriculture estimates that livestock-targeting pathogens result in approximately $17.6 billion annually in losses. Beyond the financial cost, these diseases also result in less food being available, a gravely serious issue when more than 800 million people worldwide already face food insecurity.

Given the high potential for devastating economic, ecological and epidemiological outcomes, farmers, policymakers, public health officials and researchers have collaborated for years on approaches to control the spread of agricultural infectious diseases. However, as pathogenic microbes continue to proliferate and cause damage, there is a pressing need to reexamine existing methods. Are these approaches effective? Are farmers best equipped to handle the current and future challenges facing the agricultural industry? If not, what else can be done?

Cull 'em all

In 2015, an outbreak of measles at Disneyland led to more than 125 confirmed individual cases. While the situation resulted in several immediate responses from policymakers and public health authorities, at no point was there a suggestion to euthanize the approximately 2 million people who visited the Southern California amusement park during that two-month period. Yet that is exactly the kind of blunt approach often taken when it comes to halting (or slowing) the spread of infectious disease in plants and animals. From boars in Germany to badgers in the United Kingdom to minks in Denmark, governments have forced farmers and producers to carry out broad culls of entire populations that have potentially been exposed during an outbreak, eliminating hosts (and hopefully pathogens) in the name of public health.

Unfortunately, studies of animal-culling practices have found that such strategies, while crudely effective from a cost-benefit perspective, are not economically sustainable. Besides the financial factors, there are also social and personal considerations to take into account. "It's a hard decision to have to make to euthanize an animal," says Jackie Boerman, Ph.D., assistant professor of Animal Sciences at Purdue University. "I don't know very many farmers that get into animal agriculture not really liking animals." Farmers aren't the only ones with conflicted feelings. The recent badger-culling program in the U.K., aimed at eliminating the spread of bovine tuberculosis, generated massive public outcry (drawing in luminaries including Sir David Attenborough and Queen guitarist Brian May) that has forced the government to backtrack on its plan.

Similar challenges arise when it comes to culling (or "roguing") plant crops. According to Robin Choudhury, Ph.D., an assistant professor in the School of Earth, Environmental, and Marine Sciences at the University of Texas Rio Grande Valley, "Remov[ing] diseased plants from individual farms or plots ... has been shown to be really effective when done in a timely manner." However, at larger scales, the efficiency of rouging drops, because, as Dr. Choudhury notes, "by the time that you notice a new pathogen in a region, it is too late to get rid of it completely from a new region." The decision about whether to rogue also forces farmers into daunting financial decisions. There is an "economic threshold for deciding when to cull" an infected population, states Justin Knopf, a fifth-generation grain farmer from Salinas, Kan. "A lot goes into the decision," he points out. "Is the disease hitting ahead of harvesting season? What is the yield potential of the crop? What is the weather outlook?"

NEW TREATMENT OPTIONS

Faced with the looming prospect of increased disease prevalence, researchers, farmers and regulators alike are being forced to adopt a new mindset. Instead of always defaulting to culling as a solution, Dr. Choudhury advises that a better strategy is to focus on mitigation and management practices, which generally mean antibiotics, plant pesticides and (in animals) vaccines. Unfortunately, "there's nothing profitable about mitigation strategies," according to Jeff Baker, a dairy farmer from Friendship, N.Y., "because you've already lost money." Beyond the cost of the drug treatments, Baker points out that animals who have received treatment typically have reduced production capabilities, resulting in lower yields and, ultimately, profits.

Instead, there is a growing preference for what Knopf refers to as "beneficial biology" — preventive behavioral strategies to combat disease, instead of continuing to rely so heavily on the use of drugs (whether synthetic or natural). As he explains, "The most significant measure to prevent disease is a holistic approach to [a] crop system" that relies on diverse crops, crop rotation and specialized cover crops.

Behavioral interventions are similarly effective in aquaculture. As Ted Meyers, Ph.D., state fish pathologist for the Alaska Department of Fish and Game, explains, fish-hatchery operators in Alaska "are cognizant of good fish culture." What that means in practice, according to Dr. Meyers, is that these operators "provide adequate space to rear fish so there's less density, less crowding. They examine their fish on a regular basis, watching very carefully when these fish are rearing. Adding a water supply that is free of [pathogens]." At the same time, adds Dr. Meyers's colleague Jayde Ferguson, Ph.D., there is "very, very little antibiotic treatment." The result, says Dr. Meyers, is that "we don't see as many [disease] cases perhaps as some of the pathologists do in the lower 48 [states], because our hatcheries are performing better."

