the Pathologist

SPECIAL SERIES

Infectious Disease

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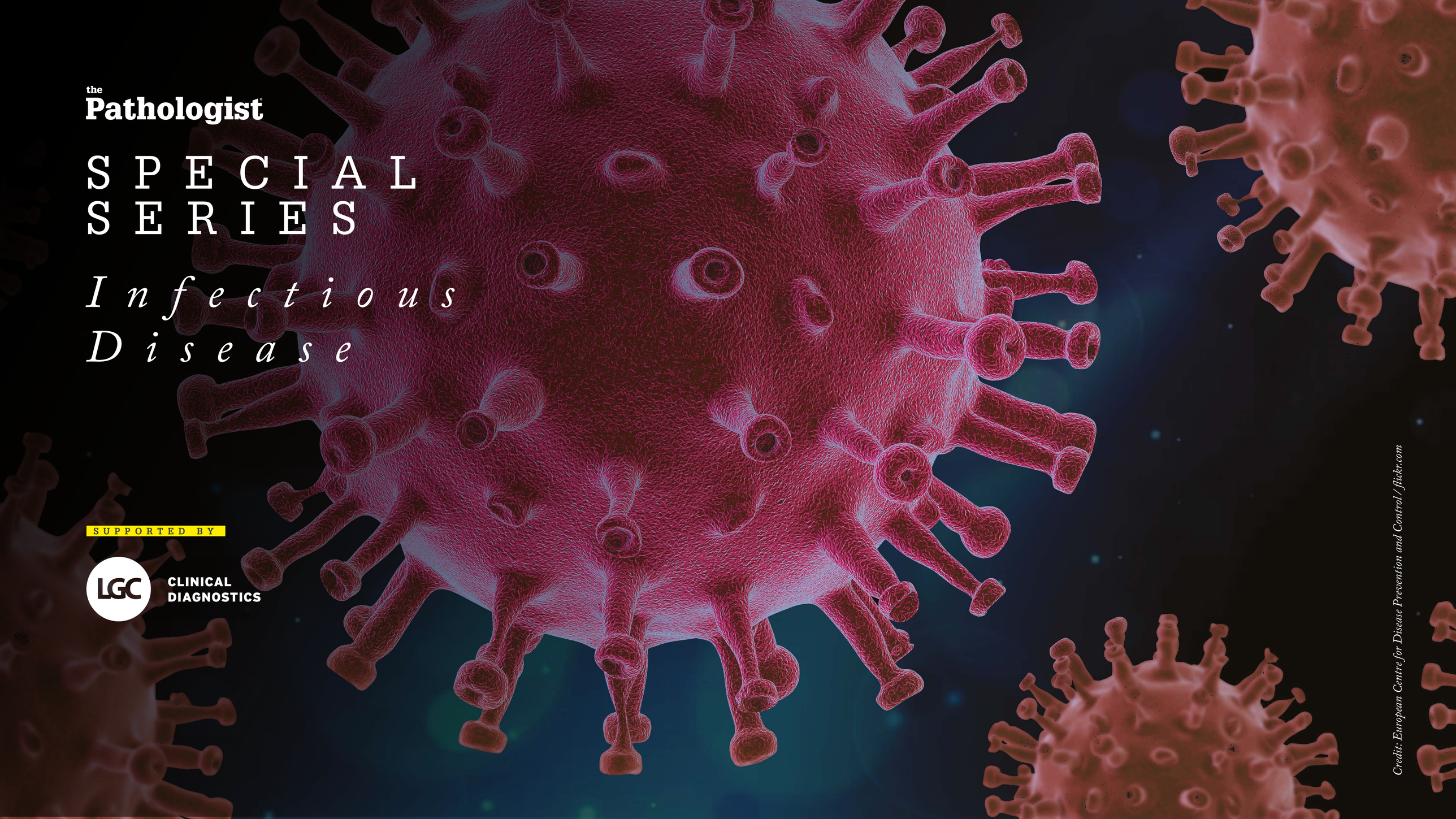

Credit: European Centre for Disease Prevention and Control / flickr.com

UPFRONT

A Weather Forecast for Disease

With recent advances in tracking, data, and surveillance technology, is an infectious disease forecast system possible?

In a world where pandemics and lockdowns are still fresh in people's minds, there has never been more of an appetite for real-time tracking of infectious disease. With new advances in genomics, as well as epidemiological and clinical data, modern surveillance techniques allow for unprecedented insight into the current status of disease. But although the technology may exist, the infrastructure for disease forecasting is a still fledgling science. One group of researchers, in a bid to support the creation of such a system, has outlined their perspective on the steps needed to design a successful disease forecast in the future.

First, it's vital to address the looming threat of antimicrobial resistance. To date, disease forecasts have been unable to incorporate predictions on pathogen diversity – but, to ensure that the models are useful to practitioners and policymakers, they must be able to describe current infectious agents and their risk of resistance diversification. Pathogens evolve fast – and we need to keep up if we want to effectively monitor our antimicrobials' ability to keep us safe.

So what can we do? The authors propose a marriage between disease forecasting and genomic data. Sequencing technology is faster and cheaper than ever, and our ability to handle large volumes of data is only increasing. We're also expanding our understanding of resistance mechanisms, causative mutations, and predictive parameters. As turnaround times decrease and access to sequencing technology increases, we can track the evolution of the most pressing pathogens and the effectiveness of our antibiotic treatments against them. Embedding this data into prediction models and refining them over time in light of real-time pathogen evolution could significantly improve the accuracy and utility of infectious disease forecasting.

Despite the availability of extensive public pathogen sequence databases and the range of projects underway to compare sequences and combat resistance, the authors highlight that differences in sampling strategies and lack of context can impact the data's forecasting utility. To remedy this, they recommend continual sampling in the context of long-term surveillance – but standardized approaches to sampling, sequencing, and reporting (including metadata) could also help.

Although mathematical modeling for epidemiology has grown significantly more accurate in recent years, there are still improvements to be made – and real-world observations, particularly in genomics, don't always match up with the math. In light of the expanding opportunities, the authors call for the incorporation of molecular data – genetics, genomics, and ultimately phylodynamics – into disease forecasting to ensure that our predictions, and the actions we take as a result, are as accurate and well-considered as possible.

UPFRONT

Feeling the Selective Pressure

Key genetic differences may have determined who survived the Black Death pandemic – and how our immune system respond to diseases today

Infectious diseases have placed intense selective pressure on the human and animal population throughout history, with many of these involving immune response genes. The problem, however, lies with linking cause and effect – which pathogens caused specific adaptations?

In the mid-14th century, the Black Death wiped out up to 50 percent of the global population – cementing its place as one of the deadliest pandemics recorded in modern history. In an effort to understand whether Yersinia pestis – the bacterium responsible for the second plague pandemic – triggered a case of natural selection, researchers have analyzed the DNA of victims and survivors who lived in Denmark or London before, during, or after the Black Death (1). By their reasoning, variants associated with susceptibility or protection should display opposite frequency patterns across the sampled timepoints; variants conferring increased susceptibility should be high in frequency in individuals who died during the Black Death then decrease in post-pandemic survivors or descendents, whereas variants associated with protection should rise after the Black Death.

By tracking genetic variants that became more common throughout the pandemic, they found key genetic differences associated with plague protection or susceptibility. In particular, changes in ERAP2 allele frequencies were implicated; people with two identical copies of the protective ERAP2 allele were about 40 percent more likely to survive the pandemic than individuals homozygous for the deleterious variant. This allele is linked with increased ERAP2 expression and production of the canonical, full-length protein ERAP2, which the researchers suggest is associated with an increase in Yersinia-derived antigens to CD8+ T cells. They also found that macrophages from individuals with the protective allele yield a unique cytokine response to infection and better limit replication in vitro.

After the Black Death, plague outbreaks continued to occur in waves up until the mid-19th century, but these often wreaked less havoc than their predecessors. Why? Possible explanations span changing health, sanitation, and cultural practices, but it could also be that, because more people with protective variants survived the Black Death, their descendents will have inherited the survival advantage and been protected against future waves of the bubonic plague.

