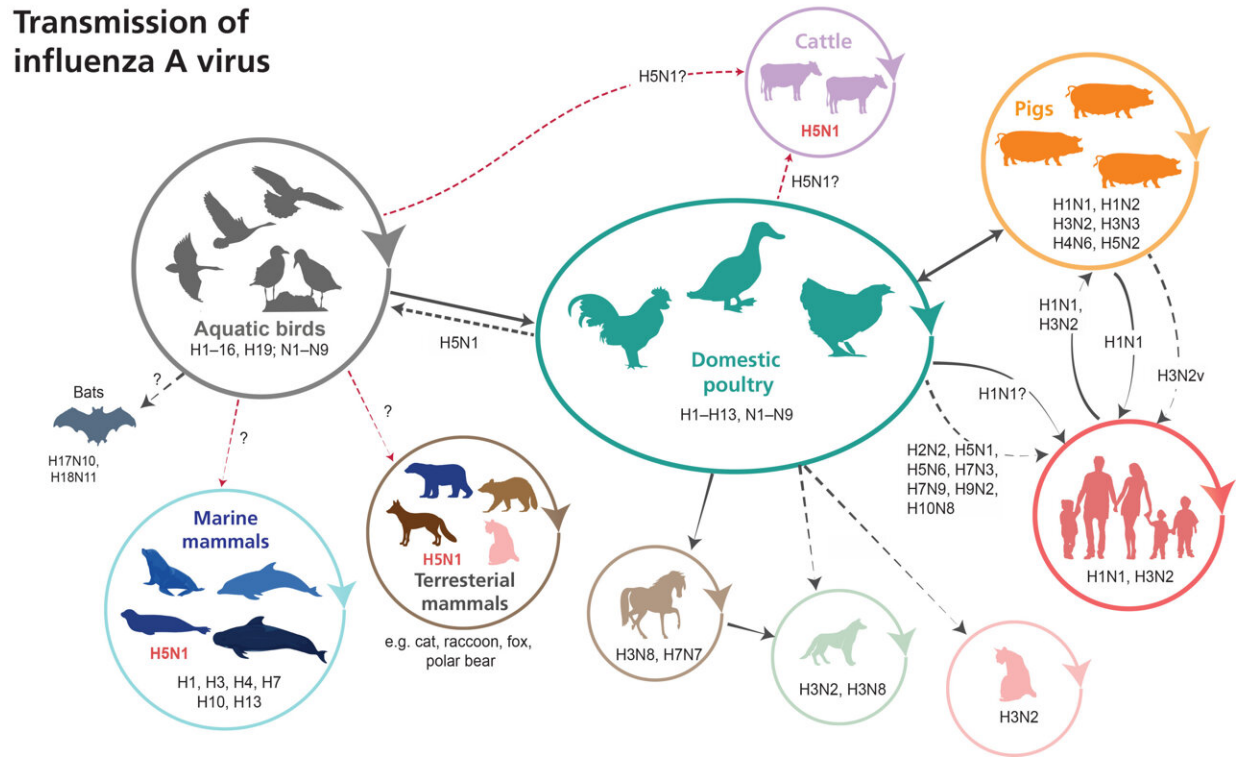
The next pandemic? How a familiar virus exploits new hosts

August 27 2024, by Nicole Lim



Influenza A boasts a wide range of natural hosts, with different strains established in different species. This wide network gives it an extra evolutionary advantage, turning it into a perennial threat to human and animal health. Credit: *Influenza and Other Respiratory Viruses* (2016). DOI: 10.1111/irv.12412

Planet Earth—a mere speck in our universe—is home to more viruses than there are stars in the cosmos. They exist at life's outer limit,

performing a delicate dance that has lasted eons but can turn in an instant. Not alive, yet entwined with the living, viruses continue to challenge and surprise us.

Like when the news broke in March 2024 that bird flu had spread to dairy cows.

"It was hard to believe," said evolutionary biologist Yvonne Su, who is an associate professor with Duke-NUS' Emerging Infectious Diseases Programme. Traditionally, cows had been thought not to be susceptible to bird flu or influenza A, the type of influenza that circulates freely in wild water and shorebirds.

Initially startled, her immediate next thought had been, "Is there direct cow-to-cow transmission?"

When we spoke in early June, Su talked me through what little data was available at that time. While the number of unanswered questions rivaled the number of herds infected, some things were beginning to emerge.

"We know it binds to the mammary glands in cows and we can see some cow-to-cow transmission," said Su.

By the end of the month, the genetic tree tracing the virus' evolution revealed an explosive epidemic. Virologist Gavin Smith, who leads Duke-NUS' Emerging Infectious Diseases Programme, summed up what he saw:

"Everything is very closely related to each other. If you look at the distribution, it comes from a background where this virus is very widespread in wild birds and the environment."

By mid-July, the virus had infected cows on 161 dairy farms in 13 US

States, along with millions of poultry across 37 farms and 9 humans.

