Sentinel Surveillance Shows Novel Hendra Virus in Horses in Australia

[Announcer] This program is presented by the Centers for Disease Control and Prevention.

[Sarah Gregory] Hello, I'm Sarah Gregory, and today I'm talking with Dr. Edward Annand, an equine veterinarian epidemiologist and a research associate at the University of Sydney School of Veterinary Science in Australia. We'll be discussing the detection of a novel Hendra virus variant from a horse in Australia.

Welcome, Dr. Annand.

[Edward Annand] Thanks very much for having me, Sarah. It's a pleasure to be here.

[Sarah Gregory] Well, it's a pleasure to have you. I've actually...you're my first Australian guest, so I'm quite excited to have you here.

Lot of things going on here in this study of yours, so let's start with a real basic. What is Hendra virus? This is new, right?

[Edward Annand] Well, it's a paramyxovirus, so that's the same family as measles and mumps and also Newcastle disease, say, in chickens. And the genus is the *Henipavirus* genus. So they...the Hendra virus is...it may seem a little new, and we do think of it as an emerging disease, but I think it might be more appropriate to think of that word "emerging" as relating to our understanding, not so much the virus. It's prob...very likely an ancient virus that has co-evolved with flying foxes. And they seem to have an incredible capacity to live with Hendra virus and many other paramyxoviruses, whereas when Hendra virus spills over into domestic animal species and then, unfortunately, sometimes also into humans as well, it is highly fatal.

[Sarah Gregory] Alright. So emerging but been around for a long time, recognized as emerging.

When was it first recognized?

[Edward Annand] So Hendra virus was first recognized when it spilled over into some Thoroughbred racehorses in 1994. And a very good friend of mine, Dr. Peter Reid, who's also one of our coauthors on the paper, was the attending veterinarian in '94 in Hendra, which is a suburb of Brisbane and a really lovely suburb near the river, and a very unique suburb where there's sort of this culture of training the racehorses from almost from sort of backyard stables and taking them down to the little beach nearby and swimming them, and passionate horse owners. Wonderful horses have been raised and trained in Hendra. There was a mare called Drama Series, incidentally enough, who had been spelling, or who had been out in a paddock (not within the city, nearby) and there had been some flying foxes frequenting the trees there next to that paddock. She developed the illness and was brought into to a stable, and actually there was 13 horses that died within a 12-hour period. And Peter sort of described those horses as sort of having drowned in their own lung fluid.

And so it was very spectacular for Peter and also for our biosecurity and infectious disease department because we had a...what appeared to be a highly contagious disease, especially when it's also affected a horse in the neighboring stable, and it affected a strapper (who became very ill) and unfortunately the trainer, who died. So it was recognized that this was very likely an infectious disease and something we hadn't seen or recognized before. And there was a big effort to work out what it was, and they ended up isolating a novel virus and originally called it an

equine morbillivirus, and it was later named the Hendra virus. And also, the flying foxes were identified as that reservoir host and the source of the infection.

[Sarah Gregory] So Hendra was actually the location, sort of like Ebola virus is named after the river.

[Edward Annand] Yeah. Most viruses these days which have been named, I guess, after their location. A close relative of Hendra virus in the pararubula family is called Menangle virus (and also from this group of Australian flying foxes). Again, that's a suburb as well. So Hendra...the people of Hendra are a little bit sensitive, of course, to the name, but...and they prefer it to be called Hendra virus disease, not Hendra disease. But, yeah, that's where it started.

[Sarah Gregory] And apparently, as you were saying, it affects horses predominantly. Why are horses more susceptible?

[Edward Annand] So this is really more theorized than known for sure, because we don't know, perhaps, the full range of species that this virus might have affected without us realizing. But hence, horses are the ones that have been the only species that have been domestic animal species in which Hendra virus has been detected. Internationally, there is Nipah virus, which has affected pigs, and we can talk a bit more about that.

Horses are very inquisitive animals, and quite inquisitive in their grazing behavior. They have very well...like, very, sort of, overdeveloped airway (upper airway) spaces—they've got sinuses, guttural pouches—and so there's a lot of surface area of highly vascularized epithelium. And they have a very high tidal volume, so when they're going around grazing, they're sniffing in and out what they're about to eat to see if it's good or not. And then they also have a quite well-developed olfactory nervous system, running down into the front of their lip area there. And this is sort of been something that's involved when they do that flehmen response. So those people who've had horses, particularly stallions after they smell a mare when she's in heat, they will put the lip up and that's the flehmen response.

So there is a source there that, because Hendra virus is both neurotropic and can be pneumotropic, which means...the first one is it can infect the nervous tissue and the second one is, you know, can infect the respiratory tissue. There's sort of two possible entry pathways for the virus. It can enter via the respiratory system from having been inhaled as a droplet from a contaminated murine that's been on the ground there, or it may also be able to track its way beginning in that...the epithelium of the lip, you know and out the olfactory areas in the upper airway system, up through the nervous system as well. And that's been shown with histology to be a possible pathway there as well.

They are also very closely monitored by their owners. So, you know, compared to a cow who's one of the herd or other sort of, you know, production animals where, you know, the veterinarian may not be called for every sick animal, horses—while they sometimes are run very extensively and not checked so regularly, there are many horses that the veterinarians attend regularly. And many owners will, well, couldn't get a horse out more readily than they'd go to the doctor themselves, you know? They're sort of...the veterinarians are attending, even for minor illnesses. And so there is...perhaps a very...they're a very appropriate sentinel species in that way.