Fast-forward to modern day, and the research demonstrates how historical natural selection can impact current susceptibility to chronic inflammatory and autoimmune diseases. When stimulated with a range of pathogens, ERAP2 was transcriptionally responsive and demonstrated its key role in immune response regulation – suggesting that the selective pressure from Y. pestis likely impacts immune response to other pathogens or diseases. The paper cites that the advantageous ERAP2 variant against Y. pestis is actually a known risk factor for Crohn's disease and some communicable diseases. Perhaps the ERAP2 variant protected our ancestors through the Black Plague, but this may have come at a trade-off for immune disorders in the present day – or "a long-term signature of balancing selection," as the researchers state.

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Lessons Learned: Pandemic Readiness & Reference Material Solutions

What can we learn from the last few years?

With Eric Morreale

In the early days of the COVID-19 pandemic, diagnostic testing was challenging. The tests were inaccurate, resulting in false results for both laboratory-developed and commercially-developed assays. This hampered efforts to identify cases quickly and contain the spread of the virus. The lack of reliable reference materials was a significant factor in these early diagnostic failures. Clinical testing labs were desperately searching for quality solutions that could help them independently verify that their assays were working properly.

Learn how we successfully addressed these challenges:

Designing reference materials for a virus with many unknowns

There are three core categories of control design: free nucleic acid; real virus (SARS-CoV-2, in the case of COVID-19); and a recombinant approach.

Developing controls utilizing free nucleic acid is simple, making development timelines short. The material is not infectious, and is relatively inexpensive. On the downside, it doesn't represent a full process control, and so is not evaluating extraction completely. Free nucleic acid also doesn't mimic a true clinical sample well, and it can have challenges with large genome sequences. Finally, storage is not ideal because, particularly in the case of RNA, it's relatively unstable.

Real virus replicates what would be found in a true clinical sample. There are no sequence restrictions, and it's relatively inexpensive once a culture is established. That being said, this approach wasn't an option in the early days of the pandemic, as access to SARS-CoV-2 virus was extremely limited at that time. Significant shipping and handling restrictions were put in place due to the many unknowns regarding the nature of the virus, including infectivity and mortality profiles.

LGC Clinical Diagnostics | SeraCare used a proprietary recombinant RNA virus technology – AccuPlex™ - to create a true full process control designed to mimic a SARS-CoV-2 patient sample. Importantly, the control is non-infectious as the AccuPlex virus has been genetically engineered to not replicate. In addition, it's extremely stable, with upwards of a two-year shelf life at 2° to 8°C storage.

AccuPlex virus-based QC reference materials have applications beyond just SARS-CoV-2, as they can be customized to contain sequence-specific RNA (or DNA) of desired pathogens. They are superior to "naked" transcribed RNA because they assess the efficiency of the extraction method. AccuPlex also is non-infectious, while still containing a viral protein coat very similar to enveloped viral pathogens like HIV, HCV, influenza, Zika, Ebola, and others. Stability makes the reference materials amenable to formulation in various formats to mimic an actual patient specimen. For example, LGC Clinical Diagnostics | SeraCare lyophilized the material when cold chain logistics were limited.

We've also formulated AccuPlex reference materials in various liquids, including simulated oral fluid for saliva-based testing and human plasma for blood-based pathogens like HIV, HCV, and Zika.

In addition, we've dried SARS-CoV-2 reference materials on swabs to make them compatible with swab-based testing systems.

Testing & quality control solutions for pandemic preparedness

Lessons learned during the COVID-19 pandemic continue to influence and improve our readiness for future challenges. Like other RNA viruses, SARS-CoV-2 mutated rapidly during the pandemic, and the new variants were of significant concern for the scientific community and the public, as it was unclear how different variants would impact infectivity and mortality rates, as well as the effectiveness of the vaccines

To support the evolving diagnostic testing around variants, LGC Clinical Diagnostics | SeraCare again utilized AccuPlex technology to develop a series of SARS-CoV-2 variant reference materials. The superior customization featured by the technology enabled a quick response to changing testing needs as new variants of concern were identified.

Supporting you and your lab

LGC Clinical Diagnostics | SeraCare has provided gold-standard third-party run controls and reference materials to clinical laboratories for over 30 years. We are committed to supporting the critical efforts of assay development, verification, and ongoing performance monitoring in response to the COVID-19 pandemic. Our quality solutions encompass molecular, serology, NGS, and antigen performance monitoring tools to support the early detection of infection and surveillance efforts.

QUESTIONS?

TO LEARN MORE, PLEASE GET IN TOUCH WITH US AT 1-800-676-1881. OR BY EMAILING CDX-SALES@LGCGROUP.COM.

IN MY VIEW

Three Mpox Challenges

Mpox testing has certainly improved, but there are still significant barriers to address and lessons to learn

Erica Frew is Product manager at Asuragen, a Bio-Techne brand, where she specializes in molecular controls for clinical tests. She is based in the Boston area, Massachusetts, US.

Since May 2022, countries around the world have been dealing with the first mpox outbreak to spread broadly beyond Africa. By November, there were nearly 80,000 confirmed cases in more than 100 countries (1).

But those numbers actually belie the tremendous challenges we have experienced in detecting mpox in this outbreak. For most of 2022, testing has been a significant bottleneck in addressing this public health threat. In New York City, for example, which has a population of nearly 8.5 million and quickly became the epicenter of the outbreak in the US, testing was so constrained that, until July 2022, only 10 people could be tested each day (2).

The US Food & Drug Administration's ability to grant emergency use authorization for new mpox tests – a development that occurred in September 2022 – should help alleviate testing issues in the coming months. Still, the clinical laboratory community has a number of hurdles to clear before it can roll out accessible, reliable testing for mpox. Many of these challenges can be addressed with better collaboration between industry and clinical labs.

Mpox-specific sequences and testing protocols

So far, the sequences and testing protocols approved for mpox testing are not actually mpox-specific — they are for the broader category of non-variola Orthopoxvirus DNA viruses (3, 4). It is acceptable to use this now because mpox is the only widely circulating member of this group – but ultimately labs will need methods that are specific to mpox. This challenge is likely to be addressed as test manufacturers bring new assays to the FDA for emergency use authorization.

Early access to reliable controls and reference materials

One key message we learned from COVID-19 is that companies need to do more to get reliable testing materials into the hands of test developers – both for clinical labs and for commercial test manufacturers. The severe testing constraints that occurred in the first few months of the mpox outbreak were largely caused by limited access to controls and reference materials needed to build, verify, and routinely run new assays. Since then, we have begun to see companies releasing synthetic mpox controls, which has helped labs better respond to unmet testing needs.

Better collaborations between test developers and clinical labs

For much of the outbreak in the US, just a handful of labs had partnered with the CDC to get mpox testing up and running. It wasn't enough. Industry should do a better job of partnering with clinical labs to help ramp up testing capacity. If these relationships are established ahead of time as part of a nimble infrastructure, it will be easier to develop new tests and materials rapidly when new outbreaks emerge. In addition, collaborations can be used to expand and enhance testing strategies, such as enabling send-out testing or a variety of sample specimen types.

Looking ahead, infectious disease experts predict that the frequency of zoonotic pathogens spilling over into the human population will continue to increase due to climate change and human encroachment on animals' natural habitats. It is more important than ever to plan ahead with new strategies for the rapid development of tests and controls. We must also establish stronger relationships between clinical laboratories and the developers of tests and controls so that we can respond quickly to new threats in the future.

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FEATURE

World Rabies Day 2022: A One Health View

What you need to know about rabies today – and how to protect yourself from the disease

Rodney E. Rohde is Chair, Clinical Laboratory Science Program and Regents' Professor at Texas State University System and Associate Director of the Translational Health Research Center.

Coordinated by the Global Alliance for Rabies Control, World Rabies Day is the biggest event on the global rabies calendar (1), occurring annually on September 28 – the anniversary of Louis Pasteur's death – since 2007. The event aims to raise awareness and visibility while advocating for global elimination of rabies. Created as an inclusive initiative, it unites people, organizations, and stakeholders across all sectors. With this concept of togetherness and unity in mind, the theme for this year's World Rabies Day is "Rabies: One Health, Zero Deaths."

