SARS-CoV-2 in Aerosol Suspension

[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. Chad Roy, a professor of microbiology and immunology at Tulane University School of Medicine in New Orleans. We'll be discussing how long the SARS-CoV-2 virus can last suspended in the air.

[Chad Roy] Hello. How are you doing, Sarah?

[Sarah Gregory] I'm doing very well, so glad to have you here. Dr. Roy, remind us what SARS-CoV-2 is compared to COVID-19?

[Chad Roy] Sure, Sarah. So, SARS-CoV-2 is the virus, and that was the designation that was given to the virus, its acronym, once it was discovered. And COVID-19 is the disease that is associated with infection from the virus. So, when we refer to COVID-19, we're referring to the disease state, clinical signs, and that sort of thing associated with…with infection.

[Sarah Gregory] And recently, I've had a couple people tell me they don't actually know what COVID-19 stands for. Can you explain that, please?

[Chad Roy] So, it's a coronavirus disease. And 19 is the designation from 2019. So, it's just an acronym that…that refers to the clinical syndrome of…associated with a viral infection. So, think of it as similar to the nomenclature around AIDS. So, AIDS is the disease associated with individuals that are infected with HIV that progress to a disease state after being infected with HIV. So, you can be HIV positive but not necessarily have AIDS, and the syndrome with late stage infection is referred to as AIDS. Same thing here, we're just referring to COVID as the disease state associated with infection from the virus SARS-CoV-2. So, we can't be infected with a disease, but we can be infected with a virus.

[Sarah Gregory] Gotcha, okay. Well, thank you for that. When SARS-CoV-2 first arrived, scientists thought it would behave like regular colds or flu and only be carried in respiratory droplets. Now we have the specter of aerosols. What's the difference between aerosols and droplets?

[Chad Roy] So, that's a little bit of a loaded question, Sarah. But they are all aerosols, and so we need to keep that in mind. It's just size makes the determination between the respiratory

droplets—and referring to it as a respiratory droplet and classifying it as such—and aerosols, which I think classified as a smaller particle than a respiratory droplet. They're all small, but aerosols are really small. And the reason that we…we don't know a lot about that fraction that's smaller, is we're…there's a lot less understanding about its behavior and characteristics in the environment during transport and person-to-person infection, as it pertains to those smaller droplets or aerosols. The respiratory droplets, which are a bit larger, we do know something about their trajectory in the air and that they can fall out of the air column quicker because of gravity and other things, than those smaller droplets. So, the movement of those…those smaller aerosols are much more opaque to us than the respiratory droplets and what we consider like close contact. And we can kind of contextualize that with diseases, like influenza, that we know a lot more about, in that case. So, when we talk about an emerging virus like SARS-CoV-2, we didn't really have a good idea of transmission as it relates to true airborne transmission, you know, over longer distances than, say, you know, one or two meters.

[Sarah Gregory] What were you looking for when you did your experiment? Did you have any particular ideas of how it would turn out?

[Chad Roy] Well, so I've been in infectious disease aerobiology for a long time, and so, first with the U.S. Army for almost a decade, and then here at Tulane University for going on 14 years now. And so, we really were interested to understand just the basis of whether or not this virus can survive in aerosols of a particular size—as kind of a…almost like a baseline—because we didn't know anything about it. We knew something about SARS-1—you know, the virus from the early 2000s—in terms of its efficiency in aerosols. So, the ability of the virus to maintain replication competence—meaning that if it gets into a human, it will or will not replicate. Because, you know, as we know viruses are the zombies of the microbiological world. They're not alive or dead, they only need that competence to replicate. And the aerosol environment is such a stressful one for microorganisms that you have to assess it to see whether or not, you know, it can…upon dehydration, it'll lose that ability to replicate, a very important measurement. And so, we wanted to do the baseline. We wanted to do what happens in that circumstance. We've done this with a number of other viruses, bacteria, and other infectious agents over the years to better understand that. So, when we saw this emerge, the very small community of infectious disease aerobiology folks were, you know, keen on getting that done. And so there

was a lot of collaboration and coordination with other laboratories—at the National Institutes of Health, at the Army labs, and places like University of Pittsburgh.