Another helpful adaptation is to use drug treatments more clinically. "On those animal ranches ... we're creating these antibiotic-resistant pathogens by overuse of antibiotics as prophylactics," states Mo Kaze, Ph.D., a fellow at the Department of Energy's Joint Genome Institute. "I've seen this in action, where the vet just goes through and hits every single animal with antibiotics, regardless of whether or not they have an infection." Dr. Boerman echoes the call for restraint. "We want to try to do our best to use antibiotics when they're going to be effective, and not use antibiotics when they're not needed," she says.

A major issue complicating these decisions is that of climate change. "Climate change is definitely going to be a factor for disease," states Dr. Choudhury. "When we have that kind of increased variability," he adds, "that means that we have to prepare our crops differently." As temperatures and humidity levels increase, Dr. Kaze points out that "we're creating these environments unintentionally that ... the microbes that are not beneficial to us are just going to love." "Parasites," she adds, "are going to be thrilled." Even geographic isolation won't be enough to stave off the impacts of climate change. "We'll be getting fish species that we don't see (in Alaska) ... extending their ranges," predicts Meyers. "They're going to bring with them, I'm sure, certain things we've never seen before as far as pathogens go."

WHAT CAN MICROBIOLOGISTS DO?

When asked how scientists could be of help to him, Baker points to tracking and detection of pathogens and disease on his farm. Respiratory diseases have been the main source of outbreaks among his cattle; unfortunately, the only way to properly identify (and treat) this kind of disease is, as Baker explains, "to euthanize [cows] and send samples to the lab." Rather than continuing to rely on this slow, costly process, he wonders whether it is possible "to develop a test that could just take blood samples from a live animal to identify and specify which disease is being dealt with."

Knopf echoes the call for better real-world applications of microbiology on his grain farm. "What would be helpful to me," he says, "is having more information and a better understanding of what … the beneficial microorganisms [are] that can help plants be healthier (naturally) so that they can overcome disease without the use of outside synthetic inputs."

Dr. Choudhury supports this natural approach. "I think we're heading into the region of actually being able to do proper biological control," he claims. Dr. Kaze agrees. "In many ways, it replaces those traditionally chemically synthesized pesticides," she says. "It's cheap, it's effective, it's used all over the world [and] it's safe for consumption."

But perhaps the most helpful advancement would be less about hardcore science and more about building better relationships between the different stakeholders. As Dr. Ferguson states, "microbiologists need to know about the culture. They need to know about the industry and how things are done so they can apply their microbiology knowledge in context with what's being produced, how it's being produced [and] what are real risks and real threats."

As with so many issues, the first step toward better disease-management may be as simple as having a conversation.

What's New at ASM

FINDING YOUR SCIENCE ADVOCACY VOICE

This September, I had the chance to speak one-on-one with my federal legislators about science policy during ASM's Hill Day. This experience taught me how to cultivate relationships with lawmakers and their staff by sharing personal stories and research, leaving me feeling inspired and encouraged in my skills as a science communicator and advocate.

My research as a Ph.D. candidate at the University of North Carolina Greensboro focuses on the honey bee gut microbiome and how lifestyle, dietary and genetic backgrounds can influence the strain-level bacterial community dynamics in the gut community. Bees are responsible for pollinating an estimated one-third of the plants we eat, and their declining health poses threats to global food security. Policies that impact science, especially those affecting the U.S. Department of Agriculture or National Science Foundation, matter to me, because my lab is directly supported by these two agencies. Without their support, I could not do the work I do to safeguard honey bee health or add to the knowledge base of microbial ecology. My lab is making the case for why the gut microbiome should be considered a pivotal piece of ensuring that honey bee populations are healthy for future generations. These bacterial communities keep honey bee pathogens at bay and are necessary for bees' growth and development into adults. My work helps support policy regarding microbiome sciences by explaining why such research should continue being investigated in the future.

My interest in science policy began in 2019, when I presented my graduate research at the North Carolina Regional ASM conference. I listened to the ASM advocacy team explain how ASM connects members to their state and federal lawmakers. This concept fascinated me and was the perfect transition for me to learn more about science policy and advocacy at the local level. Inspired by my passion for community engagement in science and desire to pursue this as a career, I co-founded a science advocacy group for graduate students at UNC Greensboro and became engaged in leadership and committee positions within the National Science Policy Network. Still, I have so much more to learn about ways to assess policy options for challenging problems and connecting my science to real-world issues.

I had my first experience communicating with local lawmakers during the NC Audubon Society's grassroots virtual advocacy day in 2020. However, even with the prep the Audubon team gave to participants about what topics we would discuss, the time limit we would have, and assurance not to worry about these meetings, I was so nervous the night before I could barely sleep. The thought of speaking to my lawmakers about birds and conservation made me feel small — like my voice didn't matter because they had so many other essential things to work on. However, this experience made me question how other students feel about speaking to their elected officials about their research if I felt so nervous to simply talk about a hobby that I am incredibly passionate about.