[Sarah Gregory] I see, okay.

So you've mentioned Nipah virus now a couple of times. What is Nipah virus?

[Edward Annand] So Nipah virus is very closely related to Hendra virus. It's similar enough that the available vaccine, which was made for Hendra virus for horses and now has been developed for humans, is cross-protective and that vaccine targets the G glycoprotein (now called the receptor-binding protein). Even though there's about only 70% similarity between the Hendra G glycoprotein (which is their...this protein is the way they attach into the cell) and the Nipah virus G glycoprotein, the immunity that immunized mammals gain is effective against both viruses. So they're that similar. They're also quite similar in their pathogenicity. They tend to cause the same range of disease manifestation, and they are both circulating in flying foxes (in the flying foxes).

So those pteropodid bats (pteropodid fruit bats) are the reservoir hosts for both viruses. So the biggest difference is where they are occurring. So Nipah virus has been identified spilling over into pigs and to humans without domestic animal intermediate hosts via the date palm. So this has happened in Malaysia and Bangladesh, so the distribution of...we haven't detected Nipah virus in Australia, but we've got a sort of equivalent, you know, a similar virus that's so similar that that vaccine works in the same way, that shows the same source and same manifestation of disease. So it's really that their geographic distribution that's the difference, there.

[Sarah Gregory] I see, okay. Yeah, I actually did a podcast on Nipah virus and the date palm trees and how they were infecting the sap being collected below it several years ago.

These viruses sound like they're pretty deadly, is that right?

[Edward Annand] Yeah. They are considered, in a way, the most deadly viruses that we know of. But it is sort of...in an individual patient that becomes sick, in those that become infected with these viruses (both horses and humans), they are highly lethal. There's been recorded fatality rates ranging from sort of 70% to even 100%. But there is another—I think coronavirus (SARS-coronavirus-2) perhaps even in comparison to SARS-coronavirus-1—reminder that how deadly a pathogen is also has to take into account how many.... how contagious it is between humans and how it's a bit of a balance. If you've got something that is really deadly, then you tend to detect it and it's, you know, and it doesn't spread as far and as quickly. And that was sort of the case for the SARS-coronavirus-1, whereas SARS-coronavirus-2, being much more mild disease in the majority of people, managed to spread across the world as we know too well. And it's a little bit similar for Hendra virus. They are deadly and they're a big concern for veterinarians and for those in...at high risk. But perhaps what would be even more deadly would be a version of these viruses that we might discover one day that was even more transmissible and even perhaps slightly less deadly in the individual.

[Sarah Gregory] I seem to remember with SARS-1 that that's basically why it died out so quickly, because it was so deadly it killed off its hosts and didn't...and wasn't so transmissible because it didn't have anywhere to move to. Is that sort of a fair assessment?

[Edward Annand] Yeah, that's kind of what I was saying. That's the idea there, and it's good to be reminded of that. But these henipaviruses have been listed consistently in those top...in those sort of top priority diseases (along with highly pathogenic avian influenza and SARS coronaviruses and Ebola viruses) as global priorities and potentially pandemic threats. But as we discussed, for Hendra virus or Nipah virus, the true pandemic threat would be a virus that was more transmissible. In fact, the canine distemper virus is in the same family and quite closely related, and it's an example of a virus that is more...far more transmissible. So something between Hendra and canine distemper virus and a morbillivirus would be the ultimate threat, perhaps.

When we talk about those fatality rates—say, 70%, 80%, 100%—a lot of the time we won't detect the milder cases, particularly in an extensive outbreak. And sort of serology can help determine that, but it's always a challenge to determine the true fatality rate because it's always a challenge to know the true prevalence of some of these outbreaks. In the case of Hendra, it's not so much a challenge because we tend to be right on top of it when we've detected it and monitor all of the horses that are in contact. But nevertheless, it's clear that there will. In that first outbreak, there were 20% of those horses that didn't show severe disease and that...but did seroconvert, and so obviously had become infected.

[Sarah Gregory] I see.

Okay, so what are the symptoms? You talked about two different ways it can transmit earlier, but how does it manifest in a horse, and is it the same or different than in people?

[Edward Annand] So yes, thanks, Sarah. We thought a lot about this as part of our research, because it is on recognizing the consistence...the disease manifestations in the horse as being consistent with Hendra that enable the detection, the testing, the laboratory confirmation. And without all of that, then the humans that are exposed will not receive appropriate, timely healthcare including, you know, potentially lifesaving treatment. So we've tried to think a lot about it. We've looked at all of the manifests...the previous cases and the symptoms that they showed, and what we found is that they...there is a lot of variation in any one time point. So a lot of the time, vets will look at horses and only have a small window to examine the horse. But what is consistent is that the manifestation through time, and what tends to happen is that the horse becomes very depressed, it may have a high fever in that early stage. So in a way, it's kind of influenza-like symptoms without so much of the runny nose. And then it will usually progress quite rapidly (it could be within that 12-hour period) to overt respiratory manifestations (respiratory distress) and/or neurological, sort of central nervous system encephalitic signs, including changes in the gait, head pressing, even seizure and nystagmus. There's also a lot of sweating, and that's sort of what it...that's a typical progression of disease. And including the changes in those sort of mucous membranes, they become quite congested, early on they might be a little bit injected weakly, we say, which is when they have prominent vasculature and it's something that we look at in the horse.

