

Global Distribution of Protoparvoviruses

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

[Sarah Gregory] Hi. I'm Sarah Gregory and today I'm talking with Dr. Eric Delwart about the global distribution of protoparvoviruses. Dr. Delwart is an investigator at the Blood Systems Research Institute in San Francisco. Welcome, Dr. Delwart.

[Eric Delwart] Thank you, Sarah. It's a pleasure to be on your podcast, which I listen to a lot.

[Sarah Gregory] Great, thank you! So, what are protoparvoviruses? Are they dangerous to people?

[Eric Delwart] Protoparvoviruses are part of the grid or family of parvoviruses. And what's interesting about 'em is they're among the simplest of viruses, both in terms of the genome size, they only have two genes, and particle size, they're very small, 20 nanometers or so across. Whether they're pathogenic is presently unknown, but the study we're going to discuss starts getting at that question. And, in terms of parvoviruses being pathogenic, there're certainly many precedents for that in... among the animal viruses. For example, people may be very familiar with canine parvoviruses, which is about 80 percent lethal when pups get infected. And most dog owners will actually vaccinate their dogs against canine parvovirus.

It's a very interesting virus. It seems to have recently switched host species from cats into dogs. And it's been a great model for studying this process, the mutations involved, the receptor switch, and Colin Parrish, who's been on your podcast, as actually, is a leader in this field. There's another example, for example, is a porcine protoparvovirus, which causes death of pig fetuses. So, there's certainly precedence for pathogenicity in this group of viruses. And in terms of human parvoviruses, until 2005, there were only two known human parvoviruses. One is an unusual one that's replication-defective, which means it actually needs a helper virus and, in this case, it recruits the help from a much larger adenovirus, to replicate. It's thought to be nonpathogenic, but it's a very good gene vector, and it's used in gene therapy, and I think quite successfully, for problems of the retina.

The other known human parvovirus, back in 2005, was B19, which was discovered by Yvonne Cossart, in 1975, by electron microscopy, the classic method for discovering viruses in healthy blood donors. So, B19 I think is an interesting example for what happens when a new virus is found. It typically might take a few years to understand exactly what it does to the host. So, for B19, it's actually a very common infection in infants. It causes a mild fever and red cheeks, and is often caused...called the "fifth disease" of children. But in certain subsets of individuals, it can cause much more severe disease, usually affecting red blood cell production in AIDS patients, or more commonly, in sickle cell anemia patients, people with genetic predisposition. Also, infection of pregnant woman can lead to fetal damage and, basically, something called "fetal hydrops." It's also been associated with temporary arthritis. So, it's a common infection, but in a subset of individuals, it has very serious consequences. And that's sort of fairly typical of most viruses that have been with humans for a long time. Most people do not get anything more than mild, mild symptoms, or no symptoms at all. But for a very few, typically the very young, the very old, or the immunosuppressed, the virus can be...lead to much more serious consequences.

[Sarah Gregory] Would you tell us about the types of protoparvoviruses included in this study and about the global distribution of them?

[Eric Delwart] So, there's been since the last decade, many new parvoviruses have been discovered. And, including parvo 4, which has been found in blood, bocaviruses, and then the parvoviruses of this study, the bufavirus, cutavirus, and tusaviruses. It's been fun coming up with these new viruses, because we have to give them names. Typically, we use the country in which we found, so bufavirus was found in Burkina Faso, tusavirus was found in Tunisia, and cutavirus, actually, was found in cutaneous T-cell lymphoma, so we gave it the name cutavirus. They're widely distributed. They've been found, the bufavirus has been found in Africa, in Europe, and in Asia, the cutavirus in Brazil, the U.S., Europe, and Tunisia, so far...tusavirus, so far, only found in Tunisia, in a single sample.

[Sarah Gregory] How were these new protoparvoviruses discovered?