The Audubon Advocacy Day motivated me to pursue more advocacy work, which I did by applying to ASM's Hill Day this year. ASM's Hill Day was my first experience advocating with a professional, scientific society. When I found out I was accepted to

be an advocate with ASM, I could feel the culmination of my advocacy work from the past two years lifting my confidence to speak with my federal lawmakers. Heading into ASM Hill Day, I felt empowered to speak with confidence and level-headedness about my passion for microbiology and the work my lab conducts. The Hill Day training provided by the ASM team gave me a broad but immersive view of policy language and the federal funding process. This overview allowed me to draw connections from my fundamental research questions to the policies that could affect that work. I was no longer afraid but excited and proud to represent my lab, my school and my research with ASM. I hope my lawmakers see that North Carolina has many leaders like me striving to make our state a hub for microbiological research.

Reflecting on my first experience with virtual advocacy, I had an idealized, almost celebrity-like, view of my local lawmakers, which made my anxiety worse and made my messages come across confusingly at times. However, I now understand that lawmakers depend on graduate students like me to provide feedback on how their districts are progressing and are sometimes even serving on committees in Congress that can directly or indirectly affect student research. For students that are considering attending Hill Day, I have the following recommendations:

- Think about who makes the decisions on funding the scientific agencies supporting your lab, or what policies change the way water quality is measured in your community. The science we conduct as graduate student researchers might not be directly related to a specific policy, but it might be supported by federal funding agencies affected by policy decisions and budgets. Advocacy experiences gave new meaning to my research, showing me how science can support people and their communities.

Connect with a local science policy group at your university, with ASM, or even the National Science Policy Network. These groups are designed to teach the basics of science policy and advocacy and provide hands-on experiences to ease newcomers into more advocacy.

- Think about the broader impact of your research. It is powerful to use your voice to explain why funding microbiological research matters not just for your research lab, but for the broader community around you and the future generations of scientists to come.

My guiding principles for continuing my advocacy work are to (1) empower students to use their scientific skills and knowledge to share their results with lawmakers, and (2) highlight the vast number of career opportunities in science outside of academia. When I had the tools, resources and mentorship to use my voice to advocate for science, I saw my future brightly and clearly in science policy and advocacy. I hope to use my experiences now to help other graduate students find their way to their science advocacy interests and encourage them to become advocates with ASM in the future as well. Knowing that I helped contribute to the overall goal of supporting microbiological sciences by advocating with ASM on Hill Day made this experience worth doing again next year, and I hope more early career ASM members will consider applying.

INFECTIOUS DISEASE IS AN INDIGENOUS ISSUE: SPOTLIGHT ON MATT ANDERSON

"I would argue that infectious disease is really a Native issue, almost independent of which organism you work on," said Matt Anderson, Ph.D., an assistant professor at The Ohio State University who is of Eastern Band of Cherokee Indians (EBCI) descent and white. Though environmental science and human genetics are front-of-mind for many Native American scientists (the first because of strong land stewardship traditions and the second because of a history of misuse of Indigenous genetic data), Dr. Anderson was drawn to the study of infectious disease just as much by his Native identity as by his own personal interest. Asked about his scientific interests, he comes back repeatedly to Indigenous health disparities. Just as the rates of chronic illnesses like cardiovascular disease are higher in these communities, so too is the prevalence of infectious disease in the few cases where it has been studied. Only with the COVID-19 pandemic is that fact becoming more widely recognized.

Dr. Anderson has family on his mother's side enrolled in the EBCI, who originally inhabited land in North Carolina, Georgia, Tennessee and Alabama before being forcibly relocated by the U.S. government. He grew up in Portland, Ore., relatively disconnected from the Native American community, at a time when the city was struggling with gang violence and homelessness. "It was not 'Portlandia' ... you kind of got hit in the face with the repercussions of infectious agents all the time," he recalled. "I really wanted to work in infectious disease because of the experience of seeing what happened with the HIV epidemic in the 80s and into the 90s where I was."

Dr. Anderson moved to Wisconsin as an adolescent. It was there that he and his sisters began to explore their Native American heritage and got involved with the Native community. "The process of being involved is really just about being present," he explained.