And in humans, it's quite similar in that they have influenza-like illness early on, usually progressing to encephalitic signs without so much of that overt respiratory distress being sort of reported. There are, of course, very few cases. There's been seven cases of confirmed Hendra infection, of which four have passed away, and a number have been veterinarians, sadly. So we realize there is a large risk for veterinarians.

[Sarah Gregory] You mentioned flying foxes. So horses are getting Hendra virus from spillover from flying foxes. Tell us how that happens.

[Edward Annand] So the flying foxes, as we mentioned earlier, sort of are understood to have coevolved with these viruses. And so that means that they live in dense communities when they're roosting; they have of course as being... the bat being the only sort of flying mammal, they have very high metabolism. They're a sort of perfect place for RNA viruses to evolve, and they've evolved with the RNA viruses. So they...they're own immunity...immune systems have evolved in ways that we're only just beginning to understand to sort of be very resilient against severe disease or disease from these viruses. They tend to...with the former...formally known Hendra virus, which we now call Hendra virus genotype 1 (so before this discovery), there

tended to be a seasonal trend (so, sort of winter months), and there tended to be certain regions that were thought to be the predominant areas where we were going to expect spillover. But ultimately, it is a sporadic event. All we need to happen is for an infected deposit of urine to land into a paddock where there's a horse that is either not vaccinated or doesn't have appropriate immunity, and that horse to engage with that—as we discussed earlier, usually smelling it or sniffing it in their grazing...part of their grazing behavior. And that's sort of what we need to happen and that....in order to see infection in a horse, and that is very unpredictable as to exactly where that's going to happen. It's a sporadic event, and that is...and it happens at relatively low frequency compared to other viruses, and yet it happens consistently sporadically with low frequency, where we've seen more than 60 spillover events resulting in more than 100 horse fatalities since we've known about this virus. So it is a very big challenge to know that we are catching all those cases. We actually have been testing around about a thousand suspect cases each year but finding less than 1% have Hendra. And a lot of horse diseases can look a little bit similar to Hendra, particularly at a particular point in time. Even some non-infectious causes of disease, such as colic, which is sort of acute abdominal disease, say, from a twisted bowel can result in the horses staggering and changing their mucus membrane and sweating a lot. And so, it can be a really big challenge to decide when we think that Hendra might be involved, and then to take the right samples, to go through that process. And there's a fantastic response system set up where veterinarians and owners can gather an answer usually within a day, sometimes it can take a bit longer, but the rapid sort of screening turnaround to allow them to manage the human health risks and the biosecurity risks.

[Sarah Gregory] And rapidity is very important, because apparently this virus is...it goes from zero to 60 very quickly, is what I'm hearing, right?

[Edward Annand] Right. So this is the progression of disease in an unvaccinated animal in an area where there are flying foxes, even if the veterinarians don't often see direct contact with flying foxes. Sometimes it might not be obvious that they are on the same property, they may just be flying overhead or visiting a dam on the property, and it might not be known to the veterinarian, and sometimes not even to the owner. So we tend to try to err on the side of caution, and veterinarians test anything that's not...any case that is not vaccinated, but could be a Hendra case, even if Hendra virus might not be our top differential at the time that we're looking at the horse.

[Sarah Gregory] So flying foxes are not foxes, they are actually bats, yes?

[Edward Annand] Right. So they are Old World fruit bats (pteropodid fruit bats). Their face is a little bit foxlike, and if anyone finds them something that's not so nice to look at, it's useful just to turn sort of around so that they are kind of there upside down, but they look kind of the right way up for us. And it's amazing how cute they look straight away, I think, too, for people that may have not found them so cute, may have found them a little bit scary.

[Sarah Gregory] I see, okay. So horses are getting it from snuffling around in the grass where there's urine, most likely. How are the people getting it from the horses?

[Edward Annand] Good question, Sarah. So all of the known cases of Hendra have occurred where there's been significant exposure to bodily fluids or through sort of the respiratory excretions or even just that sort of close proximity of the human face to the airway of the horse. So when horses are ill, veterinarians often are obliged to perform pretty invasive procedures to help them, it might be passing a nasogastric tube to administer fluids, it might be passing an

endoscope to check for the respiratory function (upper respiratory function), it might be doing a rectal examination to check for colic. And especially if horses are staggering around, there again, there can be a lot of fluid involved, be it blood or excretions, so it can be quite hard to control that. So veterinarians in areas that perceive a risk for Hendra have been trained in both themselves applying personal protective equipment and biosecurity protocols, but also in training the owners on the spot in quarantine practice, safe biosecurity, and personal protective equipment use, as well.

[Sarah Gregory] So when you're examining a horse, you're using PPEs (the vets)?