Why do we need World Rabies Day?

Rabies is a feared and neglected ancient infectious disease (2). Caused by pathogens in the family Rhabdoviridae, genus Lyssavirus, and distributed globally, this viral zoonosis causes tens of thousands human fatalities and exposes millions annually. All mammals are believed susceptible, but only certain taxa act as reservoirs. Direct access to, replication within, and passage from the central nervous system all serve as a basic viral strategy for perpetuation.

Using a combination of stealth and subversion, lyssaviruses are quintessential neurotropic agents that cause acute, progressive encephalitis. Rabies virus is diabolical in its pathology, transmission, and perseverance in nature – but no treatment exists, so prevention is key. Fortunately, in the many countries leveraging animal vaccination, pre- and post-exposure human vaccination, and public health measures, there are typically only one or two rabies-related deaths per year (2).

Present-day snapshot

Every year, rabies causes approximately 59,000 human deaths worldwide; however, it is difficult to estimate the total global burden of disease for all animal species due insufficient testing or reporting of cases in certain regions. In the most recent US research effort, 54 jurisdictions submitted 87,895 animal samples for rabies testing, of which 97.3 percent returned a conclusive test result (3). Of these conclusive results, 5.2 percent tested positive for rabies, representing a 4.5 percent decrease from 2019. Texas, Pennsylvania, Virginia, New York, North Carolina, New Jersey, Maryland, and California accounted for over 60 percent of all animal rabies cases reported in 2020.

Of the total reported rabid animals, 91.3 percent involved wildlife, with raccoons, bats, skunks, and foxes representing the primary hosts confirmed with rabies. Rabid cats, cattle, and dogs accounted for 95 percent of rabies cases involving domestic animals in 2020. No human cases were reported in 2020, but five human deaths caused by rabies were reported in the US in 2021 (4) – the highest annual case count in the last decade.

Raccoons are the most commonly reported rabid animals in the US (5), followed by skunks and bats, but the prevalent species transmitting rabies can vary from state to state (see Figure 1).

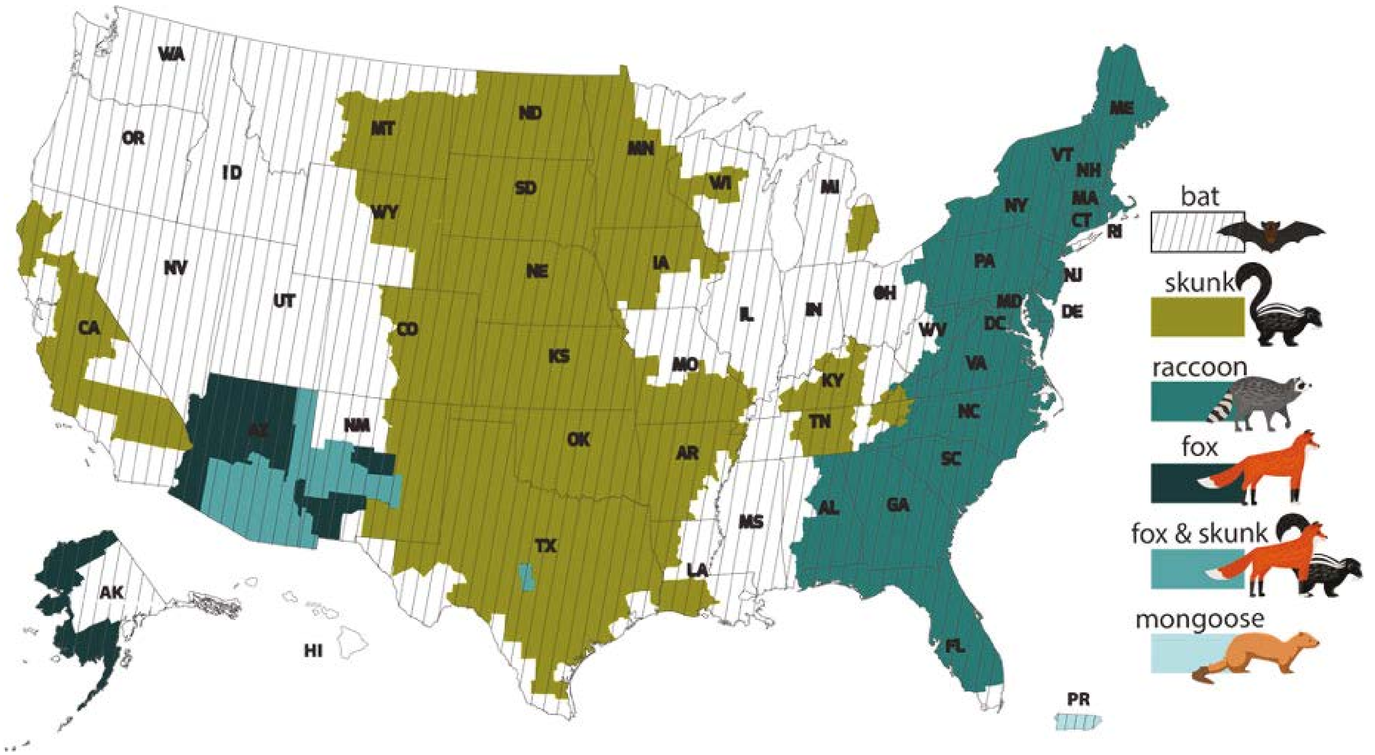

Figure 1. Common animal reservoirs for rabies virus in the US. Source: Centers for Disease Control and Prevention.

For example, in Texas, animals defined as high-risk for rabies transmission are skunks, bats, foxes, coyotes, and raccoons. If rabies infection occurs in a species other than the reservoir species for the variant – for instance, a cat infected with a skunk variant or a skunk infected with a bat variant – it is considered a "spillover event." In evolutionary terms, bats are recognized as the ultimate reservoir of the lyssaviruses (2), but despite more than 17 conspecific members, rabies virus appears to be the only lyssavirus with clear reservoir representation among multiple orders of mammals.

Protecting yourself from rabies

When people visualize a rabid animal, most picture foaming-at-the-mouth images from the movie Cujo – but the most typical signs of rabies are unexplained paralysis and a change in behavior (5). For example, a friendly cat might become abnormally aggressive, a normally playful puppy might suddenly become shy and withdrawn, or a nocturnal animal might be out during the day.

Simple steps to follow to avoid exposure to rabies

- Don't interact with strange animals.
- Never handle downed bats; as a rule of thumb, bats should be avoided altogether.
- Report bites to the proper officials, such the local rabies control authority, animal control officer, game warden, or local health department employee.
- For children, a teacher or parent is a good reporting resource.
- Though many wildlife species appear cute and cuddly, never attempt to feed or interact with them.
- Avoid handling sick, injured, or dead animals.
- If you have children or are an educator, teach them how to correctly behave around animals to avoid being bitten (not pulling animals' ears or tails, teasing them, bothering them while they sleep, running past them, moving toward unfamiliar animals, or trying to play with a mother's offspring).

What if I get bitten by an animal?

Thankfully, post-exposure prophylaxis (PEP) isn't nearly as bad as it used to be – and vaccines are widely available. If a bite does occur, wash the wound immediately with soap and water and apply iodine if available and you are not allergic; promptly seek medical attention and guidance from a physician; and take rabies PEP if prescribed by a physician (6). The single most important thing you can do is not ignore an animal bite– remember, PEP no longer involves the scary treatment of vaccinations in the stomach! Now, it consists of a weight-based dose of human rabies immunoglobulin and a series of four vaccinations (five for immunocompromised individuals) in the deltoid area over a one-month period. Sometimes, because animal oral areas also contain a diverse number of bacteria, you may also be given a tetanus vaccination and antibiotics as a precaution.