After receiving his undergraduate degree from the University of Wisconsin, Madison, Dr. Anderson went on to pursue his Ph.D. at Stanford University in the laboratory of Dr. John Boothroyd, where he studied the intracellular, eukaryotic parasite *Toxoplasma* gondii. The parasite is typically acquired from undercooked infected meat or from cat feces. *T. gondii* is also amongst a rare group of pathogens that are capable of congenital transmission, being passed by infected mothers to their babies. Though it is one of the world's most common parasites, *T. gondii* infection is usually asymptomatic in healthy individuals. However, in those with weakened immune systems, it causes toxoplasmosis, a disease characterized by muscle pain, headache and fever. Dr. Anderson's interest in the organism stems from the populations most affected by it: Before the advent of antiretroviral therapy, *T. gondii* was one of several parasitic infections that routinely killed AIDS patients. In addition, Indigenous communities have been documented with *T. gondii* infections at rates that are far higher than in the surrounding non-Indigenous populations.

In Dr. Boothroyd's lab, Dr. Anderson also tapped into his interest in genetics. Using insertional mutagenesis, he generated *T. gondii* mutants that had trouble transitioning from tachyzoites (the disease-causing stage that infects muscle and neural tissue) to bradyzoites (the stage that forms dormant cysts within tissues) in vitro. He tracked down the insertion in one such mutant to a gene with homology to pseudouridine synthase (*PUS1*). Interestingly, mice infected with *PUS1* mutants had higher parasite burdens, smaller but more numerous cysts and higher mortality. Dr. Anderson thinks that, in vivo, *PUS1* may stabilize the tachyzoite stage. He hypothesized that without it, *T. gondii* has trouble maintaining dormancy, leading to waves of inflammation that eventually overcome the host.

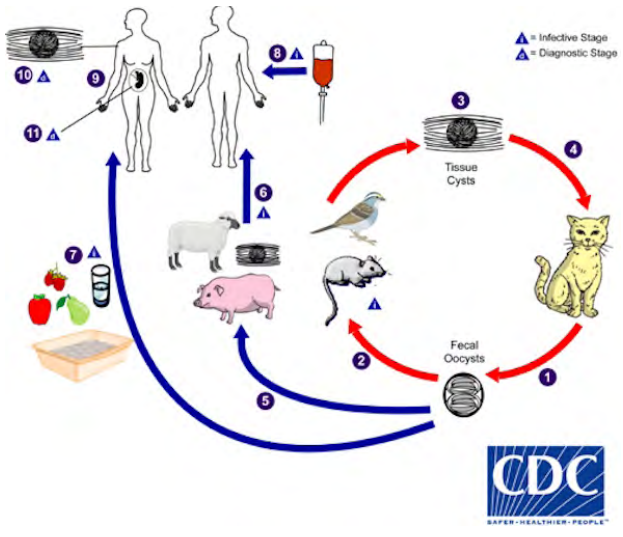


Diagram of the Toxoplasma gondii life cycle.

Source: https://www.cdc.gov/dpdx/toxoplasmosis/modules/Toxoplasma_LifeCycle_BAM1.gif.

While working on *T. gondii* in graduate school, Dr. Anderson was also supporting a family. Financial hardships meant working a second job and working with department administration to secure housing assistance and health insurance for his family (at the time, Stanford did not offer insurance for the dependents of graduate students). In reference to resources others take for granted, he said, "My personal and in-law extended family had no means to buoy us up. We were on our own."

Unfortunately, equivalent forms of assistance were not available after Dr. Anderson graduated and went on to the University of Minnesota for his postdoc in 2010. In the lab of Dr. Judith Berman, he switched to another opportunistic eukaryotic pathogen, *Candida albicans*. "I wanted to work with an infectious agent that I felt continued to stay relevant to some of the populations that were important to me," he said, referencing HIV/AIDS patients and Indigenous communities. "I also wanted to work with someone who was well established in the field," he explained, referring, at least in part, to the importance of adequate lab funding. "I needed a place that could guarantee me a certain level of stability. I couldn't afford to do a six-month appointment and then hope I landed a fellowship."

In those with weakened immune systems, *C. albicans* yeast can cause a variety of diseases, from mucosal infection (thrush) to systemic and life-threatening bloodstream infection (candidemia). Dr. Anderson focused on the telomere-associated (*TLO*) gene family found in subtelomeric regions of *Candida spp*. Though the TLO genes were expanded in number in *Candida albicans*, relative to other *Candida* species (indicating evolutionary importance), their function was unknown. Based on a conserved transcriptional regulation domain, coupled with variability in other regions, Anderson proposed that the *TLO* genes were transcriptional regulators specifically expanded in *C. albicans* to allow the opportunistic pathogen to survive in a variety of host niches. Indeed, Dr. Anderson was able to follow the evolution of this gene family during more than 4,000 generations in cell passage, capturing mechanistic detail of how specific *TLO* family members were further duplicated or deleted over time.

