Going viral in the animal facility

As metagenomics advances, virus hunters are finding novel infections in colonies of laboratory mice across the world. What that means for scientific research and the animals themselves can depend on the mouse.

Alla Katsnelson

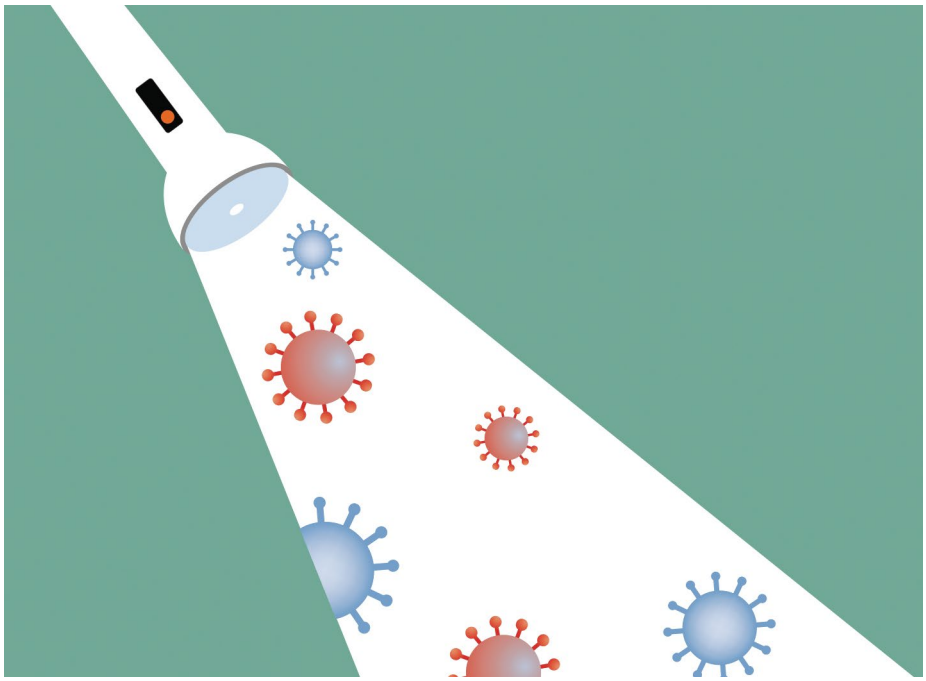
Ben Roediger is not a virus guy. He is an immunologist who studies inflammation and fibrosis in skin and lungs. But a few years ago, his team took an unplanned deep dive into virus discovery.

Roediger, then based at the Centenary Institute in Camperdown, Australia, was investigating the function of immune cells called innate lymphoid cells. Because these cells can behave similarly to T cells, he was studying them in several strains of so-called RAG-deficient mice, which are immunocompromised animals genetically engineered to lack B and T cells. The research was progressing fabulously, until he noticed that older mice from one particular strain were getting very ill.

The symptoms weren't novel. Animal staff at the institute first noticed a decade ago that breeders of immunocompromised strains would often lose weight, become hunched and sickly, and would have to be euthanized. Necropsies conducted over the years revealed kidney disease, but people generally assumed this was just something that happened to aging mice with shoddy immune systems. Roediger happened to have a burgeoning side-interest in pathology, so he did some necropsies on the sick animals himself. "It was only when my mice started getting sick that I took an interest and started investigating," he says. "And the histopathology looked classically viral."

He also found multiple mentions of a similar mouse disease in the literature, including a review that proposed a viral origin. So he and his colleagues went looking for a possible viral culprit, using a metagenomics approach to screen RNA extracted from samples of the sick animals' kidneys and searching for homology to known viruses. They hit paydirt: six sequences had distant homologies to parvoviruses, which they aligned to identify a parvovirus that had never before been described.

Initially, Roediger struggled to publish the discovery—editors just didn't seem interested in a viral infection in a single facility in Australia, he says, even though he suspected a wider problem. Then, in 2017, he read an intriguing report by veterinary pathologists at Memorial Sloan Kettering Cancer Center (MSKCC) in New York, describing a strikingly similar disease in aging immunocompromised mice with a different genetic mutation[1]. The two groups teamed up and quickly determined that the MSKCC mice were infected with the same virus, which they called mouse kidney parvovirus (MKPV).



The search is on | Using metagenomics, researchers are on the hunt for novel viruses infecting laboratory mice. Credit: RobinOlimb / DigitalVision Vectors / Getty

In 2018 they published their work characterizing the disease caused by MKPV[2]. The study had a silver lining: MPKV infection may be a promising model for chronic kidney disease in humans. But another conclusion—that MKPV has been lurking in animal facilities for decades—sounded an alarm to researchers around the world. "Within weeks of the paper coming out, we had emails left, right and center from various mouse pathologists," says Roediger. Everyone wanted to get hold of materials to test mice in their facilities for the 'novel' infection.

