

Bat research takes wing

In the field and in the lab, scientists across the globe are working to better understand the biology of the bat

Michael Eisenstein

Bats have a PR problem. In the Western world, at least, they are commonly associated with darkness and filth—and in popular culture, the supernatural forces of evil. But the researchers who actually work with these animals take a different view. “Bats are just such beautiful animals,” says Michelle Baker, who studies comparative immunology at the CSIRO Australian Animal Health Laboratory. “They’re so gentle.”

Unfortunately, their reputation has taken another hit in recent years, with the steady emergence of zoonotic viruses that exploit bats as a Trojan Horse to mount their attack on humanity. These include high-profile threats like Ebola and severe acute respiratory syndrome (SARS) as well as less well-known—but still deadly—viruses like Nipah. “That’s a bat virus that is routinely, reliably and predictably spilling over from bats into people,” says Jon Epstein, a veterinarian and epidemiologist at the EcoHealth Alliance. “In Bangladesh, it kills about three-quarters of the people that it infects.”

The steady emergence of bat-borne viruses has fueled active debate about whether these animals are ‘special’ in terms of their ability to act as disease hosts. Bats are indeed distinctive in many ways. In addition to being the only mammals capable of powered flight, they are astonishingly diverse and widespread. With over 1,200 species worldwide, bats represent roughly 20% of all mammalian species, with representatives virtually everywhere humans dwell. Bats are also an indisputably rich reservoir for pathogens¹. “Even if you compensate for research bias in the



Image: Stijn Dijkstra / EyeEm / Getty

literature, bats have a disproportionately high number of zoonotic pathogens associated with them compared with other mammals,” says Epstein.

However, it remains controversial whether this viral richness arises from unique features of bat immunology and physiology, or merely from bats’ capacity to acquire exotic viruses from far and wide and deliver them to human communities. “The word ‘special’ implies that there’s something that makes them better at this, and I don’t think that’s the case—I prefer the word ‘different,’” says Tony Schountz, a microbiologist at Colorado State University. Special or not, researchers are struggling to fill in the blanks about bat biology, and hunting for insights that might clarify why and how bat-borne infections make the leap into humans, and to such deadly effect.

Disease detectives

Historically, bats have mainly been associated with rabies, although they are only seldom responsible for human infection in North America. Bats came back on the radar in the 1990s, with the discovery of the Hendra virus in Australia. “A new virus

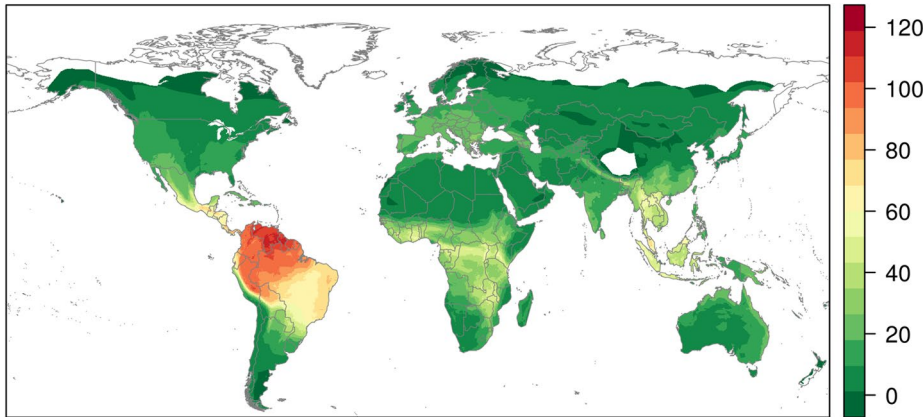
was isolated from dead horses, and a horse trainer died from the same virus during an outbreak in 1994,” says Linfa Wang, director of the Emerging Infectious Diseases Programme at Duke-NUS Medical School. “It took us about a year and a half before we realized that the natural reservoir was bats.” In the decade that followed, researchers tied numerous other viral threats to bats, including Nipah, SARS, and Ebola.

It can be difficult to identify the ‘spillover’ events where these viruses spread to humans. Each outbreak requires careful detective work to identify opportunities for bat-human interaction and obtain molecular confirmation of a shared viral strain between the two species. Most viruses are spread not by bites but through other consequences of close contact that must be pieced together through observation and field work. In Bangladesh, for example, Nipah exposure occurs through the date palm sap that villagers harvest. “Bats visit the pots overnight and lick the sap as it flows down the tree, and sometimes urinate or defecate into the pot,” says Epstein, “and a person drinking that sap a few hours later might get infected.” In other cases, the route of spillover remains unclear. Baker’s



Are bats ‘special’ in their ability to carry disease? Not necessarily, says Tony Schountz. He prefers the word ‘different.’

Image: Jackie Strong



Where to find them: A global map of bat species richness¹. Copyright 2017 EcoHealth Alliance

lab has studied a wild colony of Australian flying foxes (*Pteropus alecto*) for years, but has yet to learn what causes these bats to ‘shed’ Hendra virus as a prelude to transmitting infection.

