FIC BRAIN Webinar Full-text Transcript

"Surveillance for Infectious Causes of Encephalitis in Perú"

Presented by Dr. Joe Zunt, University of Washington
November 2, 2011

Joe Zunt: Great. Thanks. Well good morning, everyone. I would like to thank Kathy and others for inviting me to give this presentation. So it's really the culmination of my fifteen years working in Peru, just a little background. I'm a neurologist by training, did my medical school in Minnesota, and then moved out to Seattle where I've stayed, and I did my neurology residency here in Seattle as well as my infectious disease residency under the mentoring of King Holmes, getting my MPH at the same time and then going to Peru with my family and have been working in Peru ever since and love it. And today, what I'm going to do is talk about what I find the most rewarding parts of my work in Peru. And we're fortunate to have Christina Nelson online today as well, and I thank her for many of the slides that I've included, as well as Nicanor Mori, who is one of the Peruvian trainees. Both have come through the Fogarty International Clinical Scholars and Research Program and have integrated into this research project, which is continuing to grow. So as we have come out with our first publication about encephalitis, which is now going through writing the responses, we'll hopefully be published soon, I thought that it would be nice to write another article about how we developed this surveillance program in Peru because when we initially designed it, it was difficult to find much in the literature about how other people had done it, and I'm hoping that today I'll be able to show you a little bit more about how we did it, and hopefully it will be useful for others. So as many of you know, there's a lot of infectious diseases out there in the world, and there are only rare cases that cause encephalitis. So sporadic cases of encephalitis can pose a different issue when you're trying to study them as opposed to malaria where you can go into a country that has endemic malaria and pretty rapidly do an evaluation in the field, whereas with sporadic cases, you really need to form a wide network if you're going to try to capture enough numbers to make any significant statements. So we've decided that in Peru, what we would like to do, then I should acknowledge too that this was all funded thanks to the brain disorders program starting with R21 and then most recently the R01, is to set up a surveillance across the country to try to capture the geographic diversity of cases of encephalitis. So our objectives, as I've mentioned a bit, was really to find out what is causing

encephalitis starting with Peru. And for those who don't know Peru, Peru has the arid coastline, they have the Andes, they have the high jungle, they have the low jungle, and then they have the rural and urban. So there's quite a variety of conditions there that breed many different types of infection like arboviral infections as well as what we typically see in many other countries. So we wanted to find out, well what is causing encephalitis in Peru, find out the risk factors, and then also build capacity by enhanced laboratory capacity but really enhanced capacity overall, not just of the laboratories around the country but of the Peruvian colleagues to be able to not only work with us on this surveillance project but eventually design their own research. So this is the overview of what I'll be going over today, just about different factors that we were evaluating as we moved forward with designing our project. So I think the first issue was, well what are we looking for? So are we going to look at meningitis or encephalitis or meningoencephalitis? So just the case definition. So for a neurologist, it's complex to differentiate between the two, and I would say, perhaps, impossible. So meningitis is just typically an infection of the surrounding of the brain, the meninges, whereas encephalitis is when your brain is involved. And, as you can imagine, the meninges are the covering of the brain. So when you have infection of the covering of the brain, often it will affect the underlying brain. There are pure syndromes where you'll just get the brain involved, so that's pure encephalitis. And there's pure meningitis with just the meninges, but often it's a mix. So you'll have meningoencephalitis because both are involved. Encephalitis does tend to cause more focal problems, as you can imagine. So if you get the brain involved over the motor cortex, you're going to have more problems with the motor system because if you just have a swelling of the coating of the brain. So as I mentioned, encephalitis is really when you involve the cortex, and most often it's due to viral syndromes or viral etiologies. And there are other types of encephalitis becoming more and more recognized recently, and I would point out that the immune-mediated encephalitis are the ones that are, I think, of most interesting, being more described later and later as new potential causes of encephalitis, some of you may have heard of the NMDA receptor antibody encephalitis. So regardless of the cause, the etiology is really, it escapes us very often. So up to eighty percent of the causes of encephalitis, we never find out what caused them. We hypothesized that this is because the virus is going to come in and leave very quickly without leaving a trace other than the damage they've caused. So this is just to give you an overwhelming picture of what we confront as infectious disease or neurologists or physicians or anyone when you think about possible causes of encephalitis. So

