Geographic Origin and Vertical Transmission of *Leishmania infantum* Parasites in Hunting Hounds, United States

[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. Susanne Franssen, an assistant professor of evolutionary biology at the Ludwig-Maximilians-Universität in Munich, Germany. We'll be discussing vertical transmission of *Leishmania infantum* parasites in hunting dogs in the United States.

Welcome, Dr. Franssen.

[Susanne Franssen] Hello. Thanks a lot for having me.

[Sarah Gregory] What is a *Leishmania infantum* parasite?

[Susanne Franssen] So *Leishmania infantum* is a range of several species, all from the genus *Leishmania*, and they are protozoan parasites, which means they are single celled parasite, but they are not bacteria, but they are eukaryotic cells. So basically, they're different from bacteria in the way that they have a nucleus like us or other plants and animals (the type of cells they have). And *Leishmania infantum* is one species among several of the genus *Leishmania*. And these parasites in general (so not only *infantum* but also other species of it), they can infect humans and other mammals. And they do this by entering the bloodstream and infecting blood cells such as microphages.

Also, this parasite has two life stages. So there's an amastigote stage, and this is the one in the mammalian or...also including the human host. And this enters the bloodstream, and it will even enter the blood cells such as macrophages, and they will replicate in those and then come out and infect other cells. This infection can cause the disease leishmaniasis, but it can also be asymptomatic. And the second life stage is the promastigote stage, and this is the stage that is typically responsible for transmission via the vector (which is an insect vector). And this is in endemic areas the main route of transmission, and there the parasite is in the digestive tract of the insects and gets transferred by blood meals in subsequent hosts.

[Sarah Gregory] You mentioned leishmaniasis. What is that?

[Susanne Franssen] Leishmaniasis is the disease cause by *Leishmania* infection, and it belongs to the NTDs, which stands for neglected tropical diseases. And in general, for leishmaniasis, there are three main forms of the disease that are largely associated with the genus of *Leishmania* that a host is infected with. So the first class is cutaneous leishmaniasis, which basically can be disfiguring and scarring skin lesions that can range very strongly in severity. And the second class is mucosal leishmaniasis, basically where the parasite mainly infects mucosal tissue (such as in the nose or around the mouth), basically destroying these tissues. And the last form is visceral leishmaniasis, and this infects internal organs such as the liver or the spleen and bone marrow. And typically this disease is deadly without any treatment, and it also includes fever, enlargement of the spleen and liver, anemia (strong anemia), weight loss.

[Sarah Gregory] And what's the natural vector for this awful parasite?

[Susanne Franssen] Generally these parasites are transmitted by sand flies, more specifically phlebotomine sand flies. And there are several families and genera that are known that can transmit *Leishmania*, and often they are geographic region-specific, and they are also specific typically for the species of this *Leishmania* parasite that they can transmit.

[Sarah Gregory] Are there any of these infected sand flies in the United States?

[Susanne Franssen] It is known that in the United States there are some sand fly species present that have been shown in laboratory conditions that they could transmit infection with *Leishmania*. However, there have been surveillance studies, several looking at the infection status of these sand flies in the US, and *Leishmania* basically never has been detected in those. So currently there's really not evidence for *Leishmania* infecting sand flies in the US, or even transmitting it. And yes, in general, this is also kind of a complicated topic of vector competence—basically the ability of a vector (so in our case, the sand fly) to transmit a parasite—because it is a very complex trait that depends on a lot of factors. So even if it's demonstrated in the laboratory, which I have said, it doesn't mean that this occurs in the field because it would depend on the prevalence of infected animals, of abundance of the vector in the wild, how often they cooccur; even other factors like the microbiome of the parasite could play a role in infection load. So as I said, although it has been observed that in the lab the sand fly is able to do it, it has never been found in the field, basically, in the US. Yeah, the other aspect I'm just going to mention later.

[Sarah Gregory] Okay. Your study was actually kind of looking at vertical transmission. Explain the difference between vector transmission and vertical transmission.

[Susanne Franssen] Basically vector transmission always means that the parasite is transmitted between hosts only via a vector. And in our case, this is the insect vector (so the sand fly). So in order to be transmitted from one human to another or between other mammals, the sand fly has to have a blood meal on an infected host and then has to subsequently feed on an uninfected individual. So...and in this way, it's passing on the infection between hosts. But it cannot occur directly between hosts (so between humans). On the other hand, vertical transmission is a way where the parasite is directly transmitted from an infected host to another infected host. And in the case of vertical transmission, this is transplacental from mother to embryo, fetus, or baby during the pregnancy or during birth. So basically, it's directly transmitted from mother to offspring basically before or while the baby is being born.