If you happen to work in a high-risk occupation (like I did at the Texas Department of State Health Services Bureau of Laboratory and Zoonosis Control Division, where I tested animal specimens for rabies), you can get pre-exposure rabies vaccinations (three doses given in the deltoid area over the course of three to four weeks). You are only eligible if you work in a high-risk occupation, such as rabies diagnostic lab worker, spelunker/caver, veterinarian, veterinary technician or assistant, veterinary student, animal control officer, shelter employee, or wildlife worker. However, if you are traveling to a foreign area with enzootic rabies, you should consult with a physician about getting pre-exposure vaccinations (7).

An important, lifesaving reminder is that bat bites are almost impossible to see or identify – you may not even know you have been bitten by a bat due to their tiny, sharp teeth (see Figure 2). As a rule, do not handle bats; avoid them at all costs.

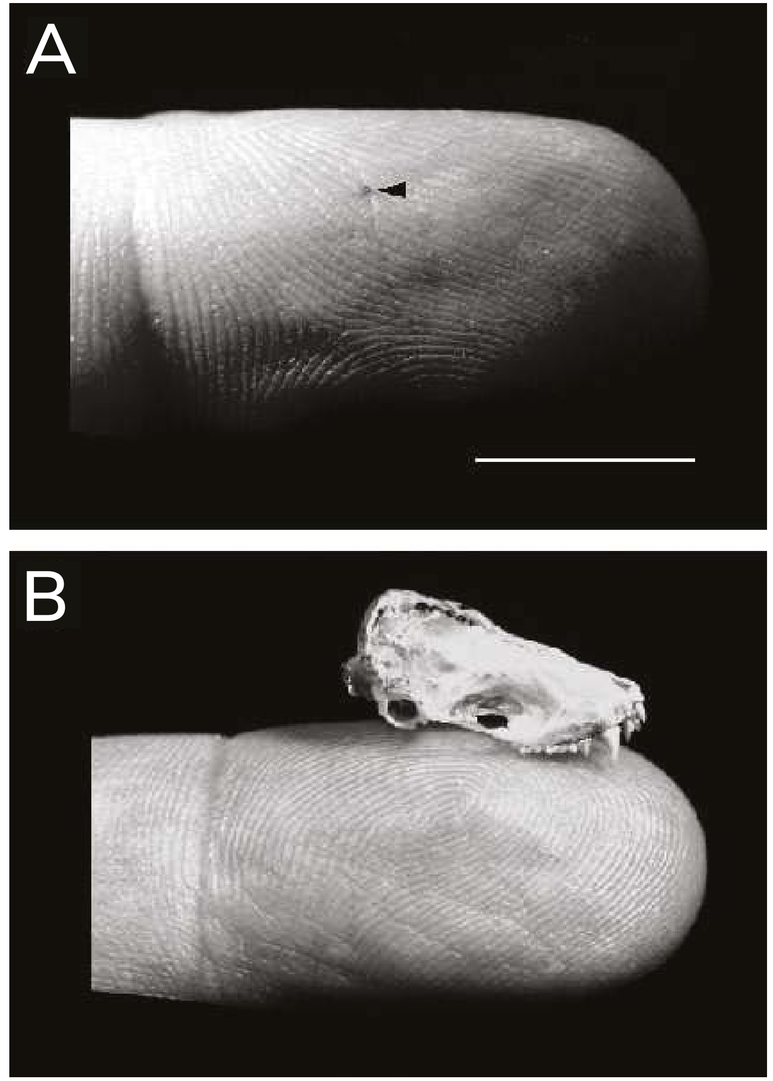

Figure 2. A) Puncture wound of a bite from a silver-haired bat. B) Skull of a silver-haired bat. Figure reprinted with permission from Elsevier (8).

"When people visualize a rabid animal, most picture foaming-at-the-mouth images from the movie Cujo – but the most typical signs of rabies are unexplained paralysis and a change in behavior ."

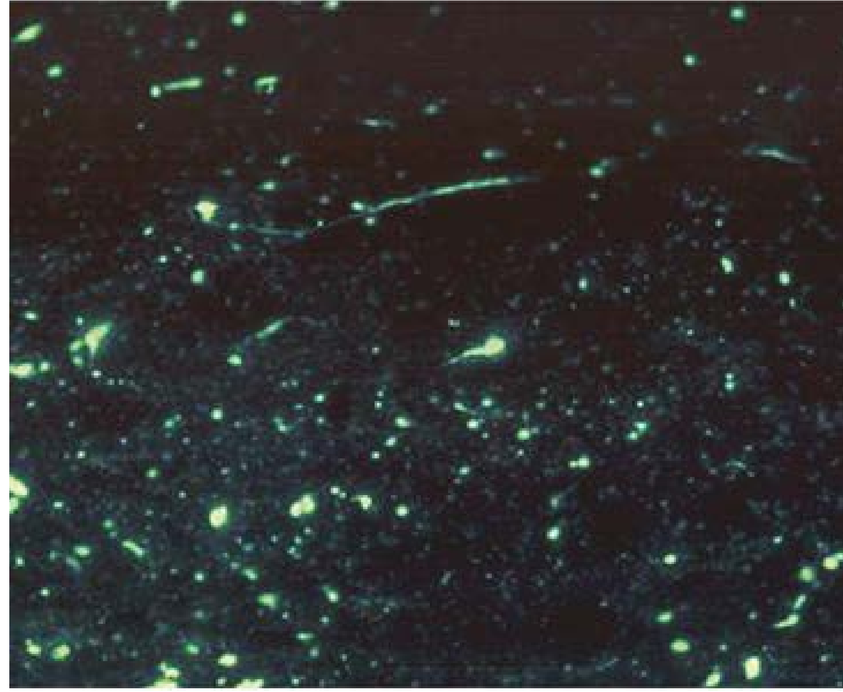

Figure 3. A specimen testing positive for rabies.
Credit: Rodney E. Rohde.

Is there a laboratory test to detect rabies virus?

In most instances, rabies is fatal once symptoms are present and specimens are tested postmortem – but there are some methods for antemortem testing in humans (9). In either case, a proper specimen (e.g., brain tissue, cerebrospinal fluid, or another specific specimen) is set up for a fluorescent antibody test (FAb), in which specific rabies antibodies attach to corresponding antigens in the tissue. If the specimen is truly positive, it results in a fluorescent green microscopic view of rabies antigens (see Figure 3). The FAb test is still considered the gold standard, though there are more current, molecular-based rabies tests and traditional classic tests that also do the trick.

What's next?

Made evident by the emergence of SARS-CoV-2 and recent outbreaks of monkeypox arriving on new shores outside Africa, viral pathogens continue to pose substantial – yet somewhat predictable – concerns to human health and welfare worldwide. In contrast to more recently appreciated threats, rabies is one of the oldest described infectious diseases and likely has an even more ancient pedigree that predates most historical accounts (2).

Beyond prevention in humans and domestic animals, rabies is the only zoonosis in which wildlife vaccination – using attenuated or recombinant biologics – has risen from an academic concept to a safe, effective, and economical long-term practice on a broad scale. For example, after the multi-year use of oral rabies vaccine (ORV) distributed in edible baits, western Europe and large parts of southern Ontario successfully eradicated fox rabies (10). There are now numerous success stories regarding ORV use in many countries, including the US. I have had the honor and privilege of working with the inaugural ORV team in Texas from 1993, during which we have eliminated canine rabies from the state (11) – just imagine what could be accomplished globally using this technology aimed at the large-scale vaccination of wildlife and subsequent reduction of mortality from this ancient, diabolical virus (12).

Over the past 10 years, substantial progress has been made on a global scale regarding pathogen discovery, diagnostics, prophylaxis, and the engagement of professionals in academia, government, industry, and international nongovernmental organizations. Further success requires maintaining this cross-disciplinary philosophy – promoting collaboration among both medical and non-medical professions within an updated One Health approach and working toward a common goal to better understand, communicate, detect, prevent, control, and eliminate lyssavirus infections within the next decade (13).

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HEV, the Blood Supply, and the Critical Role of Assay Validation

The importance of assays in the fight against hepatitis E

With Catherine Huang

Switzerland experienced an outbreak of hepatitis E virus (HEV) in 2021, and the cause still remains a mystery. More than half of the 105 cases were detected following blood donations, and almost a third were asymptomatic. Twenty-nine people were hospitalized and two died.