Initial sample collection is often done in a low-tech fashion. “In our studies of paramyxoviruses in sub-Saharan Africa, some of the most important tools we used were plastic sheets,” says James Wood, a veterinary epidemiologist at the University of Cambridge. By spreading these under bat roosts, his team could harvest urine and guano for viral profiling. However, evidence of pathogens can often only be detected in blood and saliva—and some bat species live in inaccessible locales or in small numbers rather than large colonies, making it tricky to obtain such samples. Amy Gilbert, a disease ecologist with the US Department of Agriculture, recalls the difficulties she encountered studying Lagos bat virus in African straw-colored fruit bats (*Eidolon helvum*). “They’re tree-roosting and migrate really long distances, and the second you get close to the trees they all take off,” she says.

Long-term monitoring can reveal patterns of infection and viral transmission over time, but becomes extremely complex for colonies numbering thousands or millions. Epstein’s team used RFID tags to ‘chip’ batches of 100 bats from a large population of Indian flying foxes (*P. giganteus*) in Bangladesh, and only managed 60 recaptures during a six-year-long study². “But that provided tremendous information,” he says. “We saw bats that were initially negative for Nipah virus antibodies and were positive at recapture, so we knew they got infected at some point in between.”

Safety is also a pressing concern. Most emerging viruses would normally be studied in a tightly-controlled biosafety level (BSL)

4 laboratory, and Epstein notes that field researchers are generally kitted out with full-body protective gear and a respirator, even in the stifling heat of the jungle. Baker likewise notes that personal safety

must always be at the front of researchers’ minds. “You’ve just got to be very careful and handle everything as if it’s infectious,” she says. “Never get bitten by a bat’ is the unspoken rule.” Bringing bats ‘in-house’ to laboratory-based colonies can offer a safer and more controlled research environment than working in the wild, although such efforts are also fraught with challenges (Box 1).

The best defense

Wang recalls stepping into a void when he first began his bat research. “In 1996, if you keyed the words ‘bats’ and ‘immunity’ into PubMed, you’d be lucky to get maybe a dozen papers,” he says. Fortunately, the ensuing two decades of research have yielded valuable insights.

With some exceptions—most notably, rabies—it seems that the viruses that sicken humans generally do not harm bats. “In all of the experimental viral infections that we’ve done, our flying foxes just don’t

Box 1 | A captive audience

In contrast to the countless rodent vivariums around the world, there are only a handful of such facilities for bats, including colonies at the US Centers for Disease Control and Prevention and the Colorado State University. “We have Jamaican fruit bats in our colony, and so far we’ve tested six different viruses in our bats,” says Schountz, who helped establish the Colorado facility.

Such colonies make long-term studies of viral infection and transmission safer and simpler than working in the field, and eliminate the risk of other infections that could confound the study of a particular pathogen. However, they are also costly and complicated to establish. “In many places, bats are protected and getting permission to catch them is not easy,” says Wang, who manages a colony of cave nectar bats (*Eonycteris spelaea*) in Singapore. “Then a young bat takes three years to become sexually mature, and the most active females only have one pregnancy per year with one or two babies per pregnancy.” Schountz has had less of a struggle with his colony; Jamaican fruit bats breed every four and a half months, although they still typically only produce one offspring per pregnancy.

Most colonies host fruit-eating bats, which are easy to please from a dietary perspective. Insectivorous bats make far more demanding guests; habituated to hunting flying prey, these bats are reluctant

to feed from a dish, and researchers must hand-feed these animals or even put on ‘puppet shows.’ “We had our BSL4 technicians in full-body spacesuits using forceps with these little worms at the tip, waving them in front of the bats to mimic flying,” says Wang.

Far-roaming species may have a hard time coping with captivity, requiring difficult compromises. Wood and his collaborators in Ghana have established a relatively massive *Eidolon helvum* colony. “It’s large enough to allow them to fly, and enriched enough that they can roost in relatively normal ways,” he says. This requires considerable infrastructure, however, and may not be feasible at many sites. Intermediate-sized pens can be the worst of both worlds. “Some bats will have the wrong perception and think they can fly, and then they’ll bump into the cage and get injured,” says Wang. His team uses smaller cages to avoid this problem, but this means that the bats are less active than their wild brethren, confounding research into their metabolism and physiology.

Accordingly, every new colony poses a unique challenge, where experience from one species may offer limited insights for another. “We’ve learned some things from zoos and wildlife parks that do this for a profession,” says Wood. “It’s something we’ve put a lot of background work into, and not something I’d undertake lightly.”



Bat wrangling: Jon Epstein, pictured in heavy gloves and respirator despite the tropical climate, handles a collared bat in Bangladesh. Image: EcoHealth Alliance 2018

get sick—they don't get a fever, there's just no response at all," says Baker. Epstein has also observed strong indications that many viruses are swiftly defeated by the bat immune system. "They tend to be acute, short-term infections," he says. "For example, we only find about 1–3% of bats in a colony infected with Nipah virus at any given time."