when I have someone come into the emergency room at Harborview, our county hospital where I work, I have to go through this entire list of different activities to try to narrow down what they could potentially have been exposed to. Sometimes it's very easy, sometimes it isn't, but this is just to give you an idea that there's many different etiologies out there, and if you use risk factors to help you hone down what you're looking for, that can sometimes be helpful. And then this is just another way of looking at it, so you can look at it by risk factors, or you can look at it by what types of clinical abnormalities are present. And that can be very helpful. I'll just point out, kind of two-thirds down the list, there's something called myorhythmia, which is seen with T. whipplei or Whipple's disease, which I've never seen but continue to look for. So there's some very specific findings that can help you as you're trying to sort through this. But this is really just to give you an idea that there's a lot of different potential etiologies out there that we're trying to discover. So I think the most important cause of encephalitis is herpes. So why herpes? Well for those of you who have been around a while, I was a medical student in eighty-six, which isn't that long, but back then, the diagnosis of herpes encephalitis was made through brain biopsy. So at that point, you can imagine someone who has had herpes encephalitis for long enough to go through brain biopsy is usually pretty devastated, and they were, and they usually remained devastated. So herpes, when it's not treated, is just horrendous. Its mortality is up to seventy percent, and it's one of the most common causes of encephalitis that we can detect. Since the PCR came out, polymerase chain reaction, we've really expanded the spectrum of what we're calling HSV encephalitis. So now we have people coming into the ER, they have a headache, and what has happened is, because we have PCR, we can do a spinal tap, we can send off the assay, and we can know within forty-eight hours if they have herpes encephalitis. However, at the same time, if we are suspecting herpes encephalitis, we start Acyclovir. And we see people now coming in, they have a headache, we start Acyclovir, they have herpes detected in their spinal fluids, so they have herpes encephalitis, and they do really well. They can come out of this without any permanent neurologic deficits, which, if you think about what we can do for health of brain infections, this is really, I would say, that the most stunning development in the past century. If you think about other infections like TB meningitis, yes, we can cure them, but it's a long process. This has really been quite an epiphany. So how does HSV get into the brain? There's a picture in the lower right-hand corner that shows a cartoon that was postulated by Dick Johnson at Hopkins, and the theories were one that they either, the virus either travels up the old

factory bulb and gets to the brain, but the other that Dick is in favor of is latent in the trigeminal ganglion, which you can kind of see over towards the left part of the cartoon. And then it really makes sense because once it's reactivated, it spreads to the meninges that are underneath the temporal lobe and causes the infection. And as you can see in the MRI above that, the right temporal lobe is affected. So those of you who don't read MRIs, the left is on the right and the right is on the left. It's reversed. So that theory makes sense. So it reactivates from a latent stage just like chickenpox, varicella virus. Why it reactivates, I think that's going to be a study for the future, which we still don't have a great idea. So I alluded to treatment with Acyclovir. This was the revolution that occurred in the late eighties, so Acyclovir is a wonderful drug. It does occasionally cause problems with the kidney but really uncommonly. It's typically really well tolerated. If you look at what people are recommending, the experts do recommend IV Acyclovir when herpes encephalitis is present. There are people using Valacyclovir, which is an oral medication for recurrent HSV meningitis, which is, it's probably a different entity. And for some people who refuse to stay in the hospital, I have given them p.o. Valacyclovir if they insist on leaving, but it's not an approved therapy and one that is perhaps suboptimal. I don't think it will ever be studied in clinical trials just because IV Acyclovir is so effective. So you can see the worst outcomes below, age greater than thirty, Glasgow Coma Scale lower. Another study from England noted that if you wait for two days or longer before starting Acyclovir, your outcome is also going to be poor. So going back to our whole project of identifying different etiologies of encephalitis in Peru, what we needed to do is really know what was known. And this was, I guess for those who don't know, it's all mainly Spanish-speaking literature that's in Peru about the causes of encephalitis. There are some publications by others in English that talk about more outbreaks. So, for instance, in the jungle, they have a lot of dengue, they have some malaria, and they also have some very strange arboviruses, ones called Oropouche and Rocio. So there are publications about these little outbreaks, but there aren't any real systematic approaches to defining the etiology of encephalitis. So what we wanted to do was find out, well, number one, was there any study that had been done previously similar to ours. There wasn't. And then, number two, well, what should we be looking for. So what is the list of potential etiologies that we should include? Obviously, if I'm talking on about herpes being the number one cause of encephalitis, at least in the studies that are out there, and most of those, I should mention, are from England, from Europe, from the United States, from Australia. So there aren't many large