In 2013, Dr. Berman moved her lab out of the country, and Dr. Anderson moved to Dr. Richard Bennett's lab at Brown University to finish his postdoc. In Dr. Bennett's lab, Dr. Anderson expanded beyond the TLO gene family to look at the genotypic and phenotypic diversity between patient isolates of *C. albicans*. This intraspecies diversity, as well as the functions of the TLO gene family, is a continued focus of his lab at The Ohio State University, which was established in 2016.

Dr. Anderson's Native identity and his identity as a scientist intertwine most often where he chooses to invest his time, from instruction to research to mentorship. He advises other scientists from underrepresented groups to "have a clear plan for [your] future and resist the push and pull of different people drawing [you] away from what [you] want and towards something else." Dr. Anderson is involved with several initiatives to build scientific capacity and ensure that Native American communities benefit from scientific research, including the Summer Internship for Indigenous Peoples in Genomics (SING), Indigidata and the Native BioData Consortium (NBDC). He's seen the critical mass of Native geneticists effectively organize and establish autonomy within that field. In contrast, Dr. Anderson can name only four other Native American, faculty-level microbiologists in the U.S. Raising the point that autonomy and self-determination are critical for Indigenous communities, he said, "Micro needs to catch up."

MICROBIOLOGY IS... THE SEARCH FOR NEW ANTIBIOTICS

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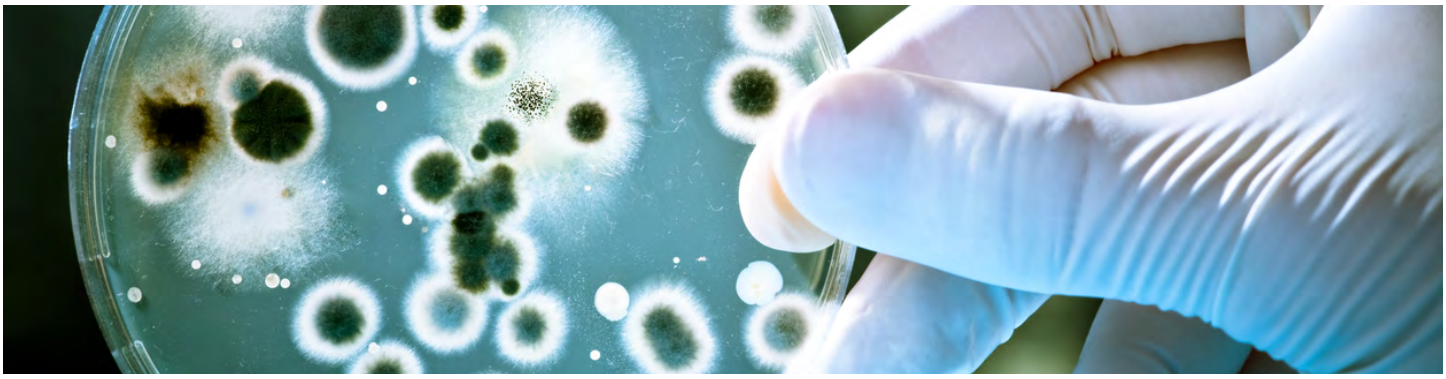
What's Hot in the Microbial Sciences

BY ASHLEY HAGEN, M.S. & STANLEY MALOY, PH.D.



MICROBIOLOGY MYSTERIES

Researchers are puzzled by the increased deaths of songbirds associated with a mysterious disease that is causing lethargy, neurological symptoms and crusty, oozing patches over the eyes of songbirds in the eastern United States. So far, tests have been more successful at eliminating potential sources of infection than at identifying the pathogen that is responsible. *Salmonella, Trichomonas* parasites and several families of viruses known to cause mass mortality in birds have all been ruled out. Some species, including the blue jay, European starling, common grackle, American robin, northern cardinal, house finch, house sparrow, eastern bluebird, red-bellied woodpecker, Carolina chickadee and Carolina wren, seem to be more affected than others. And evidence suggests that young birds are particularly susceptible to the illness. At this time, there is no known treatment or cure.

In the spring of 2021, portions of the disease outbreak area overlapped with the emergence of periodical cicadas belonging to the 17-year Brood X. Since birds eat cicadas, and cicadas can carry fungus (particularly *Massospora*) and/or accumulate pesticides or contaminants while living underground in the soil, some scientists initially hypothesized that the cicadas might be playing a role in the songbird deaths. However, sick birds have been observed in geographic locations where cicadas are rare, and the correlation does not seem to fit. More information is urgently needed to solve this mystery, but in the meantime, officials have urged people in the Mid-Atlantic region to discontinue feeding birds and providing water in bird baths in an attempt to slow transmission of the deadly disease.