Traditionally, animal facilities keep out pathogenic viruses by testing for and eradicating those on a so-called exclusion list. Monitor for pathogens that are known to be bad, the thinking goes, and you can be reasonably sure that your animals are basically healthy. Of course, MKPV shows the limitation of that approach: if no one knows a pathogen is there, then no one will test for it. Now, with sequencing growing exponentially cheaper and bioinformatics tools for identifying novel viruses growing more potent, researchers and veterinarians at animal research facilities are beginning to see the value in a broader approach.

"Most infections are caused by things we already know," says Eric Delwart, a senior scientist and 'virus hunter' at the Vitalant Research Institute in San Francisco and adjunct professor at the University of California, San Francisco. "But every once in a while, there is something that is asymptomatic, or only causes diseases in

some strains of mice, like immunodeficient mice," he says. When you've run through all the known agents that could be responsible, metagenomics allows you to probe for novel unknown ones.

And while viruses like MKPV, which clearly causes disease, definitely need to be eliminated from laboratory animal facilities, even ones that appear to be totally asymptomatic in most animals can foil results. "They're confounding factors in animal experiments that people should get rid of—or at least be aware of," Delwart says.

Viral discovery

Metagenomics—the study of microbes sampled from their environment—is not a new field. It has been around for about two decades. And as sequencing technology improves, it is an increasingly powerful way to survey the microorganisms present in a sample by sequencing all the genetic material it holds, then aligning the reads with known sequences for identification. For viruses, though, the process can be tricky. Compared to bacteria, "viruses are much tougher," says Kristine Wylie, a genome scientist at Washington University in St. Louis. Viral genomes might consist of DNA or of RNA, they might be single stranded or double-stranded, and enriching for different types of viruses in a given sample requires vastly different sample prep. "There's just a lot more complexity that you don't see with other microbes," she says. "Also, the genomes are so tiny compared to other microbes that it really is like looking for a needle in a haystack."

Still, the procedure is standard enough. Researchers filter out animal and bacterial cells from a sample to enrich it for viral particles, and then amplify the viral DNA and RNA using random RT-PCR. Then comes the sequencing. "We get millions to billions of reads," says Delwart—each only about 150-250 bases long. The real artistry is often in the interpretation of the bioinformatics. When divergence is high, for example, Delwart prefers to align the amino acid rather than the nucleic acid sequence, virtually comparing similarity between proteins rather than DNA or RNA.

To date there are significantly fewer viruses in sequence databases such as GenBank than there are bacteria, because the latter got a head start as the first focus of the Human Microbiome Project when it launched in 2007. But as more viruses are added to public databases, identifying novel ones becomes easier. "It's like a bootstrap," explains Delwart. "You use what's known to find new stuff and then that new stuff becomes part of the known and can also be used for further discovery."

On the virus hunt

And new stuff is out there, says Simon Williams, a scientist in the lab of Ian Lipkin, an infectious disease scientist at Columbia University focusing on novel pathogen discovery. Right around when Roediger and his colleagues were sniffing out the virus wreaking havoc on their immunocompromised mice, Williams and his colleagues were chasing down wild house mice in the residential cellars and restaurant kitchens of New York City, nonchalantly passing tourists and other pedestrians on the streets with bags of full mousetraps to ferry their catches to their lab and survey the pathogens they carried.

In 2018, Williams's team reported that they had found 36 viruses in the feces of these mice, many of them related but still distinct from known viruses, and six that were totally novel[3]. One of those six was MKPV. That suggests that "viruses in the wild are posing problems in lab populations—and might have been for a very long time," says Williams. "Given *mus musculus* is the most studied animal in the world, it was surprising, in a way, that we didn't know as much about it as we thought we did."

MKPV wasn't the only virus on Williams's list that had been crossing over into lab animal facilities. Researchers at the animal facilities shared by MSKCC and Weill Cornell Medicine (WCM) recently identified another of the six novel viruses Williams's team found in wild mice—this one called mouse astrovirus 2—lurking among their animals. In that case, sentinel mice—which are regularly tested for a battery of pathogens to monitor the health of the larger mouse colony—that were housed in one room of a WCM animal facility tested positive for a rare herpesvirus called mouse thymic lymphotrophic virus (MTLV) on an antibody assay.

That MTLV seemed to be present was weird enough, considering that this particular virus almost never pops up in lab mice. But the story soon took an even stranger turn, says Rodolfo Ricart, Senior Clinical Veterinarian and Head of Biosecurity at the Research Animal Resource Center at MSKCC and WCM. Sentinel mice in a room of a different WCM facility also tested positive for MTLV on the same antibody test. But when Ricart sent the samples to a different diagnostic lab, the tests came back negative by both an antibody assay and a Western blot. "Of course, we confirmed by PCR that there was no MTLV," he says. "We couldn't explain how this was happening."