surveillance studies of encephalitis in other parts of the world. Those that are out there, I think there's one from Egypt and one from Africa, did show that herpes was common, so we definitely wanted to include herpes. So then we come up with our list, well then how do we actually test for these? So if you can imagine going out to rural areas of any country, including the United States, there may not be capacity for running ELISA, let alone PCR, so you need to think about how the testing is going to be done, can you do it at the site, or will you need to have kind of a spoke-and-hub system where you're sending in samples for the more complex testing. Then the other question comes up, well how do we define our cases? So I mentioned briefly that meningoencephalitis is a mix of meningitis and encephalitis. And then there's the question of, well, what if we do the lumbar puncture, we find that there's an elevated white cell count? So typically the normal white cell count is five or less per microliter. If it's elevated, we're concerned that there's either an infection or an inflammatory process going on. But that may be the only clue we have that there's something abnormal. We may get some neuroimaging with MRI that shows that there's some focal swelling or enhancement, which would also clue us in, but we may not be able to detect the virus, at least in the spinal fluid. So what then can we do? Well we can look for it in the blood. We can look for rise in titer in the blood, and then that can give us an estimate of just how probable the infectious etiology is. This is another way of looking at how we can determine the uncertainty or certainty of encephalitis. So if we find an organism within the CNS, so if we're looking at a lumbar puncture and we find that there's herpes there, well, yes, we've made the diagnosis right there. However, if we find that there's no virus in the spinal fluid, but we do detect a virus in an otherwise sterile site, we may say, well, you know, it's pretty probable that you have this, and we would recommend treatment for it. I would say that this happens most frequently in my case with TB. So TB, as you likely know, is really difficult to culture out of the CFS. So, at times, we'll find it in the lungs, find it in the abdomen, and then we'll just start therapy presuming that it's CNS TB. And when they get better, we say, yes, that was it. And then there's other times when we don't detect anything, but we do find that there's a titer increase over time, so they've been exposed to something, and we make the link saying it's probable that they had encephalitis. So that's just another context or another layer of what we have to deal with as we're thinking of setting up the surveillance. So this is how we designed our patient selection. So we had our inclusion criteria, which changed over time. And I should say that that's something that I found that's really characteristic of most of my studies, that over time

not only our inclusion criteria but our objectives change, and we discover more interesting findings. So we initially had patients older, and then as the pediatric infectious disease doctors joined in as collaborators, they were saying, well, you know, we would really like to have our children involved in your study too because we want to know what's wrong with them. So we eventually moved the inclusion criteria to include children age twenty-eight days of age or older. We didn't include younger because they more often have bacterial meningitis rather than encephalitis. And then you can see the criteria below. As far as exclusion, we had people over four kilograms or children over four kilograms, and we were really interested in finding acute causes of encephalitis, so we didn't include people who had had neurologic symptoms for greater than two weeks. I say findings suggestive of bacterial meningitis, and that was really if there were findings on the lumbar puncture, so if we found, say, thousands of leukocytes per microliter. And then as far as identifying the potential collaborators, this took a long time. So what we initially started with during the R21 phase was to visit with neurologists in Peru, talk about what their needs were, what their typical approach was to encephalitis, what they felt the typical etiologies were because getting HSV PCR in most parts of Peru was really not done. And then we also had to determine, well, actually who is seeing these cases of encephalitis. So in many parts of the world there are very few, if any, neurologists. So it may not be neurologists who are doing the assessments and treatments of encephalitis, and this varied across Peru as well. So in some areas, ID, infectious disease doctors were doing evaluations and treatment. Other places, it was more like family practitioners. And then in some places it was neurology co-managing with others. So trying to build a team that included all of these potential collaborators took a while. And then assessing the laboratories, so when we went to visit the sites, we also looked at their laboratory, talked with their laboratory to see what they were capable of performing at the hospital or institute, whether they were relying on local private laboratories, often they were, to run CSF cultures or chemistries, cell count, glucose, protein, and culture, and then just how it would be possible to not only improve their lab but to be able to send the different samples to a central site in Lima, which turned out to be much easier than I thought it would be. So we can buy dry ice in most parts of the country very easily. We can ship on commercial airlines and get samples in the same day or the next day, so it's been one aspect that I found much easier, much easier than the IRBs that I'll get to later. So the study locations, initially we started in Lima, which is the capital city, and I'm going to try to pull a little pointer out here.