[Sarah Gregory] So, how did you go about the experiments?

[Chad Roy] So, initially what we did is we wanted to understand the dynamic efficiencies of aerosolized SARS-CoV-2 virus. And what that means is that we were generating these aerosols synthetically at a particular size. So, we were using a laboratory nebulizer that we've used in the past, we know the characteristics of that, we were using a cultured virus, and we generated an aerosol into a chamber and that chamber was run at a rate that was about…about 15 liters per minute. So, what that means is that we're just pushing air into and pulling it out of that chamber at a known rate. That's the dynamic part. So, the residence time of those aerosols were only about 30 seconds to a minute after they were aerosolized. And we knew their size, too. We measured that—we have ways to measure that as well. And we also collected a sample in real time of that aerosol when it was being generated. So, it sounds like a very complex thing, and it is, but it's something that is…was harmonized with a number of labs that I just mentioned, on measuring that.

That sample was then pulled out of that chamber, and then it was…was assessed…it was tested or assayed for a replication competence in a plaque assay, or what we call a TCID50 assay, to get some idea of the kind of remaining replication competence in that sample from that aerosol concentration that we had generated. And we're doing this in a very safe manner, I should say. You know, there's tons of engineering controls on…on doing this type of…of research in biocontainment, so, biosafety level 3 laboratory, and so we take great care in when we do this. And we've done this procedure a number of times as well to ensure the safety of everyone involved. And from that, we could derive estimates of its efficiency in aerosol. And importantly, what we did was we compared that with other beta coronaviruses that have emerged in the last 25 years—namely SARS-CoV-1, which is the original SARS from the early 2000s, and MERS, the Middle Eastern respiratory virus, as well—and compared all of those. And that's the part of the article that's being published, to see the relative hardiness of this virus in that particular set of experimental conditions.

[Sarah Gregory] That's an interesting point about the biosafety. We read these articles about testing this or that for the…for COVID, and I don't think it really strikes us how…oh gosh, you know, how dangerous that potentially is. So, glad to hear it.

[Chad Roy] Yeah, it's a healthy respect through the years for…for this, especially at the size that we're generating these infectious aerosols. It's very small. It's about 2 microns in diameter. And that's a highly respirable aerosol. So if you were to encounter a 2 micron aerosol, you could inhale it and it could make its way down to your lungs. So with that in mind when we're doing these studies…and so that's why there's so much thought and effort put into the engineering behind keeping us safe when we do these types of experiments.

[Sarah Gregory] Do certain activities have a greater impact on spreading these aerosols, you know, like singing, or shouting, or talking loudly?

[Chad Roy] Yeah so, you know, there's a big jump and it's important that everybody remember the jump from a, you know, an absolutely controlled environment that we're dealing with in the laboratory and those results, which we have to do to observe the change…to observe the phenomena. If we try to do that in, say, an outdoor environment, it's very, very difficult to observe those differences. So, when we jump to kind of real life, what you're talking about, the natural generator idea, we need to, you know, temper our conclusions with the context in which those studies were done—a very controlled environment in the laboratory where we're synthetically generating these aerosols—and the changes that we observe, which we do all the time, to someone that may be infectious that are just singing or shouting or even breathing.

We know that…and this is not just my lab, but many labs have shown that human beings, when we exhale, we exhale particles. And there's some of us that exhale more particles than others, and when we do certain activities, like shouting or singing, for instance, there's a potential there for even more particles to be generated. And the important thing to remember in that, in the assessment of those, which has been done as well by others, is that the very heterogeneous distribution of particles that are generated…they're not like the monodisperse…likely monodisperse aerosols that we're working with in the laboratory to observe these changes.

And so, the natural generator is a lot, in many ways, much more complex in terms of how they generate those particles and the size distribution of all those particles. And the reason that that's important, not to make it a really long-winded answer, but is that when we talk about

bioaerosols—so, aerosols that are caring a biologic load—remember, these particles that we're talking about, these aerosols that we're talking about, are not comprised completely of virus. And in many cases, there's no virus in those aerosols. There's a probability of a virus being in the biological load of those aerosols…and now we're getting really small when we're talking….but the viral particles are only about 120 nanometers in size. So, if you can imagine a 2 micron aerosol that's liquid, but it's an aerosol particle, and those viral particles kind of hitching a ride inside that one aerosol. And there may be one or there may be two or there may be three of those viral particles in that particular aerosol.