Essential lessons on this emerging virus came to light due to what happened in Switzerland, specifically, the serious health risk this virus poses and its threat to the blood supply. HEV Genotypes HEV is a small, non-enveloped, positive-stranded RNA virus with a genome size of ~7.2 Kb. There are four major genotypes implicated in human disease:

- Genotypes 1 and 2 are associated with waterborne epidemic outbreaks in subtropical regions of the developing world
- Genotypes 3 and 4 are typically involved in HEV infections originating in industrialized countries. They are zoonotic in origin and associated with direct animal contact or consumption of undercooked meats or transmission via contaminated blood products

HEV gt3 and gt4 infections are asymptomatic in most people, but acute infection is more likely in immunocompromised or immunosuppressed individuals. Acute HEV may present as acute liver failure and can also affect the neural and renal systems. In solid-organ transplant patients, acute HEV infection can lead to chronic hepatitis E in approximately 50 percent of cases, with a potential for rapid progression to cirrhosis.

Blood Screening

Several European studies have determined the frequency of HEV viremia in blood donations. Studies from the Netherlands and Scotland suggest that positive donations have increased significantly. A 2018 German study reported an HEV RNA prevalence of 0.12 percent (23/18,737) in blood donors, which was higher than previous German studies. The most extensive study in the United Kingdom documented a ratio of 1/2850 blood donations positive for HEV. The likelihood of developing clinically relevant hepatitis E after transfusion of an HEV-contaminated blood product was determined to be 42 percent. Since 2012, eight EU countries have implemented HEV blood screening.

HEV gt 3 transmission is frequently foodborne by infected pork, fruit, or vegetables. Boland et al. suggest that the increased prevalence may be due to changes in "agri-food industry including animal husbandry, slaughter, processing, and distribution." The risk of exposure through food is higher than exposure via contaminated blood products, leading to controversy over regular testing of all blood donations for HEV.

The mathematical risk models don't account for the potentially more severe clinical consequences of viral particles injected directly into the bloodstream versus HEV particles introduced orally that must pass the gut/blood barrier.

Testing Requirements

An Irish study from 2016 to 2017 was particularly interesting because Ireland performs universal screening on individual blood donations rather than on mini pools. Screening utilizes the Procleix® HEV transcription-mediated amplification (TMA) assay. Of the 279,879 donations screened in the two-year study period, 59 were repeat

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"The mathematical risk models don't account for the potentially more severe clinical consequences of viral particles injected directly into the bloodstream versus HEV particles introduced orally that must pass the gut/blood barrier."

reactive (0.021 percent or 1:4745 donations). Fifty-six donations (95 percent) had a quantifiable viral load, and of those, 21/56 (37.5 percent) had a viral load < 100 IU/mL. This suggests that the sensitivity of the assay is critical. (Reference: Boland et al.)

The most used assays in blood screening are:

- Procleix HEV assay (95 percent LoD 7.89 IU/mL; Panther testing system; Grifols Diagnostic Solutions, Inc., developed in collaboration with Hologic, Inc.)
- Cobas HEV PCR assay (95 percent LoD 18.6 IU/mL; Cobas 6800/8800 systems; Roche Molecular Systems, Inc.)
- RealStar HEV RT-PCR kit® for donation screening (95 percent LoD 4.7 IU/mL; Altona Diagnostics, Hamburg, Germany)

Of the eight EU countries implementing HEV blood screening, seven use a mini pool testing strategy of six, 24, or 96 individual donations. Techniques such as a larger pool size or selective testing of donations intended for those most at risk from transfusion-transmitted HEV can make screening more economical. However, pool testing puts even greater emphasis on the assay's sensitivity. For example, the Swiss Red Cross has implemented a universal HEV-RNA blood donor screening strategy using mini pools of 24 donors and testing on the Cobas HEV assay® with a required detection limit of 450 IU/mL. Since the Roche assay's LoD is 18.6 IU/mL (450 ÷ 24 = 18.75 IU/mL), screening laboratories must ensure their lab's performance is at least as good as the manufacturer's claimed limits of detection.

Laboratories need to ensure that their test performance meets sensitivity requirements. Studies have shown that low viral load samples can transmit disease, and transmission depends on the infectious dose. The median infectious dose resulting in HEV infection reported by Dreier et al. was 520,000 IU. So, while a viral load of ~130 IU/mL is unlikely to cause transmission in a low-volume procedure, Boland et al. argue that high plasma volume transfer procedures such as apheresis platelet donation (~300 mL) would result in ~39,000 IU dose and a reasonable chance of transfusion transmission.

Confident Validation

Given the critical nature of assay performance and sensitivity over time, LGC Clinical Diagnostics | SeraCare is developing a line of HEV quality control reagents to help laboratories perform robust validation studies and monitor ongoing performance through challenging, full process low positive daily QC. AccuSet™ HEV Performance Panel is a 10-member validation panel of undiluted, naturally occurring plasma samples positive for Hepatitis E. The data sheet presents data from DIA.PRO HEV IgG ELISA, DIA.PRO HEV IgM ELISA, and Altona Diagnostics RealStar® HEV RT-PCR Kit 2.0. The AccuPlex™ HEV Positive Molecular Controls kit is in development. It is a full process, low positive daily QC material targeted at less than 100 IU/mL. These materials will provide HEV screening laboratories with the tools to ensure their assays meet the patients' needs.

LEARN MORE ABOUT HOW THE ACCUSET PANEL AND ACCUPLEX KIT CAN MAKE A DIFFERENCE IN YOUR VALIDATION STUDIES BY CONTACTING US.

FOUNDATION

How Germs Shaped History

We spoke to Jonathan Kennedy, author of new book Pathogenesis, about his view that disease has shaped human history for millenia

"History is written by the victors," goes the oft-misattributed (to Winston Churchill) quote. It may be true if you only look at human conflicts – battles, wars, and the like. But one thing that is abundantly clear from reading Pathogenesis is that germs have been making losers out of us since the very beginning. Worst of all, we didn't even know it.

Much like how much of human existence was spent walking around in total ignorance of the world visible down the lens of a microscope, our many losses against infectious disease have also gone completely unnoticed; pivotal moments in our historical memory may not just be the result of feuds between people, but rather shaped and decided by the activity of germs for thousands of years.

This very argument is laid out by Jonathan Kennedy in Pathogenesis, subtitled Eight plagues that made the world in the US and How germs made history in the UK. It's a bold, striking hypothesis: Everything from the migration of hunter gatherers to the emergence of capitalism is fueled by our microscopic adversaries. I spoke with Kennedy at length about his approach to the book – and the science behind it.

Could you please introduce yourself?

My name is Jonathan Kennedy. I'm a reader in global health and politics at the Faculty of Medicine and Dentistry at Queen Mary University of London. I've just written a book called Pathogenesis that tries to transform the way that we think about history.

When we think of the natural world, we think of it as a stage. But the more that we learned about the world that we live in, we realized that we're not living on a stage. We are very much part of the system. It's a pretty precarious ecosystem in which we play a rather minor role, and in which pathogens and microbes play a really crucial one – both in the way that the planet functions, but also in the way that our own bodies, and even our own minds function.

The starting point for the book is this question: If microbes have such an enormous impact on us as individuals, what impact do they have on the body social, the body economic, and the body politic? What impact do they have on aggregations of bodies, on society, on the whole of human history? I start off by looking at the extinction of Neanderthals 50,000 years ago and wizz through history in about 300 pages – eventually looking at COVID-19 and the future.

What sparked your research into this topic?

I had quite a revelatory moment where I began to realize the importance of research done over the last 20 years – the increasing amount of evidence that showed that gut bacteria are capable of influencing our brain function. It really blew my mind, if you'll excuse the pun.

The broader context of this was COVID-19 – an event that quite quickly took on cliche to describe it: Unprecedented. As someone who has an interest in history, I knew that wasn't the case! I knew that infectious diseases have played a massive role in history and they've killed millions and millions of people at a time. But they've also created the space for new ideas and for new societies to emerge. And that got me thinking how this is one of the major driving forces of history. We often think about great men and women being the driving force of history or, if we're on the left, we may think about class struggle. But it was really interesting when I started looking at the topic of history through the lens of the pathogen. You start to see things differently.