[Edward Annand] Right. In a perfect world from a Hendra virus point of view, we'd all have our PPE on every time we see a horse that even could conceivably have Hendra. But as you can imagine, in the broad geography of Australia in some areas that are hot and dusty (over 40 degrees Celsius in temperature sometimes) —also busy veterinarians, stud veterinarians—we tend not to be in PPE as a rule. So we have the concept of staged PPE. So we might always try to put on some gloves, particularly if go look at an ill animal or if we go look at a vulnerable animal, like a foal. And then the next stage up, we, you know, we're going to be putting on a mask as well, overalls. Another high-risk scenario is examining fetal loss (abortion) for zoonotic disease, where we use a higher tier of PPE. But for Hendra virus, it's our highest tier of PPE.

When we feel that it could be the case...and as I said, we don't often realize that before we get there, we might not even realize it at the start of the examination, and sometimes we might not even realize it on the first examination. It may be a subsequent examination where we realize it. But once we sort of pull that trigger of suspicion of that virus being involved, then we're going to put on the full...what do they call it...hazmat suits. You know, the goggles and the mask, and the gloves, and take full precaution. And also, we're sort of a little bit...we have to minimize the treatments, and this is very sad actually, but we have to...we're very minimized then as to what we can do for the horse. We can't do all of those usual treatments that might be necessary or might really help. And that's been a big issue, including an issue for welfare, an issue for personal indemnity, liability, and even, you know, there have been horses that have passed away for otherwise savable conditions because of the need to exclude the possibility of Hendra virus, and that delaying the timeliness of treatment or restricting the range of treatment that can be afforded to the horse until we get the negative result.

[Sarah Gregory] Oh, that's a shame, yeah.

So since Hendra (Hendra virus) is so deadly, it seems that treatments and vaccines are rapidly in the works, or there are some already. How's that going? Tell us about that.

[Edward Annand] Yeah. So the vaccine that we have...we're very lucky to have a very effective vaccine for use in horses, and this was developed as a priority because of how deadly this virus is for humans. And particularly upon realizing around 2011, and even from 2009, we started to see more cases identified. So after '94, there was just sporadic case identification. Unfortunately, some more fatalities occurred. And then in 2009, there was another larger disease event that involved an equine hospital and the loss of another veterinarian's life. This prompted the urgent development of a vaccine for horses, as a sort of One Health approach to protecting humans and of course protecting horses. It's been available since 2012, and has been used across Australia, but predominantly where there is perceived to be the greatest risk, so that's the areas of Eastern Queensland and Northeastern New South Wales.

In the experimental trials, that vaccine...of laboratory animals, that vaccine has been equally effective for Nipah virus (both Bangladesh strain and Malaysian strain). And because of the high priority of the henipaviruses as global disease threats, there's been good support and funding, particularly in the US, to see the development of that vaccine for humans. And that is now at a point where the vaccine using the same mechanism, same protein, is undergoing a Phase 1 human trial. And there is also a monoclonal antibody that has been developed, and it's Professor Chris Broder (who is one of our wonderful coauthors and has been a huge supporter of this research) from the Uniformed Services University of the Health Sciences in Bethesda, who has been the driver and leader of the science behind the development of the vaccine and of the monoclonal antibody. And we've seen that monoclonal antibody used, I think, around 13 times now. It tends to be used on compassionate grounds, so it's not fully registered yet, but it is a lifesaving treatment and has been used 13 times with great success. But it needs to be used promptly after the recognition of disease, and that's why it's so important for us to recognize this disease in horses.

[Sarah Gregory] So we have this vaccine, and now we have a vaccine that's in trial for people. But you mentioned unvaccinated horses. Why would there be unvaccinated horses?

[Edward Annand] Ah. Well, that's a very good question, Sarah. So it is to do with the perception of risk, I guess, and that differing quite heavily between, say, a veterinarian who understands and has thought a lot about this virus and has had to really think about the...this very imminent threat that can happen in an unpredictable way and an unpredictable time (could be the middle of the night). And the veterinarians are really sort of caught between a rock and a hard place because, you know, they get the call in the middle of the night—okay, fine if they know the property and the owner and the horses and everything quite well. Say they don't, say they don't know the property, don't know the situation really very well at all. All they know is this horse is staggering around, it could just have a colic (completely not infectious) or it could have Hendra virus. If they don't go, which some veterinarians unfortunately have had to take that decision, then they would realize that there's a missed opportunity (if it would be Hendra) for those owners, and that might even include children who may be quite closely exposed to the horses.

As we know, horses can be...there can be great sentimental value to people's horses in some circumstances. They're kept for a wide variety of purposes. So the veterinarian is caught between them being sort of the only way that there can be a timely diagnosis and save people involved, and their own personal risk, which are very many—their own personal health risk, for their own family as well, and for their liability, financial issues, workspace health and safety liability, and this sort of thing.

There really is quite a challenging scenario, but the perception of risk is clear for the veterinarian. It's also clear for a very, very highly valuable horse where the insurance policy might say, you know, if your horse does not receive the right timely treatment, then we won't be able to pay out on loss of the horse. Or just someone who's got a particularly valuable horse that they really don't want to lose, and a lot of people will be taking that...opt-in to take the vaccination. But the owner is realizing that the actual likelihood of their individual horse becoming infected with Hendra virus...the likelihood of it becoming infected is quite low. There's only been, you know, a hundred horses since we've known about this virus that have become infected and died there. So it's unlikely, on the basis of probability, that their individual horse will get Hendra virus. So they've sort of realized that.