So Lima, the capital city, Trujillo, which is up on the coast, a very arid environment, and then Iquitos, which is in the heart of the jungle. The only way you can get there is by boat or by plane. So this, for me, has, I want to say, has been the bane of my existence. Obtaining study approval is really a lot of work, and it's becoming more complex as the universities require more and more documentation that prevents them from falling into more, I guess, liability. Saying that though, we definitely need the Institutional Review Board because we want to make sure that our research is ethical, but it can take a long, long time. And ours initially took two years before we got the approval, and we needed to include approvals not only from the US but obviously from the Peruvian institutions. And some of the sites where we were working were smaller rural hospitals that didn't have IRBs, so in that case, we would register them with the Federalwide Assurance. They typically had associations or agreements with local academic institutions that would then cover them as their Institutional Review Board. However, we would need to talk with the directors of the hospital to make sure that they were on board, and then they would write letters to the academic center with the IRB. So it was a very long process. And what happened over time is that we had so many different institutions that wanted to become involved that we had very many co-investigators on each page for each city. So we came up with a unique front page for the consent form that included the local investigators as well as the general investigators present in Lima and the US, and then the remainder of the consent form was the same for all groups. So building capacity, and that, for me, has been, I think, one of the most exciting parts in what I think is potentially the most useful for the countries in which we all work because we want to make sure that we're building capacity so that they can become independent investigators as well. So improving infrastructure, well when we're thinking about the surveillance, we obviously needed to have ways of processing and storing samples, so centrifuges, freezers, we needed to diagnose infections, so ELISA readers, PCR machines. But, as you know, PCR machines are expensive, but the prices are really coming down. So you can get a new nice PCR machine for fifteen thousand dollars now whereas opposed to in the past it would have been in the hundreds of thousands. And then how are we going to communicate results, so computers with internet connection. Training opportunities, obviously you want to make sure that there's good laboratory practice, and the samples you are collecting are reliable, they're not being contaminated. And then, as I mentioned, IRBs and Responsible Conduct of Research. So it's really integral to research to make sure that everything is being done in a way that respects the

autonomy of the subjects and takes into account all the different aspects of conducting research regardless of the country. So we had a series of workshops on Responsible Conduct of Research. We invited OHRP. So for those of you who are thinking about conducting a seminar in another country, you can contact the office of OHRP, Office of Human Research and Responsible...I always forget what OHRP stands for, but they will come to your presentation or your conference, and they can pay their own way. They're really fantastic. So if you need assistance with speakers for building, I guess, knowledge about Responsible Conduct of Research, think about contacting OHRP. They're fantastic. And NIH also has bioethics fellows who have participated in ours as well. And then building capacity for research, so we had workshops on research methodology, data management. And then preparation of manuscripts, which is really an ongoing task to have people learn how to write. So planning our study of procedures, so we needed to think about, well, what are we going to be collecting as far as information for our surveillance? Obviously, we wanted to know for the future when we're looking at risk factors, what are the physical findings, what neuroimaging findings were there, what CSF abnormalities were present. And for finding whether it's a probable diagnosis, we need to have CSF as well as blood. We also obtained pharyngeal and rectal swabs as well. So one of the other issues that came up when we spoke with the physicians is that, you know, I really like your idea, but I don't have much time to fill out anything, because they're really, as you can imagine, involved with clinical care. So what we came up with as our solution was, well, the physicians will fill out the clinical history and examination on a form that we have designed, really limiting the amount of time we require them to participate, and then we had study personnel who would gather the other questionnaire material that would otherwise be difficult for the physician to obtain. In Peru, many people don't have insurance, so it made it very difficult to know if they had any neuroimaging abnormalities if they don't have insurance, and the cost of a scan, although it's cheaper than in the United States, a CAT scan, for instance, you can typically get for a hundred dollars, if you can't get that because there's no insurance, but it is standard of care, what do you do. We included it as part of the protocol, so if someone didn't have insurance, we would cover the cost of neuroimaging. A lumbar puncture is considered standard of care across the world, so that came up with our IRB. If we considered it as a study procedure, it would have been more difficult just because it's seen as more risky. Although, as a neurologist, I perform thousands of them, and it isn't as risky as you would think, reading the anecdotes in the literature. But since it's a standard of care, it doesn't