And that was in the US alone. In Southeast Asia, H5N1—albeit a genetically different variant—has undergone a resurgence in poultry and is known to have infected at least 13 humans in Cambodia since 2023, with additional cases reported in Vietnam and China.

"The recent surge in avian influenza outbreaks is deeply concerning," said Kachen Wongsathapornchai, Regional Manager of the Food & Agriculture Organization's Emergency Centre for Transboundary Animal Diseases, in a statement.

"Since late 2023, we have observed a rise in human cases and the virus spreading to new animal species. The emergence of novel A/H5N1 strains, which are more easily transmissible, increases the pandemic threat. Immediate, coordinated preventive measures are essential."

How to build a pandemic

Influenza viruses have co-existed with humans since as early as the 16th century. Easily transmissible and highly infectious, they circulate the globe freely.

"It is estimated that by the age of 5, most people will have been infected with influenza," said Smith.

And it is not just a few types of animals that are infected with the virus. While influenza viruses fall into four types, it is really influenza A that has the widest reach, infecting almost all forms of life—from birds to polar bears, from seals to, now, cows.

And every one of the 14 influenza pandemics in humans is squarely the result of an influenza A virus.

While H5N1 hasn't caused an outbreak in humans on the scale of H1N1, versions of which were responsible for both the 1918 and 2009 pandemics, it does hold an ignoble honor: it is the first confirmed bird flu that successfully jumped directly from birds to humans.

"I was told that that outbreak was a total shock," said Smith of the 1997 H5N1 outbreak in Hong Kong that killed one out of three people infected with the virus. Their blood had thickened to treacle, their lungs, livers and kidneys failing one by one like a line of dominoes. All had been in close contact with sick chickens.

"Before that, it was thought that the virus needed to come from a mammalian intermediary host," added Smith, who has been working on influenza for more than two decades.

An intermediary host, such as pigs, whose respiratory tracts are lined with docking stations, or receptors, for both avian influenza and human influenza.

"That is why they are such good mixing vessels. They can be infected by a human influenza virus and bird virus at the same time, and these viruses can reassort their genes," said Su. "That's how we got the H1N1 pandemic virus in 2009."

But that didn't happen in 1997. The outbreak ended after a four-day-long slaughter, where all 1.6 million chickens in the territory were culled. As a precaution, live poultry markets were shuttered for seven weeks. Trade stood still and the world held its breath. But it was enough.

"The virus that infected people in Hong Kong has died out and never been detected in poultry again," said Smith.

But its siblings, tracked by the shared HA or hemagglutinin that they

carry on the outside, continued.

"The hemagglutinin is what binds to the host receptor, making it similar to the SARS-CoV-2 spike protein," said Su.

After lying low until the turn of the century, H5N1 re-emerged across Asia, causing widespread outbreaks in poultry, before spilling back into wild birds in 2005.

And it was that transmission back into wild water birds that helped H5N1 escape out of Asia and establish itself in poultry in Europe and Africa.

"But it didn't seem very well established in wild birds," said Smith.

As the virus mingled with other strains that were circulating in Europe and Africa, it followed the path of survival mindlessly, sampling from a vast array of possible new combinations of internal as well as external ones, including with the many different neuraminidases, or Ns. These fellow surface proteins release the virus out of the infected cells once it has made enough copies of itself.

With a wealth of 18 Hs and 11 Ns to choose from, on top of evolutionary mutations, influenza is almost as complex as the latest algorithm underpinning large language models.

"Besides the fact that you have the diversity in the H and the N, the influenza virus is different from the SARS coronavirus in that its genome is in eight separate strands of RNA," said clinician-scientist Ooi Eng Eong, a professor with Duke-NUS' Emerging Infectious Diseases Programme.

"Now if two viruses, say H5N1 and H3N2, go into the same animal, then

you can get all sorts of mixing. Out can come a virus that is H5N2, or H3N1, and the internal genes can also be mixed. It is this mixing that then opens a lot of doors for the virus to infect new animals."

This is precisely what happened. Between 2016 to 2019, H5 diversified, combining with N2, N6 and N8, until the H5N8 strain gained some dominance in Asia and Europe, causing large outbreaks in poultry.

Then in 2020, the H5 virus rejoined with an N1 gene, this time one that had evolved in wild birds.

"Suddenly, we have a virus that is very well adapted to wild ducks. And it's that virus that got into the US and South America," said Smith.

The virus was not only well adapted, but it also spread explosively. And it is deadly.

When Su lined up the hemagglutinin gene sequences from the latest descendants in this outbreak, she pointed to a particular grouping of nucleotides around the 1,000-base mark that was littered with Rs and Ks, marking them as viruses that can cause severe disease.

"When we see this pattern, we know this virus has a polybasic cleavage site, which means it is a highly pathogenic type of influenza," said Su.

In poultry like chickens, influenza viruses with polybasic cleavage sites generally infect cells more easily, causing widespread damage. Wild water birds, like ducks, suffer only transient or no symptoms at all.