There are then cost issues. Some owners will have a sort of vaccine hesitancy, in general. And in Australia, there is also an interesting thing where the owners can pick up vaccine sort of from their...from non-veterinary suppliers, and they don't...haven't always needed to have a veterinarian administer the vaccine. So that's different in the US. So in the US and the UK, every horse has got a passport, and the veterinarian has to come and administer the vaccine and write in there. And in the US, of course, you've got vaccines for West Nile virus, for quite a bunch of diseases for horses. Whereas here, we tend really to only vaccinate in the past before the Hendra vaccine. We really would have only vaccinated for tetanus, some might vaccinate as well for Strangles, and some with breeding horses would vaccinate for equine herpes virus. But they...particularly the tetanus vaccine, they can be picking this up from their feed store or pet supply store. So there's a big difference where the Hendra vaccine has to be administered by a veterinarian.

There are sort of cost issues and there's this challenge where they don't really perceive...a lot of owners won't perceive the risk as being very direct or very probable. And it's hard for them to realize that risk matrix where even though it's a low likelihood, if it's a really high consequence and fatal, it's still high risk. So a lot of owners will not understand the justification in getting vaccinated. And that's why it's been so important to educate them on the risks and where there is a risk, as well.

[Sarah Gregory] I see, okay.

Are there particular flying foxes causing these transmissions?

[Edward Annand] This is a really interesting question, Sarah. So after they identified the reservoir host of Hendra virus being pteropodid flying foxes, there was effort to identify the distribution and, you know, which flying foxes carried the virus. So serology has consistently shown that all of the four main species of flying foxes that we have on mainland Australia—the black flying fox, the grey-headed flying fox, the little red flying fox, and the spectacled flying fox—all have immunity seropositivity to the Hendra virus G glycoprotein (so, the one that our vaccine is made of). So this serology would suggest that they all have carried the virus and could be shedding the virus, and spillover could come from all of them.

So initially, it was thought that wherever there was flying foxes in Australia, there was a risk for Hendra virus. But over time, there was a failure to identify Hendra-diseased horses (Hendra-infected horses) outside of the range of the black flying fox and the spectacled flying fox. So even though not only has the serology shown that it was in the other species, but there had been isolation and molecular detection of Hendra virus in the grey-headed flying fox, for example. The detection of the horses with being infected had only occurred in those areas where the black flying fox is circulating and is living. And that is an area that has extended over time—we might talk more about that, if you're interested. But it is Queensland (in the eastern coastal areas of Queensland; so the northeast of Australia) and the northeastern region of New South Wales (so the next state down). So coastal, tropical, and subtropical regions on our East Coast, and of course this is also where there are plenty of horses living as well.

The other thing that added to what we now see as a misnomer that the distribution of Hendra spillover was limited to there, was that there had been a failure to detect Hendra virus in the urine of flying foxes. So some researchers, including some of the wonderful bat One Health researchers on this paper (Raina Plowright and Alison Peel) have been collecting urine from underneath the roost of the bat with a big plastic sheet, as there had been a failure to identify via

PCR Hendra virus in that urine when it was outside of that region, as well. So this was sort of adding to the evidence that maybe the spillover, for some unknown reason, might have only occurred from the black flying fox and the spectacled flying fox. But what our paper shows is that there was an equivalent virus sufficiently divergent in its genotype sequence (around a 15% difference, there) to fail detection on the established PCR, but sufficiently conserved in its form and function (so, much less variation in its translated immunoassay sequence), and importantly, no difference, no change at all in the critical epitope on that receptor-binding protein, and no difference in the observed disease, as well. So we're talking about an equivalent virus that was missing the molecular surveillance.

[Sarah Gregory] Okay, that's very interesting.

The way horses are sometimes tested involves dead-end testing if the horse is negative for Hendra virus. But are there dangers in this?

[Edward Annand] Thanks, Sarah. This is another very good question. So Australia is not unique in its animal health surveillance and its approach to disease management and biosecurity in focusing on a select few, in a way, well-established diseases. This is really the challenge globally for animal health. We sort of know a lot about a range of diseases and we have tests that are really reliable in detecting those diseases. And that is important because it helps us to give rapid, confident diagnostic answers to guide the health of animals, biosecurity of our countries, trade agreements, and the health of humans (when we're talking about a zoonotic disease.)

The challenge is that we are realizing over time that there is a much greater range of organisms out there, and we are only really looking at the tip of the iceberg. It's an important tip. The tips of the icebergs that we've been looking at are very important because they are the ones that over all of time, we have understood of causing significant disease. But there is, with the evolution of molecular techniques, next generation sequencing, whole-genome sequencing techniques, open-ended diagnostic approaches, we are realizing that there is a far greater biodiversity there. And with that, it is also very important to understanding health and disease. So, yeah. It has been necessary that the test is really specific and reliable and can give a very quick, conclusive answer of whether or not a horse has Hendra virus. But this study has really highlighted for this context—and of course by analogy, many others—that it's also very important that we consider divergent disease agents an emerging disease agent and have additional testing approaches when indicated. The challenge is, when do we do that? What's the justification for that diagnostic responsibility to go beyond what we know?

[Sarah Gregory] Looking at your study now, what time period were you looking at?