come under the research procedure. So this is obviously not in English, but this is just an overview of the protocol we then developed that goes through the different steps of obtaining the patient consents, completing the questionnaire. And then in the middle, it says, neurologic evaluation, and this is where the first step that was most important for the evaluation. So if there was a neurologic deficit, we would want to obtain some type of neuroimaging prior to performing lumbar puncture for those. I know there's at least one neurologist out there in the audience. For those of you who aren't neurologists, if you see a focal problem, so if there's, say, weakness on one side or someone is unconscious or there's a seizure, you want to make sure that there's not a big growth in the brain, whether it's infectious or otherwise. Because if you do a lumbar puncture in that setting, you do run the risk of having someone herniate, and that can lead to death. So the CAT scan was to make sure there wasn't that type of lesion before we did lumbar puncture. If there was, they were excluded. Otherwise, we would obtain the spinal fluid, we would get the blood, and we would initiate Acyclovir, which I think comes up in the next stage. No, I'll talk about Acyclovir in a couple slides. So spinal fluid testing, as most of you know, the typical CSF chemistry, you want to know how many white cells there are again. If it's greater than five, you're concerned that there's something infectious going on. We decided to initially look at herpes and enterovirus PCR as well as the CSF and serum ELISA and PCR for many different arboviral infections that are lurking in the jungles of Peru. And then as we thought about it, there are many people in the world who have HIV that don't know, so we decided to look also at cryptococcus, which is a fungus, and MTB as well as offer HIV and HTLV testing. I should mention, the HTLV was because although HTLV hasn't been described as a cause of encephalitis, it is very endemic in Peru. It's how I started my research career, and there are some, like me, who believe that the acute seroconversion to HTLV positivity, similar to HIV seroconversion, could cause an infectious syndrome similar to encephalitis, so that's why we added that test. So initiating our surveillance, so you can imagine that trying to get this whole system running is quite a bit of work, and I would like to thank again Nicanor Mori and Christina for really helping assist this with going. This is, up in the left-hand corner, is a picture of the intensive care unit up in Iquitos in the hospital. Fortunately now they have a respirator, before they didn't. On the right-hand side is a picture of the outside. So what we did to initiate was to get the system kind of primed before we started collecting data. So we had the IRB in submission under evaluation, going through the responses, and then we started the system going by having

physicians send cases or send CSF to the center lab in Lima. We would then run the assay, give them the results, but we weren't collecting any study data. So those patients aren't enrolled in the study, but it allowed us to make sure that the system was working, that HSV and other testing was possible. That was more run as a clinical service as we were getting the program up and going. And we did decide to run all of the advanced diagnostics at a place that used to be called NMRCD. Now it's called NAMRU, the Naval Army Medical Research Unit in Lima, and it's affiliated with the United States military, and their objective is to look at infectious diseases of regional importance. And they have a wonderful advanced diagnostic laboratory, and they've done a fantastic job at training local researchers to bring that technology throughout the country. So patient benefits, I mentioned Acyclovir. As alluded to earlier, if you don't treat with Acyclovir, people die. So seventy percent of the people will die. And this is what we heard uniformly as it went across the country, well what can we do for our patients, we know that there's HSV encephalitis out there, or we suspect there is, and we want to treat them, but there's no IV Acyclovir in Peru. So what we did was, we said, well, you know, we agree, if we're going to be diagnosing herpes encephalitis, we really need to treat it. So that was one of the benefits of enrollment in the study. So if a patient was enrolled, if we suspected it was herpes encephalitis, which was most often, we would start IV Acyclovir that we imported into the country, we'd run the HSV PCR, if it was positive, then we continued for the recommended fourteen days. If it's not, we stopped the Acyclovir. And getting the Acyclovir has been another, I guess, work in progress. There's a lot of issues that come about that, but I think it's been a great value for patients. And as you'll see, I think it's probably saved some lives. So these are the initial results, and, again, I'll thank Christina. Many of these slides are from her presentation that she developed. So this was our initial look at the test results, so a hundred and fifty-seven subjects. Most were female. Most were young. And Trujillo, up on the coast, was really vigorous about their enrollment, so they were the site that enrolled the most patients. And what we found was not really surprising. So the people that came in, well, a lot of them had headache, and a lot of them were confused, some were comatose, and they also had fever, so as we would expect. Down below, you can see the mean white cell count was about two hundred and thirty-seven cells, which for an infectious disease person is most consistent with, what, with virus, with fungus, with TB. If it were bacterial, you would expect the white cell count to be up in the thousands. And then this is what we found. So we were very excited to find that herpes was the