"Unfortunately for us, highly pathogenic influenza in chickens happens to also be quite deadly in humans," said Smith.

And while the strain from 1997 has not been seen since, sporadic cases

of human H5N1 infections continue to occur, with 889 cases reported to the World Health Organization between 1 January 2003 and 3 May 2024. Its fatality rate? More than 50%.

'Teatering' on the tip of the next pandemic?

For now, the strain of H5N1 detected in the US has not made its way back to Asia. But this could just be a matter of time. Apart from geographic spread, virologists and public health officials are also on alert for other danger signs.

"The receptor binding profile. If that changes, that is a concern," said Smith, because for now, H5N1 is still fundamentally a bird flu. But with the viruses replicating exponentially, it wouldn't take a better adapted variant long to muscle out its weaker brethren.

"Another concern is that it acquires some specific mutations in the polymerase genes that are associated with improved replication in humans," added Smith.

Unlike the two surface genes, the polymerase genes are tucked away inside the virus. While they don't affect the virus' ability to bind to cells or to infect them, they determine how well the virus can copy itself. Even if an individual is infected with a virus whose polymerase genes don't carry that mutation, by the end of their infection, the mutation more likely than not to have developed spontaneously.

"So if these viruses come pre-loaded with this mutation that is a bit of a concern," added Smith.

Widespread infections among pigs are another concern because so far H5N1 seems to have shunned pigs. In fact, throughout the decades of circulating between birds and poultry and on occasion into humans,

H5N1 seems to have struggled to replicate in pigs.

Of course, so far pigs—and cows—have only had their noses swabbed for virus samples.

"All the previous surveillance looking for infections focused on the respiratory tract. No one looked at what happens in the teats," said Smith.

Even though, Smith was quick to point out, scientists from Canda's Department of Agriculture had demonstrated that a type of human influenza A could infect the mammary glands of cows back in 1953.

"Why they started in the largest animal, no one knows, but it's funny how quickly we forget," he added.

When asked whether this could be the beginning of the next pandemic, Su whispered "I hope not," as if her words could become a self-fulfilling prophecy if uttered too loud.

To do her part, Su is going back over all her samples collected not just from pigs, but goats and sheep too, testing them for the shadow presence of a virus once alive. Particularly in pigs, these viruses are known to persist for decades, always lurking in the background ready to reassort with other viruses. But she's also thinking about the implications of this outbreak on future work.

"We need to think about changing where we swab different animals when we do surveillance. Because the route of transmission will be key to helping us understand not just how an animal got infected but how we can control an outbreak and prevent it from escalating."

How cows in the US got infected remains an unanswered question. The

latest evidence, published by *Nature* on 25 July via early access, leaves room for a respiratory route as well as direct infection of the mammary gland through contaminated floors and bedding, or mechanically via milking equipment.

Expanding those early observations Su made in June, the researchers observed that their investigation "provides evidence of efficient intra- and inter-species transmission."

And if this virus isn't just well adapted to cows but spills freely from cows back into poultry and from there back into wild birds or establishes itself in pigs, it is anyone's guess what will come out.

Feeding the next pandemic

Such rampant spread through the food production system is what makes the current situation so perilous.

"The more parts of your food production system that you have infected and the more people who are possibly exposed, the greater the risk," said Smith. "Regardless of whether that is a risk of reassortment or an adaptation, that scenario provides more opportunity for either of those."

And because the number of individual virus particles is so high during an infection, population level changes can occur within a single replication round.

"So, if you are infected with viruses, where 20% bind to human receptors, and 80% bind to bird receptors, in the next round, you will only have viruses that bind to the human receptor," said Smith.

Catching the virus before this happens is impossible. The best thing that can be done is to reduce the amount of viruses out there through culling

of infected animals and vaccinating at-risk populations. Vaccinating high-risk farm workers and updating the latest pandemic vaccine strains that need to be on standby are other precautions.

For Renzo Guinto, an associate professor of Global and Planetary Health with the SingHealth Duke-NUS Global Health Institute, this is an opportunity to strike. "Since zoonotic outbreaks push us to find ways to improve infection safety in the way we produce our food, particularly animal meat products, we have an opportunity to transform food systems more comprehensively—to make them environmentally sustainable, resilient to shocks and accessible to all."

With the recent resurgence of different H5N1s around the world, the chance of a variant that is fit to spread in humans freely remains a constant threat.

But regardless of how events unfold on a human stage, H5N1 has already wrought untold damage.

"We have seen large die-offs among many species, it's spilled into marine mammals, it makes pigs sick, wreaks havoc in poultry production and now it affects cow's milk production," enumerates Smith. "It is an ecological disaster."

An ecological disaster with humanity's fingerprints all over it. So understanding this interface, where humans, farmed animals and wild birds collide, will be essential as we seek to trace the evolution and spread of H5N1. And in doing so, we may just discover how to fortify life's borders against these invisible threats.

Provided by Duke-NUS Medical School