[Eric Delwart] So, there's been a great revolution in the field of viral discovery due to next-generation sequencing, which is the ability to generate millions of DNA sequence reads. And the technique we use is called viral metagenomics, or another term for that is "shotgun sequencing." And the way we do that, is we will take any biological samples, be it feces, be it part of the intestine, a tissue, we homogenize it, we filter the homogenates to basically exclude cells and bacteria, and then we take what goes through that filter, we nuclease it to remove free-floating nucleic acids. Turns out blood and feces is just loaded with RNA and DNA from dead cells. And we want to remove that as much as possible, so what we end up with is what's protected within the viral particles. So then we extract that, we convert that into material we can sequence, and we put it through the sequencers. This technique was basically started before next-generation sequencing, when people would just take that nucleic acid and Sanger sequence it. And I think the field was started by Breitbart and Rohwer in about 2002. They were looking at sea water and, you know, many viruses present and also at the NIH looking at bovine serum. So this is a very attractive method due to its simplicity and its power, where you can recognize, through computational mean, any sequence that looks like a previously sequenced virus. So you can find all the relatives of all the viruses we already know.

At BSRI, when I started this BioDiscovery program, I asked the local scientific community what samples might be interesting to look at and people suggested I look at acute...people with symptoms of acute HIV infection, but that were not HIV positive. So, we received plasma from these high-risk cohorts with fever and rashes, but that are HIV RNA-negative. And using this shotgun sequencing, we found our first parvovirus in people with these symptoms. And then other people, of course, became interested in this virus, and a lot of work by Peter Simmons, then at Edinburgh, now in Oxford, started to look at the epidemiology by developing antibody tests, and analyzing a very specific cohort.

And it turns out that the virus is likely transmitted by blood-to-blood contact, 'cause it's heavily concentrated in people who are hepatitis C or actually HIV infected. Not only that, but it's a very tough virus, so that even Factor VIII and the coagulation factors that are heat treated, which is a very effective way of removing HIV and hepatitis C, even that heat treatment does not remove or deactivate parvo 4, because people who are being treated are still serum reverting to this virus. There's been anecdotal association of this parvo 4 with fetal hydrops, the same disease caused by B19, and also it's been reported in a few cases of encephalitis, in the cerebral spinal fluid. So, that's how we found our first parvovirus, parvo 4. The other viruses we found in feces, that is

bufavirus, and cutavirus we found in silico, by putting new parvovirus sequences through our computer pipeline, looking for homologues, and we found related sequences in a transcriptome from a skin tumor. Once we had that genome, we derived from the material from the skin tumor, we then used that to look for related viruses and we found some in feces from Brazil.

So, one of the consequences of this rapid progress in viral discovery is that now we're having a lot of viral genomes, some of which we're pretty sure are human viruses, but because we find them in just one or two cases, or even sometimes in healthy individuals, we're just not quite sure what they do. So, then it becomes important to understand the epidemiology of these viruses, and one of the methods, the most traditional method, is to develop antibody reagents and start screening a lot of people for antibodies.

[Sarah Gregory] Okay, your study looked at blood samples from people around the world. What were you looking for?

[Eric Delwart] So, we were looking for antibodies that are specific to these parvoviruses. During an infection with a parvovirus, the viremia, or the detection of the viral particles themselves, can only be done within, usually, typically, a couple of weeks postinfection, because the virus infects the individual, reaches very high titer, whether it's in the gut or in the blood, and then the body develops antibodies and cellular immune responses, and typically clears the virus. That's the scenario for most viral infections. So, when you're trying to understand the epidemiology or the distribution of this virus, looking for the virus itself is not going to give you a very clear picture, because you have to catch people at just the right time when they're sick. People don't always give blood when they're sick. So, a more precise way of determining how prevalent these infections are, is to look for antibodies, because once you develop antibodies in response to infections, you keep these antibodies for years. So, the study by Elina Väisänen, of...in the lab of Maria Söderlund-Venermo and Klaus Hedman, this lab is specialized in parvoviruses and other infections, but specifically in developing what I think are very specific antibody assays, which they can then apply to many different individuals from all over the world, and answer the questions, "How common is this infection and what kind of people are infected?"