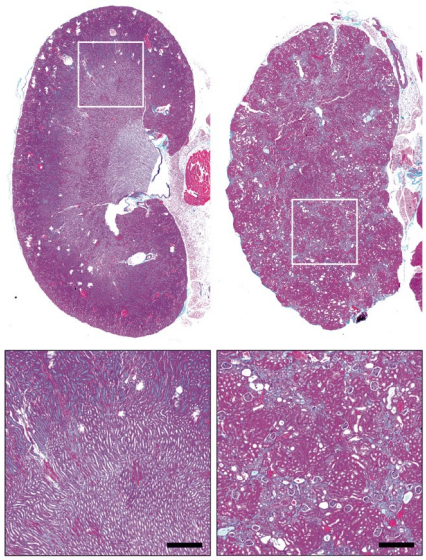
Some further sleuthing revealed that cross-reactivity with another virus was producing the positives. To find out what that virus was, Ricart and his team brought in the big guns: Vitalant's Delwart, who pegged a previously undescribed astrovirus. Then, a deep analysis of the sequence revealed that the virus was identical to one described in Williams's paper, murine astrovirus 2 (Ricart's study has been accepted for publication but not yet published.) Ricart suspects that the laboratory mice were probably infected by feral interlopers that gained access not to the housing rooms but to the laboratory areas where scientists conduct experiments. "While we do not have evidence that this is what happened, all the information we have is highly suggestive that this was the case," he says.

Williams says his study suggests this kind of infiltration is not especially unusual. "I think there's a really good chance that a lot of these viruses have found their way in, and we're just not looking," he says. "It's a 'hear no evil, see no evil' situation."

Considering the consequences

Unlike Roediger's viral find, MKPV, murine astrovirus 2 doesn't seem to cause any symptoms in mice, and Ricart has no evidence that it affects either the animals' health or the results of experiments they are used in. Murine astrovirus 1, which was discovered in animal care facilities around the world over the past decade, seems similarly benign. That raises the question of whether such viruses might simply be considered commensal— freeloaders, but harmless ones. Although researchers are beginning to identify commensal bacterial species present in the microbiome, much less is known about how that works for viruses. But where and how to draw the line between commensal viruses and ones that must be eradicated is still very much a matter of debate.

A tale of two mice? | Novel viruses found in city rodents aren't limited to the streets—in New York, viruses may have made their way into 'clean' laboratory mice. Credit: Marjorie Santos / EyeEM / Getty



Is that MKPV? | Whether or not murine kidney parvovirus can cause complications in a mouse can depend on the strain that's infected. Reprinted with permission from Roediger et al. (2018)[2]. Elsevier Inc.

Mice bred for research are typically much cleaner than wild mice, but evidence suggests that animals need some exposure to viruses in order to develop normal immune systems. For example, a 2019 study reported that inoculating mice with one virus— in this case, murine astrovirus 1—makes them more resistant to subsequent pathogen exposures[4]. Similarly, recent studies have shown that laboratory mice whose microbiomes were fortified with microbiota from wild mice exhibited immune responses that more closely approximated that of humans.

Indeed, it's hard to predict how a viral infection might affect results for different strains of mice and under different conditions, says Delwart. MKPV causes disease in immunocompromised mice, but immunocompetent lab mice may carry it too without showing any clinical effects. What's more, he says, "'asymptomatic' is in the eye of the beholder." For example, if you're running a cancer study and using anti-cancer drugs to immunosuppress your mice, the asymptomatic infection they're carrying might suddenly become symptomatic. And even while it is asymptomatic it may be affecting immune responses in a subclinical yet significant way.

Wherever you fall in the debate, knowing the identity of the viruses your animals carry is crucial, says Williams. In fact, ideally, researchers who find a virus in their population should experimentally determine what it does. "Until those experiments are run to try and infect naïve animals, we really don't know whether it's part of the normal flora or whether it actually could cause disease," he says.

Roediger, who is now an investigator at Novartis Institutes for Biomedical Research in Basel, agrees that to really understand how newly identified viruses affect a mouse or other model organism, researchers will have to do the hard work of characterizing them. But first they have to find them—and he concedes he lucked out with a virus that has such a big pathological and geographical footprint.

But even if it's not as obvious, he recommends that researchers who see something funky in their mouse studies reach out to veterinary pathologists, metagenomics experts and other colleagues to investigate what might be hiding in their animals. "Don't just ignore it because it's not germane to the experiment you're working on at the time," he says. "Understanding the pathology of the mice is really, I think, a part of our job."

It can also be exciting, he says. In January, he and his colleagues published a follow-on study to their original MKPV paper reporting that close relatives of MKPV on other species also attack the kidney. For the study, they worked with scientists around the world to identify the kidney virus in multiple species—a vampire bat from the Brazilian rainforest, a wild mouse found in China, a capuchin monkey killed by a car in Costa Rica[5]. Collaborating with researchers around the world to solve a mystery that was born in his lab felt like "the start of an Indiana Jones movie," he says. "I still think of that as the most fun project I was involved in." ❐

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