most common etiologic agent that we were able to detect. But surprisingly, a lot of it was HSV type 2. So if you know about HSV, HSV type 1 is what we typically see in causing sporadic encephalitis, whereas HSV-2 is typically the virus that causes genital herpes and typically causes recurrent meningitis. So this was a surprise for us, finding that there's HSV-2, and I'll get to our theories a little bit later. But we're still not sure if this is just a variance that we'll see go away as we collect more patients or not. So patient followup, I alluded to Acyclovir being a lifesaver, only for subjects died, so two and a half percent of the patients though. That is, for us, fantastic, so people were doing fairly well. We don't have long-term convalescence data. We do have this next line, which is typically about two, three weeks. Patients still had problems, but they aren't very severe. You can see twenty percent had persistent cranial nerve dysfunction, five percent had recurrent seizures. Hopefully, in the future we'll have more data about long-term outcome. So results, so compared to subjects without HSV encephalitis, you can see all these factors were not any different. And then if you look at those factors that were significant in patients who had herpes encephalitis, they were more often going to have nausea, cranial nerve disorder, vaginal or penile discharge, which would go along with HSV-2, causing herpes of the genitals. So this is more of the, I guess, maybe information about HSV-2. So in other large studies, like the California Encephalitis Project or a study in England by Granerod, HSV-2 was much less frequent than it was in our study. Again, we just have a hundred and twenty-seven patients, so we need to see if this will pan out in the future. There were some differences between the patients who had HSV-1 and 2. One is the site where they were enrolled, the other was age. And then this is the distribution of the subjects. We won't go into that in detail. So what we found was that herpes was the primary cause of encephalitis in Peru, which was, I think, important to know because we can treat it, and this is something that we need to convey not only to the physicians and people treating encephalitis but also to the government because if this is the major cause of encephalitis and it's treatable, and they don't have IV Acyclovir available in the country, they should. So this is a stage where we're working now in trying to get the government to realized that it's of importance and trying to change the policy. I suspect that this is similar in many other countries, that they also have herpes as a major cause of encephalitis. And if we can also help other countries define the etiology and get Acyclovir available, I think that we will be helping a lot of people. And here's a picture of Christina and Nico presenting their initial findings at a conference in Vienna. We had a lot of interest. So now expansion of the network. So we started

off with just the sites in Iquitos and Trujillo and Lima, and as we started doing more and more testing, just word of mouth spread and also we did our presentations about our study at various conferences. And other areas of the country said, you know, I would really like to participate, we do have these cases, we'd love to know what's causing them. So this has been kind of the continuous process of IRB modification, so we need to, as we had sites, make sure that they're capable of processing the samples, add them on to the IRB application, which is probably enough work for two people. And then also thinking about, not only is this a great study for determining the etiology, but we can also start looking at risk factors. And this is where Christina came in as a fellow, and she has now added a nested study, which I'll talk about in a few minutes. So going back to building capacity. So, again, I mean if you can build capacity as you go along, that's wonderful. We were able to renovate this reference laboratory here. This was in conjunction with a company in Seattle going under called Icos. They donated a few hundred thousand dollars worth of laboratory equipment as well as our center for AIDS research and the Fogarty International Clinical Research Scholars Program, which provides some infrastructure money. So we were able to renovate. This is an architectural drawing, but it looks about what it actually turned out to be. It was a vacant space, now it's turning into a reference laboratory. And then on the flip side is what used to be an empty library at the tropical medicine institute. And with the Fogarty International Clinical Scholars infrastructure money, we were able to convert it into a distant classroom, and now they have the capacity for doing distance education, which they didn't have before. And it's also been a site where we can do other workshops. We also have continued with the Responsible Conduct of Research, and we've made sure to include not only Lima but also other areas on this case. So these are the current study sites, so we are growing rapidly. And this is the most recent compilation of enrollees. We're now up to three hundred and thirty-six. Again, Trujillo, up here, has really been amazing, a hundred and forty-seven. So we have just been enrolling since the past February, so we can't really say anything about temporal trend, but we hope to in the future. But you can see that we have all of these cases from different parts of the country. I'm looking forward to seeing what comes out with analysis over time. I'm not going to delay on these signs and symptoms. I'll go through them fairly rapidly, but this is the larger group of symptoms. And, again, you can see headache and fever are pretty common. This is what we anticipated we would find, so unknown cause. The majority of cases still evade our ability to diagnose them, granted we did start off with not the complete list of potential