[Sarah Gregory] Going back to leishmaniasis or *Leishmania infantum* parasite not being found much in the United States that's infecting people, where is it usually found?

[Susanne Franssen] Leishmaniasis in general, which I said can be caused by several species of *Leishmania*, the foci where it's most prevalent is really Brazil, India, and Africa. However, it can also be present in the Mediterranean Europe, or Asia. And the different geographic regions that *Leishmania* is endemic are really associated with different species. So *Leishmania infantum* that we are discussing mainly here is otherwise present in Mediterranean Europe where it is nowadays mainly infecting dogs or also other wild animals and not so much in humans, and is also present in Asia and otherwise mainly in Brazil.

[Sarah Gregory] Your study was in looking at hunting dogs. Is this relatively new to the US?

[Susanne Franssen] Well, relatively maybe yes, but the first case was identified in the 1980s where they identified a dog with no travel history outside the United States that had been infected. And then subsequently, in....so it wasn't much of an issue afterwards, I guess, but in 1999, there had been a large disease outbreak, again, in hunting dogs in the US. And in this case, the CDC started to do more, prompted basically an investigation to determine the burden of the disease in US hunting dogs. So relatively new, but it has been around several years already.

[Sarah Gregory] So again, your study was about vertical transmission in hunting dogs. How was it discovered?

[Susanne Franssen] I think initially it had been discovered by these two events that I had said earlier basically where it had been detected in dogs that really had no travel history outside the US. It was clear that it must have been transmitted in the US. So the first one was in 1980, and in 1999, it was even a much larger outbreak, and here it was kind of obvious by abnormal deaths in these hunting dogs that could not be described to the normal tickborne diseases that are found in the US that could cause other deaths.

Originally, also one of my collaborators and also a coauthor on the current study, Dr. Peterson and her group, they looked at...in 2008, they studied a bitch that was seropositive for *Leishmania* (so basically, there were antibodies against *Leishmania* found in her blood), and this was also the case for her offspring. And they also confirmed it with other means that in fact these pups were also...also had *Leishmania*, and here they could first identify also directly in the lab that it must have been this vertical transmission.

[Sarah Gregory] What were you looking for in particular when you did this study?

[Susanne Franssen] Vertical transmission, generally that had been known before. But in this study, we wanted to look at the genomes of *Leishmania infantum* parasites from the US to be able to kind of identify via this genetic information from where they might have been introduced. So basically, we wanted to pin down the geographic origin directly by genetic means.

[Sarah Gregory] Clarify for me here: how did you go about establishing geographic origin?

[Susanne Franssen] Basically, the general idea here is that we analyze the genomes of *Leishmania infantum* from the US and we would compare them with *Leishmania infantum* samples from all over the world (some of these other foci that I've described before, where they occur) that were already available. And then based on.... while comparing these genomes, basically we can determine the relatedness between the US parasites and the parasites from other origins and then they are.... but where this genomic information, there are phylogenetic methods that help you reconstruct the ancestry (who's related to each other). So basically, by this means comparing it with parasites from all other regions we could see to which they are most closely related—so basically where they originate from.

[Sarah Gregory] Your study actually mentions ethics because there were dogs involved. What kind of ethics were involved in your study?

[Susanne Franssen] My coauthor and collaborator Dr. Peterson has the approval (which is called Iowa Institutional Animal Care and Use Committee approval), and this allows her to study *Leishmania* in these dogs, including...and this includes sampling of blood tissues and when the owner requests also euthanasia and necropsy. And they've been working with these kinds of

dog populations over 15 years, and they have made up some relationships with the owners and caretakers, so always when they do a particular study in these kennels where the dogs are being bred, they always describe the study to the owners and obtain the consent for all the work that they do. And I think for our study particularly, it was that licensed veterinarians collected one to five mL of whole blood and serum samples from these dogs in the kennels. So that was basically what was relevant for our study.

[Sarah Gregory] How was your study conducted?