Credit: Jonathan Cole

What research surprised you while you were writing your book?

One of the really striking things for me as a layperson was reading about retroviruses and the way in which retroviruses could insert their DNA into ours, and if they infect a sperm or an egg, this gets passed down from generation to generation. Scientists talk about something like 8 percent of DNA in the human genome coming from these retrovirus infections. This knowledge was mind-blowing enough, but then to learn that there are studies that show this isn't only junk DNA that humans seem to have acquired. Things like the ability for the placenta to bind to the uterus or the ability to form memories. The way in which information seems to pass from brain cell to brain cell seems to have been acquired from retrovirus infections.

We are taught the Darwinian idea of evolution through natural selection. This is an important aspect of evolution, but it wasn't just through conflict between and within species that evolution occurred. It was also through collaboration between our distant ancestors hundreds of millions of years ago and viruses.

Why should we be studying and recontextualizing the history of disease?

For one, it's fascinating. It's a really interesting story that overturns the way that a lot of us think about the world. And it contains some really important lessons. Even if we go back as far as the first written histories, Athens went to war with Sparta and thought it would win. But the plague of Athens struck. Nothing did them more harm, it killed about a third of the population, including the army. It killed Pericles, the great general and statesman. Eventually Athens lost and Sparta won. I think there's a lesson there for our own times in a way that we shouldn't be too hubristic. In many ways, the success of our own society has created the kind of conditions that might bring about its downfall, whether that's AI, climate change, or infectious diseases. Certainly, it seems like we're living in a new golden age for infectious diseases. Think of the unprecedented population growth, the encroachment on animal habitats, the industrial scale of factory farming, the kind of ease in which we can travel across the world. These have all combined to create almost perfect conditions for pathogens to hop the species barrier and spread quickly throughout the population.

How aware were people in the past of having immunity advantages?

That's a really good question. Certainly it must have been bizarre for the conquistadors. We often underestimate how advanced and how strong some of the civilizations were in the Americas before the European conquest. The capital city of Tenochtitlan, which is on the current site of Mexico City, had a population of something like a quarter of a million people – four times as big as the biggest city in Spain.

Pandemic after pandemic literally decimated the population of the Americas; you see the population falling from 60 million to 6 million within a century. In the days before germ theory, the best ways to explain this would be God or racism, or both. Terrible explanations in my opinion, but we can again understand a lot about the contemporary world by realizing how long lasting the legacy of those explanations have been.

One review said the book was more "marxism than microbiology." How do you respond to that?

The book certainly looks at history from the perspective of pathogens and infectious diseases, but it would be too simplistic to say that pathogens explain all of history. Often they come in on the side of one group or another and play a crucial role. I think that review in Nature was particularly pointing towards the explanation of the transition from feudalism to capitalism.

My explanation of history draws really strongly on the work of Robert Brenner, a professor of history and sociology at UCLA. Brenna doesn't really touch on infectious diseases, but he does mention briefly that, in the middle of the 14th century, you had this catastrophic demographic collapse where 60 percent of the population of Europe died. All of a sudden agricultural land is plentiful and agricultural labor is really scarce. This created a kind of crisis in the feudal system. In England in particular, it triggered a series of events that ended up with the decline of feudalism and the emergence of capitalism.

How does your research affect your teaching and mentorship role?

One of the key conclusions of the book is that, although advancements

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in medical technology over the last 50 years – even the developments of vaccines during the COVID-19 pandemic – have been fantastic, it's not enough on its own. If we look at what happened over the last couple of years, we see that particular groups suffered much, much more from the pandemic. They got sick at much higher rates and they died at much higher rates. And that really points to the fact that you cannot just see the pandemic as a virus spreading. You also have to think of it as a virus taking advantage of a habitat that human society has created for that virus to thrive.

The optimistic thing to take home from this is that we can look back to the late 19th century where politicians in the UK first started to tax the middle classes directly and tax the working classes indirectly to improve public health by building sewage systems pumping clean water into the cities. The past shows us that visionary politicians are capable of transforming public health and helping societies deal with the challenges that pathogens create for us. And I certainly think we can see that the political response to the pandemic in the UK and the US wasn't perfect, and that inequalities made the pandemic much worse. There are some pretty easy gains that can give us cause to be optimistic when we deal with the next pandemic.

What lies ahead for your work?

Oh, I'm not sure. I have to catch up on some sleep! I'm really interested in this interaction between the social world of humans and the world of microbes. So I don't think I'll move too far away from this topic…

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FOUNDATION

Colistin? Colist-out

Craig MacLean talks about how antibacterials are driving resistance – even among host AMPs

Antibacterial resistance is one of the biggest emerging threats to health across the globe, but the relationship between drug usage and growth of resistance is a delicate scale to balance. The use of therapeutic antimicrobial peptides (AMPs), for example, is met with concerns that we are running the risk of creating resistance to naturally occurring AMPs in the human immune system.

A recent study led by the University of Oxford's Department of Biology seeks answers to this problem, and presents some striking results regarding the use of the antibiotic colistin in agriculture (1). I caught up with lead author Craig MacLean to find out what these results mean for the resistance crisis.

Before we talk about your recent study, could you introduce yourself?

I'm Craig MacLean, Professor of Evolution and Microbiology at the University of Oxford. I started my research career as an evolutionary biologist, trying to understand the kind of mechanics of evolution, how populations adapt by natural selection.

A really cool example of adaptation by natural selection is antibiotic resistance. I started using that as a model to test evolutionary theory, but as I've worked on it more and more, I've become interested in resistance for its own sake and, effectively, in bacterial disease. That's what we work on in my lab – trying to understand what are the evolutionary drivers of antibiotic resistance. How can we use evolutionary thinking? How can we combat it? Why does it go away? Those are the main questions we tackle using a number of approaches.

In our experiments we challenge bacteria with antibiotics in controlled environments and watch how resistance evolves, trying to understand resistance in the real world. Sometimes we take samples from patients before and after they've been treated with antibiotics and use that to infer the processes driving resistance during infections. That's the experimental side, but we also do genomic work where we use bacterial genome sequences to help us understand resistance.

How did you get involved in the study?

It was down to an antibiotic called colistin, which was discovered in the mid 20th century. It wasn't really used in humans; it's quite toxic and has side effects. However, it could be produced really cheaply and the side effects on animals weren't bad. In fact, researchers found that if you put it in the food of farm animals, it would be economically beneficial because they would fatten faster.

From there, colistin started to be used on a really big scale in agriculture. As resistance to other antibiotics increased, colistin emerged as an important last line of defense for treating infections in humans. I became interested because of this crazy situation where an antibiotic that was the last line of defense to treat serious infections in humans was the same one being used at a massive scale in agriculture, largely as a growth promoter.

The way colistin works is quite different from other antibiotics. It's a peptide; it has a chemical structure that's similar to the chemical structures of some of the compounds that our immune system uses to fight bacterial infections. And the way they attack bacteria is similar to how components of our immune system attack bacteria. In our case, it's suggested that perhaps the resistance that eventually spread in agricultural settings is mediated by a gene called *mcr-1*.

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Is the use of colistin limited to a few countries?

It's mainly used as a growth promoter in Asia. The EU banned the use of antibiotics as growth promoters in 2006, and some other countries have followed suit. But at one point it was being used on a very big scale in China, which is where the best data comes from.

When the mcr-1 gene appeared, the Chinese government banned the use of colistin as a growth promoter. That's another reason why I became interested in it – because we had samples that were taken before and after colistin usage, which is a good way to study the consequences of reducing use.

What were the overall findings of your study?

We found that the mcr-1 gene – which spread because of the use of colistin in agriculture – confers increased resistance to antimicrobial peptides from humans, but also from pigs and chickens. This is important because these are important reservoirs of colistin-resistant bacteria.

We found that colistin also increases resistance to some other components of the immune system. The gene actually makes bacteria more virulent toward moth larvae. Wax moth larvae – Galleria mellonella – are being increasingly used to study how virulent bacterial pathogens are.