etiologies. One, for instance, is varicella zoster that we hope to add this year, but you can see that herpes was the most common identified virus we were able to detect. And then we found, we did have some patients who died. So now out of hundreds we've enrolled, only fourteen had died, some of them with HIV infection, which obviously has a higher mortality, and I've already talked about Trujillo being that number one area. I know that time is limited, so I won't delay much on the difference. These are just the patients who had HSV encephalitis. Headache is common. This one is in Spanish. I couldn't translate it in time, but they did have abnormal gait most commonly and headache. So herpes encephalitis, we did find that, again, the majority that we could diagnose or had an etiology, did have HSV encephalitis. Of the ones we were able to sequence, ten had HSV-2 and twenty-five had HSV-1. Three patients with herpes died. Two of them had HIV co-infection. So that was a fairly low mortality rate compared to what I had mentioned earlier, seventy percent of causes of encephalitis by herpes died before Acyclovir. So in summary, we had HSV as the most common cause of encephalitis. They did have some abnormalities that were associated more commonly with either HSV-1 or 2, as you read here. And then what are our strengths and limitations? Well, I was really happy that this was the first comprehensive surveillance, although we are less than a year, so you may not call it surveillance officially, but our first evaluation in any Latin American country, and hopefully this will be published very soon. We were able to cover diverse areas. The bias is, looking at encephalitis, they were obviously sick enough to come into the hospital, and those with the ability to pay to get into the hospital were the ones we enrolled. So this was hospital based, which was really the only way we could design it to get it done officially. So you can imagine, there are some people who can't afford to get into the hospital or in areas so rural they don't have a hospital. We aren't able to enroll those patients. That would require much larger network to be able to actually find those patients. And we didn't look for non-infectious etiology. So I mentioned earlier, NMDA receptor antibody encephalitis, or other types, like the acute demyelinating encephalitis, we didn't look for, although hopefully, as we expand our ability to diagnose not only infectious etiologies, we'll be able to start looking at non-infectious etiologies in the future with thanks to other collaborators. So I mentioned this before, I mean, what does this mean? Well I think it means that we need to start working more with the government in ensuring that they're not only testing for HSV but supplying Acyclovir because this is a research study. And although we were able to provide Acyclovir as a benefit to patients, this won't continue forever, and there's other

countries that also won't be doing research, but they do need IV Acyclovir. So this is where we start working with the multidisciplinary teams trying to get policy changed, and I suspect this will be the work of my life. As I had alluded earlier to Christina Nelson, being able to look at nested case-control studies, so this is wonderful because we have this surveillance going on, and adding her study to start evaluating, well what are the risk factors of HSV encephalitis made all the sense in the world and was fantastic to be able to use the surveillance to be able to further the knowledge available. So she had designed a wonderful study using two control groups. You can imagine that control groups are difficult to determine which ones you want. You don't want someone who has the same infection, but you do want to try to get someone who may have the same genes or comes into the hospital for other reasons. And some of the other factors we're starting to look at with the assistance of other collaborators are genetic mutations that may predispose people to getting HSV encephalitis. So we really don't know why some people develop HSV encephalitis and others don't. So I'm hoping that with Christina's help we'll get further along in that direction. So I've mentioned this, we're really trying to increase the availability of IV Acyclovir as well as built capacity. And then building capacity, we've had a lot of trainees up here in the corner. You can see one of our mentoring workshops. So now we've had so many trainees come through the Fogarty system that they're turning into junior mentors, and, we, like many of my colleagues, merely learn to become mentors on the run. So we try to be more intentional and plan a mentoring workshop where we talk to people about how they can mentor the next generation as well. We're working on developing CSF reference laboratory at the neurologic institute, which is the reference laboratory or reference institute in the country for neurologic disease. And we've used that computer classroom I've showed you to start Adobe Connect research methodology course to also train not only Lima but in rural areas of Peru. We also had a cerebrovascular diseases methodology workshop, so we're kind of expanding our network of trainees to not only include infectious but other causes of chronic neurologic disease. And here's a picture of Nico, me, and Silvia. I didn't thank Silvia, but Silvia Montano is my Peruvian colleague who has really been instrumental in all of my collaborative work in Peru and wouldn't have been able to do it without her. And then this is a list of our meningoencephalitis group, and finally a picture of the hospital in Belen, founded in 1551. And there's the end of my talk. So thanks for your attention, and I'd be happy to answer any questions. Oh, and then I see a note from Kathy, would like to get in touch with me afterwards. Sure. So probably the easiest