So, the study in question looked at a population of about 800 individuals, mostly healthy, except for a subset of Kenyan individuals with fever, unexplained fever. It's a rather interesting study because one of the populations analyzed were veterinarians attending a congress in Finland, I believe, and they were asked to provide serum samples, with the intent to understand whether people exposed to animals have more frequent infection with these viruses. That goes to the issue of whether these viruses may come from animals, so you would think or expect these vets to be...have a high rate of antibody production. So, this Finnish group has extensive experience in developing these assays. The way these assays are made is you first take the genome of these parvoviruses and you express their capsid, or their outer shells, in insect cells. And, surprisingly, what happens is, in these cells, they form empty capsids, or viral-like particles. So these make great antigens to look for antibodies, 'cause these are properly folded proteins. So, you take these viral-like particles from your production system, and you attach them to the bottom of tiny plastic cups. And then you take your human serum from your population of interest, and you react that with these viral-like particles and you measure the level of antibodies that sticks specifically to these viral particles. You do that basically by washing away those that don't stick to the particles. And then you can measure the bound antibodies. So you can take a population and see how many or what percentage have been infected over their entire lifespan, since

antibodies last often for your entire lifespan. I've seen this virus and you can get an idea of the distribution of infection, whether kids get infected at a higher rate, whether it's seasonal, whether certain professions get...have a higher level of prevalence of antibodies. You can get some basic epidemiology on these viruses.

Now, a major problem with antibody measurement is cross-reactivity, especially this case, 'cause the viruses that we're looking at—the cutavirus, the bufavirus—are closely related. They're all protoparvoviruses. And therefore, the way they address this problem is they first hybridize the antibody...sort of react the antibodies to the other viruses and remove those that are bound to the soluble antigens, so that, if antibodies now bind to your plate, you can be ensured that they're specific to the virus and are not cross-reactive to the other viruses. And that is a very nice way of making sure you're not overestimating the prevalence of your antibodies in the population. So, what they found was that, first of all, they did measure quite a high level of antibodies to bufavirus, especially, and then to cutaviruses. They did not find any reactivity to the tusavirus, the third protoparvovirus in their study. But bufavirus and cutavirus are therefore now confirmed to be infecting those humans who develop antibodies. They also found, interestingly, that there was no apparent cross-protection between the different parvoviruses, so that some people had multiple reactivity to two or three of those viruses. They also found that the bufavirus, the genotypes, are actually serotypes. That is, even though they have the same name, bufavirus 1, 2, and 3, they are actually behaving differently, so that having antibodies to one does not seem to protect you against developing antibodies to the other ones. It's like you are, can be infected with all of these viruses at different time in your life.

Now, the results were that the bufaviruses were actually much more common than the cutavirus infections. And the rate of infections was quite high. For example, in, I'm gonna say, developing countries, or less-developed countries, like Iraq, Iran, and Kenya, the rate of antibody detection was over 50 percent, as high as 84 percent in Iraq. But the rate of antibody detection in Finnish individuals and from the U.S. blood donors was much lower, at about one to three percent. So, you see a difference here that I think is fairly typical of more-developed countries versus less-developed countries. Now, the cutavirus was at much lower prevalence, but that was a more even distribution across different countries. So, that's a bit of a surprising result, which will require more study. There was no antibody to tusavirus, except for a prior study from the same group, where they had found antibodies to a single Finnish child. So, it's hard to make a definite conclusion on a single positive reaction, but if tusaviruses do indeed infect humans, it seems to be a much rarer infection than the other two parvoviruses. It may well be that this virus is less adapted to human-to-human transmission, or it may even be simply a dietary contamination, since it was originally found in a fecal sample that may have originated from a, say a eaten animal, meat that, from an infected animal. So, bufavirus, cutaviruses, seem clearly to be human viruses, bufavirus at a very high prevalence of infection, and the third may or may not be a human virus.

[Sarah Gregory] Okay, so what makes these viruses interesting to researchers?

[Eric Delwart] So, there is a lot of disease that remains unexplained and people are always looking for infectious causes. There are a large fraction of enteric respiratory disease and diarrhea, where you test for all the known culprits and nothing comes up. So, it may be that there are viruses that are causing these diseases, and they may be directly causing those disease or they may be worsening those disease, if they occur on top of other infections. So, we're looking for

pathogens and, as I mentioned previously, it may be that pathogenicity is only occurring in a rare subset of individuals, so that most infections will be asymptomatic. But rarely, you will have a very nasty consequence.