In short, the mass use of colistin in agriculture has driven the evolution of bacteria that are both more resistant to colistin and more resistant to some important components of our immune system.

What can we expect to happen as a result of using colistin in agriculture?

That's kind of an open question. I think the good news is that when China imposed a hard ban on the use of colistin, consumption dropped by about 90 percent, which was followed by a reduction in the prevalence of colistin-resistant bacteria, both in agriculture and in humans.

So – reduce consumption, reduce the prevalence of resistance. It suggests that if we stop using this antibiotic, resistance will go down. The worry here is that antibiotic resistance is becoming a bigger and bigger problem. It kills somewhere between about 1.2 and 5 million people a year, and that number is increasing. One of the ways we need to deal with it is with new antimicrobials. There may be all kinds of peptides out there that are effective antimicrobials.

The colistin story warns us that if we're going to use antimicrobial peptides to treat human infections, we may end up driving the evolution of bacteria that are resistant not only to those peptides but to our own immune system. This is really important because our immune system provides us with an important first line of defense for fighting off bacterial infection.

How would you go about solving this issue?

People are excited about these peptides for good reasons. A journalist asked me, "Should we be banning the development of these as antimicrobials?" I said, "No, we're in a position where we desperately need new antimicrobials." What we need to be doing is assessing – what are the risks in terms of resistance to our own immune system? So, we need to think about this carefully before we use any of these antimicrobial peptides.

Where does your research go from here?

That's a big question! We have three main ongoing projects in my lab. One is trying to understand what drives resistance during human infections. Another is developing new antibiotics, especially using phages – viruses that infect bacteria – as a potential alternative to antibiotics, and this is something that can complement antibiotics. The final line of research is trying to understand the mcr1 gene, how it is spread, how to stabilize it more. This has been a really interesting project and we'll be publishing a few more papers this year. It's like an onion – every layer we peel off, we find a new, interesting puzzle underneath.

We'll also be publishing some work showing that, initially, the resistance gene declined quickly when colistin use was banned because it's really costly to bacteria. So, if there's no colistin around, having this gene really harms them. But we've found that bacteria have evolved to offset that cost, which has helped to stabilize colistin resistance. So, it's unclear even if this ban led to a big drop off in the prevalence of mcr-1, and it's unclear whether it's going to disappear or persist at a lower level.

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FOUNDATION

Don't Kick the Dust: Disease in Climate Change California

A relatively obscure disease could thrive and spread in a warmer, drier United State

An interview with Royce H. Johnson

California is hot – and it's getting hotter.

The Golden State is used to periods of intense heat and drought, but man-made climate change is making central California hotter and drier for longer than before. Predictions on the lower end suggest that the state's annual average temperature will rise by 3–4°C by 2100 (1). This is bad news for the environment, agriculture, and general human survival. But there are some who will benefit from this change: two humble fungi named Coccidioides immitis and Coccidioides posadasii. They are present across the southern United States (though most prevalent in California) and thrive in hot, dry conditions. Unfortunately for us, the pair are also responsible for coccidioidomycosis, a fungal disease also known as cocci, desert rheumatism, and valley fever. Soil disruption – from construction to earthquakes – ejects infectious fungal spores into the air, where they can be inhaled to cause disease. Symptoms of valley fever are often mild – but not always.

In September 2022, the National Institutes of Health (NIH) reported on how valley fever cases have shot up in recent years (2). The numbers are still small, but this relative rise is predicted to increase further. And though the disease has, until now, lain in relative obscurity, one organization – the Valley Fever Institute – has been working since the 1950s to better understand and combat it. Now, with climate change taking center stage, the VFI is in greater demand than ever.

We spoke with Royce Johnson, Chief of Infectious Disease at Kern Medical and Medical Director at the VFI, to learn more about this insidious disease.

Could you please introduce us to your work?

My major research activity has been in coccidioidomycosis. One might ask, "Why would someone who had an NIH virology training grant in his fellowship at UC Irvine turn his attention to a fungus?" Two reasons: one, that's what we have, and two, the mentorship of Hans E. Einstein, among others. I joined Kern Medical in 1976 and have chaired the Department of Medicine, led the Division of Infectious Disease, and chaired or participated in almost every committee in the hospital during my tenure. With the establishment of the VFI, my duties have shifted to focus more strongly on directing our research and education efforts.

How long have you been involved with the Valley Fever Institute?

For more than seven decades, Kern Medical has cared for those suffering from valley fever. Our long history with valley fever care began in the 1950s

Credit: Eric Sonstroem / Flickr.com

"As the endemic areas expand, this could become a problem for some communities in states that do not offer access to healthcare and safety net healthcare."

with Hans E. Einstein, who led efforts that included treatment, research, education, and awareness. In 2015, Kern Medical formalized those efforts by institutionalizing the VFI. In the summer of 2020, the VFI opened its new state-of-the-art research and treatment facility.

For those unfamiliar with the California climate, how is it changing?

California's climate has always been varied due to the state's large geographic area. Central California has always experienced hot summers and periods of drought – but, recently, the periods of drought have been increasing in frequency and duration and the number of triple-digit days during the summers seems to be increasing as well. Additionally, the population in the Central Valley is growing due to the area's affordability relative to California's coastal and metropolitan areas.

Are there other endemic diseases in California that might worsen with the changing climate?

I can't predict the future, but I have concerns that climate change and the arrival of Aedes aegypti (a type of mosquito known to be a disease vector) could bring dengue, chikungunya, Zika and yellow fever to the region.

Is treatment for valley fever accessible (and affordable)?

Treatment is accessible, generally speaking. Because we are the safety net hospital for our community, our staff is experienced in helping patients access the available assistance programs. We also do our best to ensure that all patients receive care and treatment, regardless of their ability to pay. As the endemic areas expand, this could become a problem for some communities in states that do not offer access to healthcare and safety net healthcare.

If the fungus is present in soil, are there health implications for people working in agriculture and farming?

Not specifically those occupations, because the two Coccidioides species do not survive in irrigated and fertilized soil. However, people in outdoor occupations overall do have some level of increased risk due to the amount of time they spend exposed to ambient air, dust storms, and wind events. Workers involved in large-scale commercial green energy jobs may have an elevated risk because these projects frequently occur in areas that were previously undisturbed and may contain pockets of valley fever.

Does the VFI have plans to expand to accommodate rising cases?

Yes, the Institute hopes to continue to expand not just our clinical services – of which a key aspect is our new Infectious Disease Fellowship – but also our research, awareness, and education efforts.

What are you working on in those areas?

The Institute has a Patient and Program Development Coordinator – also a valley fever patient – who leads our awareness, education, and advocacy programs. For awareness, we are hosting the 2023 Valley Fever Awareness Walk on February 25, 2023. The most recent walk was held in 2019; we have been unable to host since then due to the pandemic. This event provides an opportunity for patients to interact with other patients and also brings awareness to the community. We often receive requests to speak at or participate in community events to help spread awareness even further.

When it comes to education, through a Susan Harwood Grant from the Department of Labor, the VFI offers training to outdoor workers through their employers at no cost. This training is required to be provided annually to construction workers in several California counties, including Kern County.

Finally, for advocacy, the VFI has recently targeted antimicrobial resistance (AMR) and the importance of education, research, and the development of new therapeutics. By linking valley fever with the broader topic of AMR, we have been able to collaborate with a broad community of stakeholders and speak with a louder voice.

The VFI is clearly making progress against valley fever, but it's not alone in its fight. Researchers from Northern Arizona University and Washington have stated that they aim to create a valley fever vaccine within the next 10 years (3). With the prospect of cases rising across the southern states in the coming decades, it seems wise to adopt a cross-country approach as soon as possible.

A lot lies ahead for California and the southern states when it comes to dealing with climate change-led disease. Joyce has answered some burning questions, but it's perhaps his shortest answer that carries the most weight. When asked whether he was concerned about the future of climate-led disease, he offered a stoic, yet simple, "Yes."

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SITTING DOWN WITH

Infectiously Radical

Sitting Down With… Ayesha Khan, social justice activist and Clinical Microbiology Fellow, Department of Pathology, Vanderbilt University Medical Center, Nashville, United States.