[Susanne Franssen] Generally, when the blood was sampled from dogs and they were found to be infected, then these blood samples were used to initiate *Leishmania* culture in the lab. And this is the purpose to amplify *Leishmania* so that you have enough material to basically get the genomes of them to sequence them. So they are amplified by a culture in the lab, then we extract the DNA, and we perform whole-genome sequencing on this DNA. And basically with these whole-genome sequencing methods, we obtain very many fragments of the genome that are sequenced, and we do bioinformatics analysis (which was also the main focus of this study) to assess their genomic information. And then we have phylogenetic and population genetic analyses by whom we can answer our questions of interest.

[Sarah Gregory] Dr. Franssen, what did you find?

[Susanne Franssen] For the first question about the geographic origin that you wanted to answer, we found that the US *Leishmania infantum* samples, they are most closely related to each other. So this...basically, this tells us that for the samples (at least that we sequenced), we assumed that there was only a single introduction into the US. The second point is they...the next closely related samples came from Mediterranean Europe and not from Brazil, and this is interesting in a way because of course Brazil would be geographically closer. But we can definitely say that the closest relatives were in Europe. And also for *Leishmania infantum*, we know that originally the *infantum* parasite in Brazil also originates from Europe, but already they had been introduced around 500 years ago (which previous studies suggest). But for the US samples, we know that they have been independently introduced, and we could also date this independent introduction from Europe, and this was only roughly 1900 or even more recently than that.

So this was one of our main aims. But additionally with our whole-genome sequencing data, we also wanted to address with this data if we can find any signatures about how the parasites are transmitted in the US. And for this we did some population genomic analysis to look for signatures that might be consistent with vertical transmission in the US parasite only. And answering this question got a little bit more into depth into the population genomic analysis. It was a bit more technical. But basically, we finally found a signature that in the US parasite, we have a great access of heterozygous. So heterozygous basically means that in your genome, you always have two copies of everything. And if you have a position where one copy differs from the other, this is basically a polymorphism, so we call it a heterozygous position when there are two positions different. So in this population, we had a great access of those, which could be an indication that no sexual reproduction is occurring in the parasite.

An additional thing that is suggested that no sexual reproduction is occurring in the parasite is a lack of evidence for recombination, and recombination is when parental copies are stitched together when they are from both parents when they are passed on to the offspring. And this also only occurs during sexual reproduction. So basically, we had two indices that sexual

reproduction is not happening. And this is significant because we do know from previously that the *Leishmania* parasite in the human or mammalian host only reproduces clonally and never sexually. And this sexual reproduction can only happen in a sand fly vector. So by observing lack of sexual reproduction of the *Leishmania* parasite, we also have some further genetic evidence that we think that indeed, it is not transmitted by the sand fly vector because this kind of reproduction could only happen in the sand fly vector.

[Sarah Gregory] Were there any surprises?

[Susanne Franssen] Yeah. I was quite intrigued by this very high heterozygosity rate, which formed several questions that we could look at in further studies. And I think for those, generally, some more sampling also of European samples would be required given the number of parasites that we sequenced from the US, which was unfortunately not very big which it's not surprising. But I was a bit surprised that they were all of a single origin, because I could have imagined that hunting dogs, there's quite a trade...or they are quite more frequently exchanged, and I would have thought that potentially more than one introduction could have happened. But I guess as we didn't sequence a huge number of US parasites yet, if more were to be sequenced, that picture could change. So I was a bit surprised by that, but I think it also shows that there's some more research that could help us understand even.... the dynamics even better.

[Sarah Gregory] I imagine there were challenges in finding this information. What were they?

[Susanne Franssen] I mean, as for bioinformatic analysis, I would say there were just the typical challenges that always when you find a very unusual signature in the genomic data, that you have to check that it cannot be due to any technical artifacts. Then, I mean, maybe interpreting the results, this is a little bit challenged by maybe the limited sample numbers. As I explained before, we found a single origin, but if we had looked into many more samples from also different breeds of hunting hounds, could we have found multiple introductions?

I mean, another challenge is maybe a little bit the extent to which we can interpret the results. As we said, we didn't find any evidence for sexual reproduction of the parasite. This means no sand fly vector was involved, but unfortunately this information is not completely quantitative so we cannot completely rule something out. This is a bit challenging in the ways of how you can interpret the data, basically.

[Sarah Gregory] What do you think is the most important public health aspect of this study?