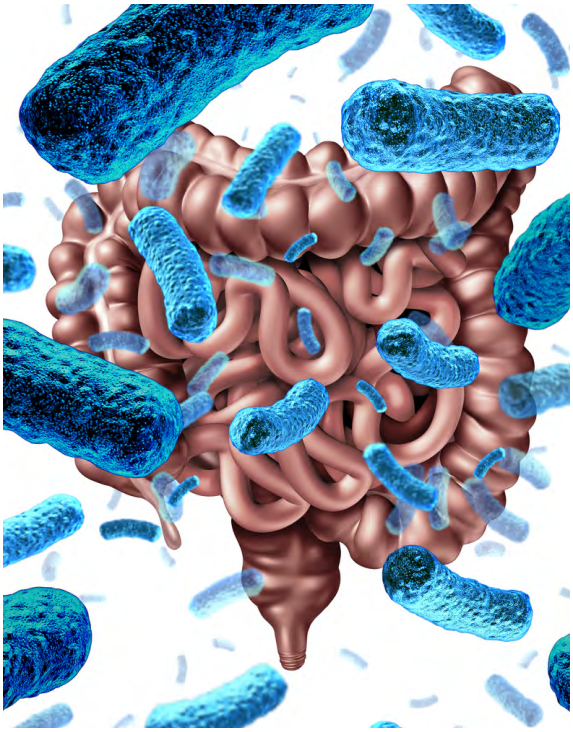
Mysterious die-offs of African elephants are also making headlines. During the months of May-June 2020, approximately 350 elephant deaths occurred in Botswana, Africa. An additional 39 deaths were reported in the Botswana Moremi Game Reserve from January-March 2021. The casualties are especially concerning in light of the African Elephant's standing on The International Union for Conservation of Nature's (IUCN) Red List of Threatened Species.

Evidence points to a common cause of death, and microbes have been implicated. At a news conference in September 2020, the principal veterinary officer with Botswana's Department of Wildlife and National Parks stated that lab tests had identified cyanobacterial neurotoxins as the culprit.

Cyanobacteria, photosynthetic bacteria found in water and moist soil, are capable of producing toxins that are harmful to humans, wildlife and the environment, especially in high concentrations, or blooms, which result from overgrowth in warm, stagnant, nutrient-rich water. Remote-sensing and data obtained from water samples collected in the area showed that cyanotoxin concentrations (ranging from 0.36 to 124,460 µg L–1) were much higher than the provisional guideline value of 1.0 µg L–1 recommended for mammals and humans by the World

Health Organization (WHO). At high concentrations, neurotoxins produced by cyanobacteria can cause paralysis, cardiac or respiratory failure and death, and reports of elephants seen walking in circles before suddenly collapsing to death in Botswana have been used to support the cyanobacteria hypothesis.

While cyanobacteria contamination in pools of drinking water is a plausible explanation for the deaths of these elephants, many questions still remain. Other animals in the surrounding area, including scavengers who fed on the carcasses of the affected pachyderms, appeared to be unharmed. This observation, combined with the fact that the government has yet to release full test results from their analysis to the public, have led some scientists to suggest that the elephants may have been targeted. Although poaching has been ruled out as a possibility due to the fact that the dead elephants all had intact tusks, some have questioned whether the agency's tests were designed to rule out neurotoxins that may be available to farmers eager to prevent the giant herbivores from trampling and eating their crops. Additional data and transparency are needed to solve this microbiological mystery and prevent further die-offs of the endangered species.



NATURE VS. NURTURE? HERITABILITY OF THE MICROBIOME

Commensal bacteria are known to be more similar between relatives than nonrelatives, but it is less clear whether gut microbiome traits are heritable or more likely to result from shared environments between related individuals. Research published in *Science* characterized changes in the microbiomes of 585 wild baboons from fecal samples collected over 14 years. After controlling for diet, age and socioecological variation, scientists found that nearly all (97%) gut microbiome taxa are heritable in baboons. Although baboons have a microbiome similar to humans, these results contradict previous work, which found few heritable taxa in humans. The magnitude of heritability was small (mean = 0.068), aligning the results of this study with prior observations that environmental effects have a larger impact on gut microbiome composition than additive genetic effects. Overall, the findings of this study indicate that it is important to consider host genetics when evaluating microbial landscapes and imply that host microbiome traits are subject to natural selection with the host genome.

While commensal bacteria are found in adult butterflies, resident microbial communities are almost entirely absent in butterfly larvae. It is known that parental exposure to environmental conditions can exert transgenerational effects on the phenotypes of offspring, but it is unclear whether the microbiome of the parent has any direct effect on the organism's offspring. A study published in *Applied and Environmental Microbiology* tested the hypothesis that disturbance of parental microbial communities via antibiotic treatment would affect the ability of first-generation (F1) larvae to cope with transgenerational shifts in host plant species. In support of this hypothesis, researchers found that in 75 percent of experiments, larvae derived from antibiotic-treated parents gained less biomass than control larvae when feeding on a different plant species than their parents, but not when they fed on the same plant species as the parent generation.