What led you into infectious disease?

I was born in an impoverished rural town in Bangalore, India. And I noticed early on that there was a disproportionate amount of people in my community specifically dying of a cold or drinking bad water. I came to realize there's a strong social-political context that explains why certain communities are disproportionately targeted by infectious diseases. And to this day it's the number one cause of death in the Global South.

I saw very early on that governments are terrible everywhere, but there are also people doing good work everywhere. I understood that a lot of communities were struggling with the same sorts of issues in terms of human health outcomes. Marginalized communities everywhere had similar health outcomes, died of similar things, and had higher morbidity or mortality for similar reasons regardless of location. This remained very obvious when I came to the US.

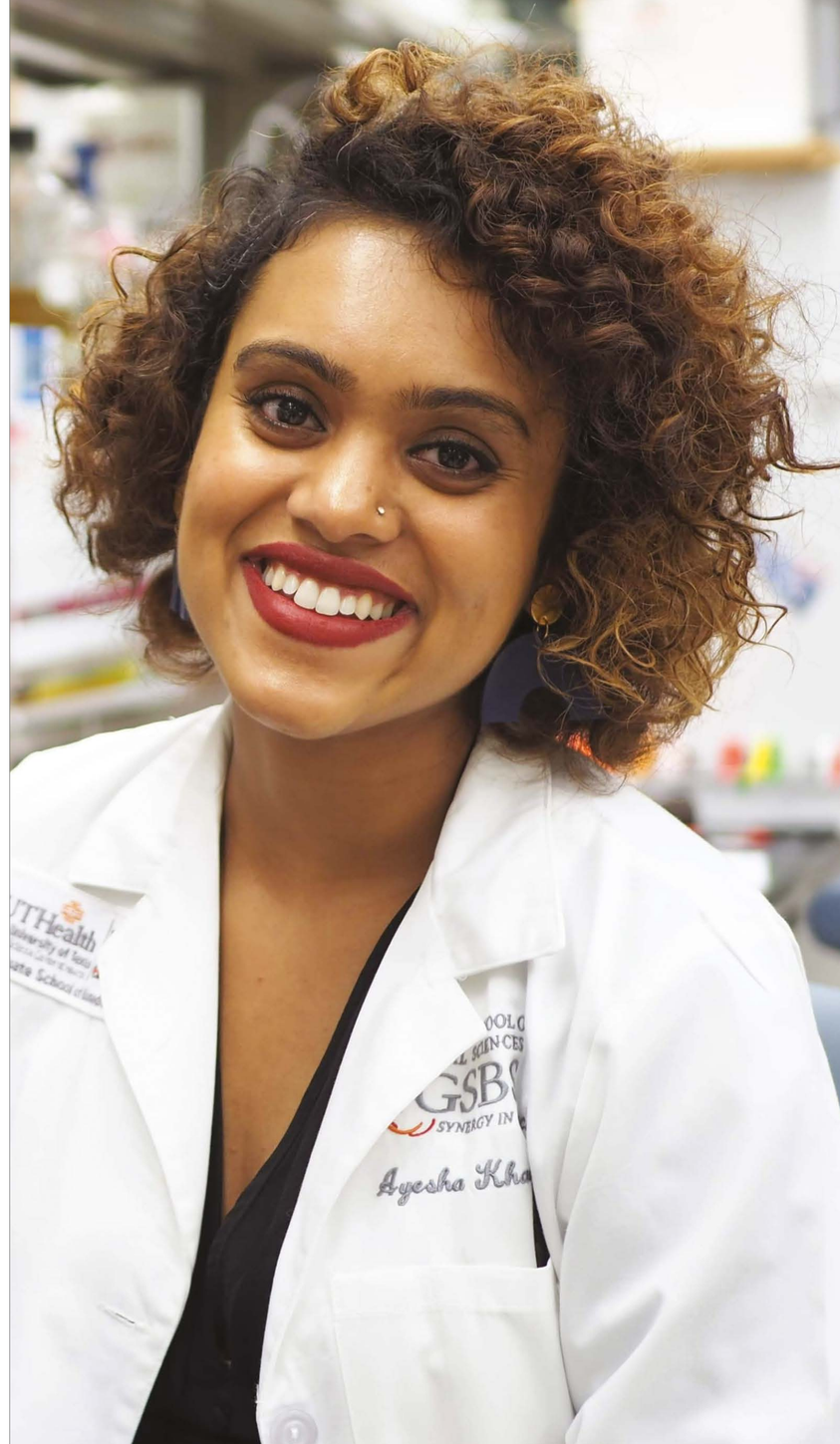
As I grew older, I had to deal with the fact that capitalist healthcare systems, almost everywhere, are profiting from sickness – without ever addressing the core root to social conditions that are making people sick in the first place.

What was medical school like as a neurodivergent person – and how has your experienced changed?

I think what's changed is my understanding of the colonization of very logical human responses to correction – or, to put it another way, the pathologization of divergence in general. Without going down the biological route, we exist in multitudes. And many of us are now even rejecting the label "neurodivergent" because it has more biological implications; instead there's a move towards "divergent" because you can't really separate the mind and the body. It's all one system. But you can't separate me from my environment either. So I've somewhat let go of the illusion of individuality. I'm always thinking about the impact of being socialized under oppressive systems and what it does to our global health.

Where do you focus your attention?

I focus a lot on trauma and how it manifests in our bodies and systems as infection, but also in how they trickle down. I'm also focusing on decolonizing medicine, and specifically psychiatry because I think it's a beautiful example of an entire branch of medicine that's based on social constructs. It's made up of diagnostic criteria that are impossible – even today – to validate with biomarkers. In infectious disease, I do



"I believe everyone, regardless of what they do, has to ask themselves about the choices they have to make."

actually have to culture something to be able to say what the etiology is. But in the case of psychiatry, it's just an arbitrary list of criteria and boxes that we have to check. What hit me is realizing that medicine pathologizes the individual right to fall out of line in some way.

So you think society, politics, health, and science are inextricably intertwined…

I believe everyone, regardless of what they do, has to ask themselves about the choices they have to make. For example, if I care about providing care, am I really achieving that in the systems that I'm working within? Am I using the tools offered to me by the system? So far, the answer has been no. So much of my work has been focused on looking at healing through a much more politicized, collectivist lens.

One pattern that I've recognized is that capitalism reduces everything to overly simplistic binaries – good and bad, right and wrong, positive and negative. And it's essentially the same in the medical system. Our approach to healthcare is reductive – even though there's plenty of research to support different kinds of public health measures.

There's a reason that medicine around the world focuses solely on public and community halls. If you think that we really care about keeping people healthy, it makes sense to say that we need to provide them with the basic social conditions that are required to have a baseline level of health, right? Everyone needs food, water, shelter, and community. So the answers are already there.

In terms of antimicrobial resistance, how scared should we be for the future?

Most AMR is not due to overuse of antibiotics in hospitals, but overuse of antibiotics in agriculture – industrialized capitalist factory farms that mass produce brutal abuse of animals, because they are also objectified. The fertility of soil everywhere is dramatically decreasing because we're pumping herbicides and pesticides into the ground because it's the best way to maximize yield.

Unless we acknowledge that our health is inextricably tied to the health of every living being within our ecosystem, I don't think we're actually going to be able to fight or defeat AMR. We need to get to the point where we see ourselves as in sync and in collaboration with microbes.

Do you see any sort of professional pushback for being politically vocal?

Until three or four years ago, I was very much the "good diversity" hire. It was a good story. Institutions loved me because I was the person who came from nothing to climb the colonial ladder. My work today has really required me to step back from all of that; I no longer speak on career panels (because I'm no longer invited to do that sort of stuff!). The political work for sure has limited my opportunities in terms of where I can apply for fellowships. But it's also led to me having to do a lot more work on myself to be successful.

If you could say one thing to the entire world, what would it be?

We need each other. We should care for each other. We should take care of each other. We should protect each other, keep each other safe, and feed each other. Whichever way you can, embody our right to interdependence.