[Edward Annand] The time period that we were focusing on with our cases was really from 2015-2018. Our study actually was conceived and began in 2014 off the back of my experiences with Australian bat lyssavirus and the detection of that virus, which is a relative of rabies virus, in its first time being detected in domestic mammals (so it had only previously been detected in bats and then had caused three human fatalities). So off the back of those important cases (two horses dying of that) and communicating...sort of interpreting the science, writing that up with Peter Reid, as well as many wonderful scientists from the state laboratories and national lab. I became familiar with some incredible virologists, particularly Dr. Ina Smith, that were looking into the wider diversity of bat-borne viruses, and we were sort of realizing that they had a lot more viruses than the disease significance was known for. And as veterinarians, we had a lot

more severe, concerning cases than we were getting diagnoses. So it was really a matchup of that.

And so, by 2015, we had the support of the Australian veterinarians, and particularly the Queensland state laboratory (Biosecurity Sciences Laboratory) and their biosecurity department. We were beginning the sample bank of sort of rescued samples after they'd gone through their window of sort of investigations within the usual system and with the usual tests.

[Sarah Gregory] And on that note, what is different about how you suggest surveillance be done?

[Edward Annand] I think that the key difference is that we need to incorporate active surveillance activities in addition to passive disease reporting and investigation, and that we involve very much transdisciplinary teams, involve the researchers, so that we're getting that cutting edge, forefront approaches from each of the relevant disciplines and sort of combining that in constructive, proactive ways with the routine government-based disease surveillance. And it's very challenging to do this, but if this study can highlight that it can be done and can be very successful...and the active surveillance activity here might be described in a technical way as risk-based, because we were sort of focusing on the most concerning cases, both targeted and general—so we were targeting with some of our approaches a range of related viruses, but we also had an open-ended aspect to our approach, and so we could detect completely novel viruses—and sentinel, because we are detecting...like the canary in the mine, you know, we're detecting something earlier than it would otherwise be detected and getting that early warning both for One Health and animal biosecurity benefits.

These sort of active surveillance initiatives can be coupled and integrated with the routine surveillance and disease management approaches that happen in a government-run laboratory if you have the right framework, network, and funding and collaboration to enable that to happen, importantly while protecting all of those things that are so important to keeping engagement from those stakeholders at ground level—those to whom the horse matters most, the owners and the vets. So they have to be confident that by engaging with that surveillance system, they're not going to have too much burden or negative repercussions. And so, we focused on that as well. So, you know, leaving the sensitive information with that state lab and just taking what we needed to do the research, and then giving back to the state lab updated assays, updated information, updated capacity.

[Sarah Gregory] So you had an unusual surveillance employed to detect this case in your study. Is... sort of that everything you had just talked about, how you went about detecting this case?

[Edward Annand] Yeah. We gave a pretty good summary, and there's been a lot of talk about...well, I should just say that another form of active surveillance has been sort of wildlife surveillance. So a lot of the time...and we've talked a bit about that through this interview as well. So you have under roost testing of urine, is an active surveillance activity, again, designed to guide routine surveillance. There's been a lot of talk about the benefit of interdisciplinarity trends, disciplinary approaches both in research and in surveillance generally, including in the government sector. I really like the term "convergence" and convergence research, which is something that the United States research embodies. I've highlighted that it should be a big aim, now, since 2016. And actually, the term "convergence", it really sums up that transdisciplinary approach that's drawing from deep within each discipline that's relevant to the problem (to usually to a vaccine problem) persistently over time and reviewing their focus on that problem over time. So sort of inquiry-focused or hypothesis-driven research that is transdisciplinary, not

just the benefit of the junction and meeting of the disciplines, but actually forming those into disciplinary teams and drawing from deep within them to use our best tools towards a vaccine problem that is a real significance for society.

[Sarah Gregory] Now briefly, tell us about your study and why you did it.

[Edward Annand] So we called it Horses as Sentinels for Emerging Infectious Diseases, and as I mentioned, it really came out of my personal experiences on the farm (that ground level experience) with the detection of Australian bat lyssavirus (so, a relative of rabies virus there in horses). And then, as a result of that, meeting with the bat virologists, including Dr. Ina Smith, and also being connected very generously with Dr. Peter Reid, who extended his support to me when he heard about the detection and he knew firsthand, of course, what it could be like to consider such a diagnosis. And so, from that, we formed a group, initially the three of us. We found great support within the state laboratory and amongst our colleagues as veterinarians, who saw this problem and felt it firsthand as well.

So then we formed a team (and Dr. Chris Broder, who was a key founding member as well there of that team), and we basically were looking to resolve that hypothesis—could there be similar viruses closely related, likely, to Hendra virus, causing Hendra virus-like disease in Australian horses, posing similar zoonotic human health risk but failing detection via routine surveillance? So that was our hypothesis. And we looked to resolve that by extending the known assays, developing new assays, both serology approaches where we used some innovative approaches, such as both the nucleocapsid proteins (which are far more conserved), so actually looking for cross-reactivity as well as more specific proteins like the G glycoprotein or the receptor-binding protein. And then using the latest, best methods of Bayesian latent class analysis, information theory approaches to epidemiology and to test performance to interpret those results. Because of course we were sort of forging ahead a new path here, and there wasn't always going to be a Gold Standard reference test to check out our work with. So we were using these novel assays as tools, interpreting them as best we could.