way is via email. That's just jzunt@uw.edu. We do have a website that we started for the Fogarty International Clinical Research Scholars Program that I'd be happy to provide that goes over some of our approach to mentoring and training. I don't have a specific website for this particular study, although I'm hoping that with Christina, Nico, and Silvia, we'll develop one in the near future. I'll be happy to answer other questions. Okay. I will type in my address.

Steve Schiff: Oh, great. Steve Schiff here. Dr. Zunt, great talk.

Joe Zunt: Good morning. Thanks.

Steve Schiff: Two questions.

Joe Zunt: Sure.

Steve Schiff: The exclusion of neonates and the impression that they don't get viral encephalitis, are we sure about that?

Joe Zunt: I think, yeah, that's a great question. I don't think we can be certain, but as far as our resources, we were talking back and forth with pediatric ID physicians in both Peru and the US about where we should draw the line. Christina is a pediatrician, which was very helpful. And I would say that our initial line that we drew was at two years, and it is an artificial line. So then we moved that down to twenty-eight days. Should we be moving it down to the newborn period? Perhaps we should. A part of it is just the resources available, but I think that's...I think that there certainly could be causes of encephalitis in children due to viruses in neonates, and that's an area that would be interesting to look at in the future.

Steve Schiff: Thanks.

Joe Zunt: Now there's a question from Michele, any ongoing or future plans for followup study of long-term neurological sequelae? I think that is really imperative that we do long-term followup. So with our initial IRB, I mentioned it took us two years to obtain approvals, and they

recently asked us to rewrite the consent form, which those of you out there who have written consent forms can know the pain I'm feeling. So we currently have permission to reevaluate at two to three weeks. We wanted to get that initially approved before it went into long-term evaluation, but that's next on our list to include long-term evaluation because I think that is really important that we are able to address that. So with this initial run baseline where we know that there's enough cases out there, and now we'll add to that and include long-term sequelae or evaluate for them.

All right. Another issue...this is another question from Steven. Another issue that troubles me, we are working with brain infections in infants in Uganda, are the large number of unknown etiologies of such brain infections, so what are your thoughts on the two-thirds of patients that you have studied without identified organisms? Boy, I wish I knew. I think that, for me, regardless of the country, the US or Peru, has always been really, really difficult because more often than not we don't come up with an etiology. So one comment we had with our publication we're trying to get through is, well, why didn't you check for varicella because it's another common etiology. So we're going to. So we're going to start adding different potential pathogens. One other collaboration that we're working on is to do these new micro DNA assays, so you're running the CSF for any type of RNA or DNA that's potentially infectious. However, even with that assay, they're still coming up with many that don't have any identified pathogen. So what can you do? Well we can start looking for noninfectious etiologies, and as you probably know, for instance, the NMDA receptor antibody encephalitis is a fairly new entity and becoming more and more widely recognized. And now that we're testing for it in Seattle, we've seen, I would say, a half dozen cases in the past two years. So, I think, as our expertise increases, we're going to be able to detect more. I don't think we'll ever reach a hundred percent, but we'll continue building collaborations to try to detect those new etiologies that are emerging.

It looks like we've reached the six o'clock hour here for me, whatever hour it is for the rest of you. I know, I think we end at six. But thanks for your attention and your questions, and I'd be happy to answer other questions by email or hang on for a few minutes if other people have questions. So thanks again.

Jeff McAllister: I'd like to thank you, Dr. Zunt, for an excellent presentation. I know it's pretty early your time, and I see we have some applauding from Christina Nelson, so I believe she welcomes your presentation. So I would just like to thank you for that.

Joe Zunt: All right. Well thanks again.

Krystyna Isaacs: And I see that Kathy raised her hand, and I think she would like us to read out loud, if there are no more questions, we'd like to thank Dr. Zunt.

Joe Zunt: Great. Thank you.