Investigations of bat immunity have uncovered several possible explanations for this apparent tolerance. Baker has found that her bats maintain relatively high levels of interferons, signaling molecules that rouse the initial immune defense against infection and are normally only generated after host immune cells detect a virus. "We think this is giving them a bit of a head start," says Baker. "Then, when they're infected, they can clear the virus much more rapidly." Her bats also seem to mount a different kind of interferon response from humans and rodents, lacking a strong inflammatory component that could otherwise inflict serious tissue damage. Schountz hypothesizes that some viruses survive in bats by churning out interferon-blocking proteins, which could in turn accelerate the evolution of deadlier viruses that can essentially overwhelm the human immune system before it can react. "Human cells may have little chance to combat the virus, which then gets free rein," says Schountz. "That leads to virus replication, and subsequent pathology and cell death."

Wang believes bats may have evolved improved resistance to disease as a consequence of adaptation to the metabolic

demands of flight. "During flight, bats' body temperature can go to 38–42 °C, depending on the species, and their heart rate can go up to 1,000 beats per minute," says Wang, who notes that such sustained activity would inflict punishing stress on most organisms, rendering them more vulnerable to disease. "Bats need to have a much more efficient and more finely-tuned defense system," he says.

Starting from scratch

Nevertheless, progress remains slow in untangling the workings of bat immunology due to the limitations of the laboratory toolbox. On one hand, the falling costs and soaring speed and accuracy of DNA sequencing technologies have yielded a steadily growing collection of genome sequences for different bat species. Emma Teeling at University College Dublin is spearheading the ambitious 'Bat 1K Project', which aims to collect genomic data from every bat species on Earth.

However, bat researchers lack many standard reagents that rodent labs take for granted. Cell lines are a powerful resource for gleaning biological insights without the complexity of live animal models, but only a handful are available from bats, and none of these represent the immune cells that respond to viral infection. This has left researchers scrambling for alternatives, such as harvesting and cultivating fresh immune cells from animals for each experiment.

Many experiments rely on antibodies that bind specific proteins, which can be used for applications ranging from imaging to the selective isolation of different cell types. With few bat antibodies commercially available, labs must derive their own. This is time- and labor-intensive, and the result

may not be useful across species. "If you were to suggest a researcher should use an antibody developed for a cow in a horse, they'd laugh at you," says Wood. "That's the situation here—these species are very different, and just because we call them all bats doesn't make them close genetically or immunologically."

This highlights another critical challenge. Wang notes that bats diverged evolutionarily from land mammals roughly 100 million years ago, with a second split 30–35 million years later that produced two radically different sub-orders. "The differences between the two can be as big as between mice and humans," he says. Most research is focused on known reservoirs of disease, but Baker hopes that heightened interest in bats and the influx of data from projects like Bat 1K will help the community converge on broadly representative 'common denominators.' "We need to get a few model species so we can start sharing reagents and be more productive in what we're doing," says Baker.

Collision course

The extent to which bats are 'unique' as viral reservoirs remains open for debate, but it is indisputable that research into these animals is extremely important from a public health perspective in terms of both known and emerging diseases. "We are discovering loads of new viral sequences, but we don't know about their potential to infect people," says Epstein. And unfortunately, human activity continues to create opportunities for spillover, with ongoing urbanization and agricultural expansion steadily pushing bats and people into closer proximity. Climate change could also increase the risk. For example,



Is a bat a bat? The Jamaican fruit bat (left), cave nectar bat (center), and Indian flying fox (right) are just three of over 1200 different species that can be as varied genetically as mice are to men. Images: Tony Schountz / Linfa Wang / EcoHealth Alliance 2018



Infectious diseases aren't the only reason to study bats. "Modern medicine can learn a lot from bats," says Linfa Wang, in areas like aging and cancer. Image: Linfa Wang Lab, DukeNUS Medical School

Schountz is monitoring Jamaican fruit bats (*Artibeus jamaicensis*) infected with Tacaribe virus—a pathogen that can sicken and kill these animals. Tacaribe is closely related to numerous dangerous viruses, and although it does not currently infect humans, it could acquire that potential. "The Florida Keys is as far north as they get

right now, but climate change seems to be driving this bat species further north," he says. "I get nervous about that."

But some experts also hope the field will move beyond focusing on bats as couriers of disease to explore other unusual characteristics of these animals. For example, Wang notes their exceptional lifespan relative to what scientists would predict based on their body mass and metabolic rate. "A seven-gram bat can live for up to 43 years—that would be roughly equivalent to a human living 1,000 years," he says. He also notes that bats seem to be less prone to cancer, a possible fringe benefit of their finely-tuned 'innate defense system'. "Modern medicine can learn a lot from bats," says Wang.

More generally, Epstein hopes that fears of disease will not cause the public to lose sight of the positive contributions and

ecological significance of bats—or the responsibilities humans have towards them. "They're such important animals in terms of pest control and for pollination and seed dispersal," he says. "People are disrupting their environment, and that's causing wildlife pathogens to jump and spill over... it's squarely in our hands to think about that and adjust the way we do things." □

Michael Eisenstein

*Michael Eisenstein is a freelance science writer in Philadelphia, Philadelphia, USA.
e-mail: michael@eisensteinium.com*

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