And on the molecular side, we have a lot of challenges there for viral discovery when it comes to open-ended, next-generation sequencing because the actual amount of genetic material in the sample, particularly a clinical sample of variable quality, will be at most, in an infected animal, 1%. So you really are looking for a needle in a haystack. The RNA itself is very, very fragile, so we have to really take care of the samples, keep them at negative 80, think about the biosafety issues, and then develop a pipeline. We tried viral enrichment methods as part of our extraction, but in the end, we found a great high-throughput extraction approach and coupled up with the leaders in that sort of research, particularly Dr. John-Sebastian Eden, who had been working with Eddie Holmes around that...the full biodiversity, but also with pediatricians Professor Cheryl Jones and Dr. Philip Britton that had done very similar research in children looking for novel causes of encephalitis in Australia.

So we sort of formed a team, and then there was a lot of early career members of our team, as well that joined. And we sort of focused on this issue, kept revising our approaches, and also gaining funding. So we had various sources of funding, philanthropic donation from the Dalara Foundation for Horse and Human Health, which was our big opportunity to develop all those assays, trial our approaches. And once we had some initial findings, particularly serology findings, that this was likely to be a...that the hypothesis was correct, that there was other concerning viruses. Of course, we communicated that (with our government's approval) to our

veterinarians, so they became aware. But everyone realized we had to keep going and try to get that molecular evidence, and so we were very grateful our national government agency recognized the value in this research and funded it as part of their Biosecurity Innovation Program.

We then used all our approaches that we developed on our 300 most priority, higher suspect cases from a biobank of 1,700 cases, and that effort happened in 2020-2021, and we had the viral discovery here January 21st, 2021 (my son's birthday, actually). And then I think on the 24th of February, we had communicated to the government agencies everything about the finding and the interpretation, as well, of the expected vaccine efficacy being equal. Thanks to the amazing collaboration afforded through Chris Broder with Kai Xu, we were able to model the protein based on our sequence. And then we were able (sometime in March) to notify veterinarians and the public via media release.

We had also shared the capacity for the updated testing to the state and national laboratories for animal and human health. And quite incredibly, in October, prior to the ultimate publication in your wonderful journal...so we were...we did host the manuscript in pre-print as well on bioRxiv to get it to make sure everyone could read it while it was going through the rigorous process of peer review, which I commend your journal on, again. You have wonderful reviewers; they were really engaging and wonderful. And we then found that there was another prospective case detection, so a contemporary detection of a case, and it was further south, actually, near Newcastle (so further south than ever before). And it was of this novel, previously unrecognized Hendra virus variant genotype, now called Hendra virus G2. So extraordinary to see that happen as well in the timeline, and a great example of the benefit of proactive, transdisciplinary, convergent research that is supporting the government sector and routine surveillance.

[Sarah Gregory] Why is this all so important?

[Edward Annand] For us, it was so important because of the very real risk for our colleagues (the veterinarians on the frontlines). It is just really difficult for the veterinarians that, while the chances of a single individual horse or an individual farm or individual owner experiencing Hendra infection and being confronted by that deadly risk, for veterinarians it's really not at all such a low probability. They are spending their time going around and seeing all the sick horses in the area and having to treat them, sometimes in the middle of the night, sometimes without any prior knowledge of the likelihood of Hendra spilling over on that particular property due to say, you know, flowering trees or waterways or bat roosting areas. So it's very, very difficult, and that was really our justification. It was about saving the lives of our colleagues and of those that love working and caring for horses in Australia, so that's why it has been important to us.

But of course, then we realized over time that the research had a broader importance that can extend from routine surveillance and give back to it. Looking back, it seems to be a really good example of convergence research, of active surveillance activities coupled with routine surveillance. But, I mean, it's important to realize that Peter Reid and I didn't really know the meaning of any of these terms when we set out on our journey. We see that they apply now, having studied epidemiology in my case, and him having come all the way along this journey. But for us, it was just about making the job safer. We've actually seen a lot of veterinarians leaving equine practice just because of this region or going and practicing somewhere where they thought that there was negligible risk for Hendra.

[Sarah Gregory] Which is a shame for everybody—for the horses, for the vets, everybody.

[Edward Annand] Yeah. And very sadly, we've also seen a loss of the relationship between owners and vets. The relationship between horse owners and vets is different for different horses in different countries, and they are all unique. But we had something special here, as a relationship between veterinarians and horse owners and horse trainers and horse carers and breeders. And that relationship has suffered due to the challenges in managing Hendra and the difference in perceived risk, and the perceived pressure to vaccinate, and all of this sort of thing.

[Sarah Gregory] Yeah, that is a shame.

Are there environmental factors that are affecting the spread of this virus?

[Edward Annand] So the distribution of the flying foxes has been changing over time and also the pressure on the flying foxes. The main reason for this anthropogenic cause is from, you know, human related causes, particularly land clearing, both for development of housing and also for farming. And that has meant that those crucial East Coast forests (coastal forests) that have got those native blossoms that the flying foxes seek, have greatly diminished. And actually, this is all stuff I've learned from these incredible flying fox researchers, such as Ali Peel and Raina Plowright on the paper, that it's not just like any tree would work for them. They really were...used to be quite nomadic, so they would go up and down the coast with the seasons and they would actually visit particular species of flowering trees. So we've also had bush fires, and there's been a lot of changes...even changes in the climate and microclimates. Really, they have changed the way that the flying foxes are living and where they are living.