[Susanne Franssen] I think generally, it says that (or it indicates that) it is very important to screen dogs that are imported from *Leishmania* endemic countries for their *Leishmania* infection status. And this is not only because the dog itself would be infected, but it's also possible that it's transmitted to further offspring and causes suffering in the dogs, and also I think some financial problems for the owner. But it can also be passed on to other dogs, and generally we have seen that it didn't cause any further problems which could have occurred when the parasite was also transmitted by sand fly vectors. But our signature (genomic signature) basically that they found and differentiating if a parasite might be vertically vector transmitted, this could be a possibility to look into these kinds of signatures further to potentially even identify some diagnostic marker where you could say this parasite that we observe here was vertically versus vector transmitted, and if you would know this information, you could have some more targeted intervention

method in the future. But this is something that could be potentially done in the future if we were to put some more research into this direction.

[Sarah Gregory] Can these vertically transmitted infected dogs infect people?

[Susanne Franssen] Theoretically, it is possible through blood-to-blood contact. So for example, there have been very rare case reports where *Leishmania* has been transmitted between dogs horizontally, which means they are not related to each other, by during...which could have happened during dog fights where maybe they have wounds, and they exchange blood in this way. However, in the US we don't find any people that are infected. And, I mean, generally there's no blood contact and also...it would also depend on several factors, like the infection load and how much blood would be transferred to get infected. So far, it has not been detected as a problem, but I think it would be sensible to avoid blood contact with infected animals.

[Sarah Gregory] Is there a treatment for these infected dogs?

[Susanne Franssen] Yes. So there are some treatments available, but in the US, it is allopurinol or miltefosine. However, miltefosine is quite costly and therefore not usual treatment in this cohort that my collaborator is working with.

[Sarah Gregory] What about for people? I know people aren't getting it in the US, but in other regions of the world.

[Susanne Franssen] So in general, there are also several drugs available, which include pentavalent antimonials, liposomal amphotericin B, and also miltefosine, however...and also a range of some other drugs. However, the problem here is that all of these drugs have very toxic side effects.

[Sarah Gregory] You are in Europe. How did you become involved in a study about US hunting dogs?

[Susanne Franssen] So this is basically through our collaborator, Dr. Christine Peterson from the University of Iowa. So as I had several times mentioned her during the study, so she has been working with the dog cohorts for over 15 years and she has been looking at also a multitude of aspects of *Leishmania* in these dog populations. And when I started working on *Leishmania*, I just started in the UK at the Wellcome Sanger Institute and there was a group for parasite genomics. And we did a lot of population genomic studies and we specialized on this particular parasite.

[Sarah Gregory] Tell us about your career path and your job.

[Susanne Franssen] Originally, I started the study of bioinformatics in Frankfurt in Germany. And then, I went on to do my PhD (also in Germany), and here I focused on evolutionary genomics, and particularly genome-wide sequencing to understand evolution in natural populations. I also continued on this general topic in my first postdoc, which I did in Vienna. And here, I looked particularly more at population genomics and also experimental evolution, basically where we used *Drosophila* as a model species to have them evolve over several generations. And then when I did my second postdoc at the Sanger Institute in the UK, I used this population genomics expertise to study *Leishmania*. And basically, now in my current position I'm an assistant professor at the university in Munich. I've basically combined the population genomics of *Leishmania* and experimental evolution, but also continued to work with

collaborators that work with *Leishmania* in particular settings (like the US setting in the field) to have some more also....to also have some data from clinical and epidemiological relevance.

[Sarah Gregory] What's the most interesting thing you've ever worked on?

[Susanne Franssen] That's a little bit tricky to say. As a bioinformatics and person doing genomics analysis, what I'm generally always fascinated about is observing evolution and its dynamics in real time, even, by looking at the genome sequences and using population genetic models to predict what should be happening if something is beneficial, and then seeing how things like this are what we expect happening in real time. And this is what generally interests me. And then, when I started also working with *Leishmania*, I was quite interested in this kind of health relevance. Though what I'm concerned with is rather the basic research that is not directly relating into clinical settings or something the like. And therefore, also very much like the collaborations with Dr. Peterson, for example, about the US hunting hounds in the US, but also have a collaborator (Dr. Kropf) who is directly working in Ethiopia and has more immunological and clinical expertise. And he kind of complements this with using genomes and population genomics to answer them...some complementary questions that they are also interested in.

[Sarah Gregory] Well, thank you so much for taking the time to talk with me today, Dr. Franssen.

[Susanne Franssen] Thank you very much for having me.

[Sarah Gregory] And thanks for joining me out there. You can read the June 2022 article, Geographic Origin and Vertical Transmission of *Leishmania infantum* Parasites in Hunting Hounds, United States, online at cdc.gov/eid.

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

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