[Sarah Gregory] So, you sort of talked already about these viruses being endemic in geographically distinct, undeveloped areas, like Iraq and Kenya. So, what are...again, what are the regions of interest and do we know why this, why this is the case?

[Eric Delwart] So, it's likely, as in most of those enteric infections, that it's due to lower general health care and less sanitary conditions in developing countries. For example, Iraq, in this study, had the highest prevalence, and these samples were actually collected in 2013, so after the beginning of their civil war. And I think people can appreciate that war usually impacts health care, the food supply, sanitation. So, any one of, any breakdown of these, of food supply or sanitation, I think, can increase the prevalence of enteric infections.

[Sarah Gregory] What do these results mean for, generally, for public health?

[Eric Delwart] The more people are infected with a virus, the more likely this virus is likely to land in somebody who's susceptible. So, if only a small fraction of, let's say, infants or older people or immunosuppressed will come down with a disease as a result of this infection, the more people get infected, the more likely you are to have a health consequence and cause damage.

[Sarah Gregory] Are there precautions people can take to protect themselves?

[Eric Delwart] I'm not sure there's much interest in developing a vaccine, due to the cost, and these only typically get developed for very common and very severe pathogens. There is not much one can do besides good health, good diets, maintaining good immune system, besides washing your hands and general hygiene, there is really not much one can do.

[Sarah Gregory] Are you aware of any next steps?

[Eric Delwart] Some of the outstanding questions regard tusavirus, for which no samples were found with antibodies in this study. But, the questions, typically, for those newly discovered viruses is pathogenicity. If they infect 85 percent of the population, as shown in Iraq, for example, even a one in a thousand persons, would still be a very large number. So, the way to address those is usually to do what's called case control studies, where you take a thousand unexplained cases of diarrhea, a thousand controls of the same age, the same region, but not sick. And then you measure the prevalence of either direct virus detection or recent infection with these viruses, which you can do using, again, serological assays looking for IgM, which are the first antibodies to come up. And, because of this study now, we know where these studies are more likely to yield useful data, in developing countries, where there's a much higher prevalence of infection.

[Sarah Gregory] I know listeners would really be interested to hear about your job as a blood researcher and how it relates to this particular research.

[Eric Delwart] So, at Blood Systems Research Institute, our primary mission is the safety of the blood supply. So, we collaborate a lot with blood banks across the U.S., in South Africa, and in Brazil, particularly for...on the, on the subject of emerging viruses, such as the many arboviruses that have entered the blood supply recently, such as Zika virus and West Nile. And so, for

example, one of the things we do here is we look at people with unexplained fever, which is a typical symptom of an acute viral infection, and we apply this shotgun sequencing to look for new viruses. And therefore, once we have a genome sequence, we can start addressing these questions. Is it likely to penetrate the blood supply? Is it likely to cause symptoms if transfused? Does it survive the different fractionation procedures used to make different blood products?

So, historically, BSRI was very involved in the response to the emergence of HIV and hepatitis C, and more recently, the arboviruses. And one of the missions is to develop nucleic acid tests, which are much more sensitive and allow you to identify viruses, even before the person's serum converts, when there's only the virus and not the antibodies. And working with biotech companies, they institute checks to how sensitive these assays are, how good they are at detecting infected blood donations.

Also, if you look at a, sort of a longer time scale, most human viruses originate from animals. For example, HIV came from chimps in the last 50 to 100 years. So, we also sequence material from animals with unexplained disease. We have many collaborators, for example, IDEXX, which is a vet diagnostic company, so we receive their genome, their material where unexplained disease, and we look for viruses and we sequence them. And just adding these viral genomes to the database of GenBank will help other researchers also looking for new viruses to more rapidly find and identify them. And, if these are found in human samples, it will allow a more rapid response to determine how pathogenic are these viruses and are they a concern for blood transfusions.

[Sarah Gregory] Thank you for taking the time to talk with us today, Dr. Delwart. I've been talking to Dr. Eric Delwart about his July 2018 policy review, Global Distribution of Human Protoparvoviruses. Listeners can read it online at cdc.gov/eid.

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

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