So the black flying foxes have been coming south, also the grey flying foxes, where we now have flying foxes in our most southern areas. And even black flying foxes, which traditionally or typically were not coming so far south, are coming all the way down as well in some numbers, while the numbers will remain higher further up. We're seeing that sort of change. And also, the way that they're living, so they're living now in larger roosts and really close to cities. So they're living on, let's say, the outskirts of cities and even in the middle of little towns, and they're less nomadic. So they may see each other, populations would meet perhaps less frequently. And again, the seasons themselves will influence that.

So in 2011, we had an extraordinary number of spillover events occur that year, an unprecedented number of individual spillover events to horses of Hendra virus. And one theory would be that bats that hadn't seen each other for a while had caught up with populations there, and then that virus had sort of spread around them. Certainly, they're also sometimes less well-nourished than they were in the past. They may be, in fact, more likely to visit and use as food sources via trees and the gardens of homes in cities and in sort of commercial fruit-type orchards, and in the trees where, you know, where horses are living that aren't native. That type of thing is happening. And these, apparently, are not their preferred foods, and so they can also be quite stressed. And they have also had heat waves. So there's a lot of influences, and we're just beginning to understand all that, but it's incredible to watch the research that people like Alison Peel and Raina Plowright have been doing on that.

[Sarah Gregory] Do you have any recommendations for further investigations?

[Edward Annand] We have some proposals in to do further research on the viruses we found. So we have found some additional paramyxoviruses that we're working on interpreting, that are more divergent than this one but seem to have been found in diseased horses, and have also been found in flying foxes. We're looking to investigate those viruses with some further research. We

are also definitely—for our recommendations, as well—is involving veterinarians on the frontline in research, and involving, you know, wildlife and ecology researchers in research. And so, in acting the One Health aim in a very meaningful way, by...and it's not easy to do, so I think there's got to be a lot of thought on how to do that, including funding, and also making sure that we meet the various aims of the different disciplines, while also meeting a common aim. Actually, I drew quite a lot in my research inspiration from my wife, who is an ethnographic anthropologist. So, again, they're very different disciplines, but there is incredible benefit to considering what is valuable from a very broad range of perspectives to a problem like this.

[Sarah Gregory] Tell us about your job, what you do, and how you got there and what you like most about it.

[Edward Annand] Well, I definitely thought of myself as an equine veterinarian. I've been practicing as an equine veterinarian in England and a little bit in France, and all the three eastern states of Australia since 2007. And I absolutely love horses and really enjoy working with horse owners and carers and producers and breeders and trainers, and just the breadth of the purpose of the horse. I really enjoy that job. Because of this research, I was obliged to study epidemiology, and I gained a qualification in that. Also, to have studied virology and pathology, I felt I probably should put some of that stuff to use. And I really am pleased to have been employed by the same federal Department of Agriculture, Water, and the Environment that funded our research here, in a very exciting role that sort of is sitting within their epidemiology and One Health section, but also engaging with a very promising section that they have that is all about biosecurity strategy and reform.

And so, this new role is really an exciting opportunity for me, perhaps to try to integrate some of the perspective we gained through this research into other disease scenarios for the benefit of animal health and One Health and our nation's biosecurity

[Sarah Gregory] And what part of Australia do you live in—I know you're teaching in Sydney—and what are some of your favorite activities there?

[Edward Annand] Right. So I was born in Sydney, as it happened, but I grew up in Queensland. I met my wife in Sydney, and we've raised our children there. But recently, we've moved to the Great Ocean Road. So my role for Sydney and for the federal department are remote, which is lovely to be...able to be around the children as much as possible. And actually, you know, my eldest son is only seven years old, so he doesn't really know what it would be like to have me as his dad without this enormous research project. So I'm really looking forward to spending more time with my kids, and they're just wonderful kids (two boys, there).

So we live on...in a little town called Aireys Inlet on the Great Ocean Road, and it's called "the surf coast" and it is some of the best surf in Australia. So I love surfing and the sea and the water. But I also love working with horses, and my wife made sure I've downsized my herd a little bit. So I've only got, I think, six left now (I had 24 at one point). But I love seeing them every day. I also love brass instruments—the trombone, the cornet, and even the flugabone and jazz.

[Sarah Gregory] You play those?

[Edward Annand] Yeah, yeah.

[Sarah Gregory] Nice, nice...jazz. Yeah, I play in a folk orchestra. Not even remotely similar, but...

[Edward Annand] I think that my playing developed alongside my research as a little bit of a... what do they say...for mental health.

[Sarah Gregory] Oh, absolutely.
Well, thank you for taking the time to talk with me today, Dr. Annand.

[Edward Annand] Oh, it was an absolute pleasure, Sarah. We covered a lot of ground, but one thing we probably missed out on was just the gratitude to each and every one of those coauthors, in particularly my co-lead author, Bethany Horsburgh, who is an incredible early career scientist who made this discovery possible.

[Sarah Gregory] And thanks for joining me out there. You can read the March 2022 article, Novel Hendra Virus Variant Detected by Sentinel Surveillance of Horses in Australia, online at cdc.gov/eid.

I'm Sarah Gregory for *Emerging Infectious Diseases*.

[Announcer] For the most accurate health information, visit cdc.gov or call 1-800-CDC-INFO.