The effect was linked to higher prophenoloxidase activity (a modified form of the compliment response that is found in some invertebrates) and downregulation of a major allergen gene (*MA*) involved in the detoxification of glucosinolates, secondary metabolites of plants that can be activated to produce toxic products, such as isothiocyanates, when damage occurs to the plant.metabolites that potentiate interferon-γ production.

EVOLUTION OF ANTIMICROBIAL RESISTANCE (AMR)

Compared to the magnitude of the ongoing threat of antimicrobial resistance (AMR), relatively little is known about the de novo emergence of resistance genes or the role of microbial physiology on resistance. It is known that AMR often results in a fitness cost to the microbe, which may be mitigated by compensatory mutations elsewhere in the genome. One of the better-studied examples of that fitness cost is rifampicin resistance. Rifampicin targets the bacterial RNA polymerase (RNAP). Rifampicin resistance is commonly due to mutations in the β subunit of RNAP. These RNAP mutations affect how different genes are transcribed, thereby influencing the amount of various proteins in the bacteria. Mutations in the β' subunit of RNAP can compensate for the fitness cost of resistance mutations occurring in the β subunit. were not found to be neurotoxic.

In a study published in *Antimicrobial Agents and Chemotherapy*, researchers sought to identify molecular mechanisms underpinning the fitness cost of rifampicin resistance in *Mycobacteria tuberculosis* by subjecting a collection of rifampicin-resistant *M. tuberculosis* strains to genome-wide transcriptomic and proteomic profiling. Analysis revealed a signature of physiological changes that may alter the fitness cost of rifampicin-resistance. The most common clinically conferring rifampicin-resistance mutation, *rpoB* $_{Ser450Leu}$, causes measurable fitness defects, which vary in different *M. tuberculosis* genetic backgrounds. Increased abundance of proteins involved in central carbon metabolism correlated with the fitness defects, leading the authors to conclude that posttranscriptional modulation of gene expression occurred in most of the strains carrying *rpoB* $_{Ser450Leu}$. Based on the data of this study, researchers asserted that the fitness cost of rifampicin resistance and its compensation are mediated by differences in gene expression conferred by the *rpoB* mutations, suggesting that expression of the mycobactin biosynthetic cluster is the most likely cause of growth rate differences of resistant *M. tuberculosis* strains.

In order to better understand how de novo resistance genes emerge, a study published in *PLOS Genetics* tested whether random DNA sequences can generate novel antibiotic-resistance determinants. Scientists expressed over 100 million randomly generated sequences in *E. coli* and identified six *de novo* colistin-resistance-conferring peptides (*Dcr*) that were auxiliary activators of the two-component regulatory system, *PmrAB*. The newly identified peptides conferred resistance by modifying the cell envelope and causing reduced antibiotic uptake. This is the first example of random-expression libraries being used to select for peptides that lead to an AMR phenotype via direct peptide-protein interactions *in vivo*. The results of the study support the idea that noncoding DNA can serve as a substrate for *de novo* gene evolution and suggest that a new class of peptide could potentially evolve to be resistance-determinant in nature.

HOW DOES PHAGE DNA ENTER A BACTERIAL HOST?

When you learned about phage in your general microbiology class, you probably learned that the DNA is injected into the cell via a pre-assembled hypodermic-like structure, a dogma based on the well-characterized phage T4 that has a long, beautiful tail. However, phage T7 and many other phage have puny little tails that don't fit the hypodermic model. A paper published in *Molecular Cell* combined biochemical, structural, biophysical and modeling approaches to generate a composite model of the periplasmic channel used by the phage T7 DNA to transfer the phage DNA from the capsid to the cytoplasm of a bacterial host.

These studies showed that phage T7 has an "ejectosome" composed of phage proteins: gp14 forms an outer membrane channel connected to a periplasmic channel composed of gp15 bound to gp16, and a large cytoplasmic hub formed by gp16 has DNA-binding activity. This work nicely shows how structural studies can inform an understanding of functional questions that have been difficult to resolve.

WHY DO RESPIRATORY INFECTIONS OFTEN LEAD TO INTESTINAL SYMPTOMS?

Although influenza is primarily considered a respiratory disease, influenza virus infections are frequently associated with complications outside of the respiratory tract, including intestinal symptoms like nausea, vomiting and diarrhea. Previous studies had indicated that influenza alters the gut microbiome, but the cause, nature and consequences of influenza-associated intestinal symptoms had not been not understood.

In a study published in *Infection and Immunity*, researchers examined signs of intestinal injury and inflammation, altered gene expression and compromised intestinal barrier function in influenza A virus (IAV)-infected mice. The composition of gut microbiota was altered, and this was accompanied by two important changes: (1) a decrease in the production of short-chain fatty acids (SCFAs) derived from fermentative gut microbiota; and (2) up-regulation of inflammatory markers in the liver of the infected mice, suspected to result from translocation of bacterial products across the gut barrier. Scientists concluded that influenza virus infection can remotely impair intestinal barrier function and thereby trigger secondary enteric infections. Moreover, when IAV-infected mice were treated with SCFAs, systemic infection of *Salmonella typhimurium* was reduced, supporting this hypothesis.

NEW INSIGHTS INTO MICROBES AND BIOGEOCHEMICAL CYCLES

It has been suggested that of all of Earth's biogeochemical cycles, the methane cycle is the most tightly linked to climate, and approximately 1 gigaton of methane is generated by methane-producing (methanogenic) archaea in oxygen-depleted environments every year. Methane-oxidizing microbes, or methanotrophs, play an important role in balancing atmospheric methane. A team of scientists have recently discovered large (~1 Mbp), linear DNA sequences containing genes that expand redox and respiratory capacity. Results of the investigation are not yet peer-reviewed and are currently in preprint on the bioRxiv server. Data suggest that these sequences are novel extrachromosomal elements (ECEs) that coexist and replicate within *Methanoperedens*, a methane-oxidizing archaea. Because these elements have the ability to scavenge and "assimilate" genes from microorganisms in their environment, scientists have named them "Borgs" after the fictional "Star Trek" aliens who sequester the technology and knowledge of other alien species.

Still, *Methanoperedens* has yet to be cultured in a lab, and the findings are based on sequence data alone. Finding Borgs in cultured *Methanoperedens* is an important step to verifying the conclusions of this study. The team is currently investigating the role of DNA repeats present in these sequences in the hopes that Borgs could be a useful tool for gene-editing and reduction of methane emissions.

Fe(II)-oxidizing microorganisms and Fe(III)-reducing microbes are key drivers of Earth's biogeochemical Fe cycle. A study published in *Microbiology Spectrum* identified the first single organism capable of both Fe(II) oxidation and anaerobic Fe(III) reduction at circumneutral pH. Researchers isolated a novel neutrophilic Fe(II)-oxidizing *Rhodoferax* bacterium from an iron-rich wetland in Japan. The novel strain, MIZ03, can grow chemolithoautrophically at nearly neutral pH (6.5-7.5) by oxidizing Fe(II), H2 or thiosulfate as the sole electron donor under (micro)aerobic conditions and can reduce Fe(III) or nitrate under anaerobic conditions. This discovery identified a novel model organism that will provide insights into the molecular mechanisms of microbial Fe redox-cycling and the Fe cycle in the environment.

The clamp-loader complex has two accessory proteins, HolC and HolD. Together, these proteins help assemble and stabilize the clamp-loader complex, but HolC is the only protein of the complex that binds with single-stranded binding proteins, an interaction that directs DNA polymerase to RNA primers and stabilizes the interaction.

Although HolC is not essential for viability, deletion mutants exhibit poor growth and acquire suppressor mutations. One of those suppressors reduces the stability of RNAP. Another duplicates the ssb gene. On the other hand, transcription factors, DksA and Rho termination factor NusA remain viable, even when HolC is absent, but loss of DksA and NusA leads to synthetic growth defects with HolC.

Transcription elongation complexes can impede the progress of the replication fork. In the absence of HolC, it appears that DNA replication is incomplete and Rho-dependent termination is critical to maintaining chromosome integrity. This suggests a new role for HolC in preventing collisions between transcription elongation complexes and the replication fork. However, the mechanism by which it accomplishes this task has yet to be fully defined.

HOW DO FUNGI BREECH PLANT SURFACES?

To better understand how oomycetes breech plant surfaces during infection, a publication in Nature Microbiology analyzed how *Phytophthora infestans* invades potatoes. *P. infestans* was responsible for the devastating Irish Potato Famine and is still a global threat to potato and tomato crops. The authors used three-dimensional confocal imaging to monitor the invasion of etiolated stems of potato plantlets by strains of GFP-expressing *Phytophthora*. The authors observed that, following spore germination and germ tube growth, the oomycete hyphal tip indented and invaded host surfaces at an oblique angle of attack, suggesting a slicing mechanism of invasion. Fracture imaging confirmed that surface cracks in front of the growing hyphae allow pathogens to invade. This study indicates that *Phytophthora* hyphae slice through plant surfaces to initiate infection. It is the first time this novel mechanism of plant invasion, which does not rely on appressorial structures, has been described.