Now, because we're dealing with aerosols, we deal with cubic volume, right? So a 10 micron aerosol can contain potentially a thousand times a biologic load as a 1 micron aerosol, because it's cubic. And so, the 10 micron aerosols can harbor a lot more, potentially, viruses than the 1 micron aerosol. And so, when we talk about heterogeneous distribution natural generators, that's the potential that we're talking about. So, it gets really complex there compared to the relatively simple way that we approached it in the laboratory with a kind of a single-sized aerosol, just to observe that change on that particular circumstance. And so that's where you, you have to allow the individuals that have a collective of the data—all the data that's being generated—to understand the public health implication and think about it in such a way that they can consider all the data out there as it pertains to transmissibility in aerosols.

[Sarah Gregory] Okay, so along those same lines, what do we know about the impact of conditions? I was…Were you able to look at that, say, humidity versus dry air, heat versus cold, windiness and so on?

[Chad Roy] Yeah. So…well, there was two things. So, to answer your question, we didn't do that here at the Tulane site. But some research has just come out in the last month, month and a half, maybe, from Homeland Security's laboratories located at Fort Detrick, the NBACC facility, that published two just absolutely wonderful articles that did just that. And they compared humidity versus dry air, they compared heat versus cold, and importantly ,they compared the effect of sunlight. And they used the same sized aerosols, viral aerosols, that I was using here, and…which was really nice because we can kind of compare apples to apples there. And sunlight has a dramatic effect on replication competence in aerosol and drops the virus's essentially "half-life" to less than a minute once it's in aerosol, which is kind of comforting if you think about it.

[Sarah Gregory] Yeah.

[Chad Roy] And all the data—and because of the data that I generated is, you know, or the collaboration of folks that I worked with—it's a little sobering. And that's the second part, which is the suspensions—which I understand we're going to talk about a little bit here—and looking at longer-term survival of this…this virus.

[Sarah Gregory] Okay. So…so sunlight has an impact, but not, say, humidity or rain or wind?

[Chad Roy] I don't know about rain…I don't think they…they simulated rain, but these…these articles were published in the *Journal of Infectious Diseases*. And it's clear that there is an impact on the replication competence of the virus, but that's kind of buried in those…those graphs that are included in that article, or those two articles. I do remember that sunlight has a dramatic impact on that.

[Sarah Gregory] Alright. So, say somewhere sunny is better off outside than say, a rainy or dark…or not. I guess I'm not a fan of rain, so, I don't know. Okay, so how does this SARS-CoV-2 aerosol suspension compare to SARS and MERS, which are also coronaviruses, as you mentioned earlier.

[Chad Roy] Yeah. So, that…that's the interesting—I mean, it's all interesting—but that's the interesting part of the story with SARS-CoV-2. So, in a second set of experimentation we artificially suspended those same size particles in a rotating drum. So, a rotating drum is a small device—well, it's small here; there's other labs that have much, much bigger ones, and this one's about 11 liters in capacity—that allows us to overcome a terminal settling velocity of those particles as we…when we generated them, they were about 2 microns. So, think about a…kind of a washing machine set up, to where you have a horizontal agitator, much smaller in our hands, and…and rather than the particles naturally settling based on its terminal settling velocity—which is very slow, by the way, for a 2 micron aerosol—it will take hours and hours and hours and hours for it to settle out, but it eventually will settle out. With a rotating drum, we can overcome that. And if you can imagine that particle kind of corkscrewing…and it can corkscrew for an indefinite period of time, so not necessarily contacting the walls but not necessarily settling at the bottom either.

And so, we've run our rotating drum in prior experimentation for hundreds of hours, and…and has been successful in…in suspending these particles for a long period of time. So that's just what we did in those experiments, and we had timed points where we evacuated the entire chamber and sampled the entire chamber and assayed those viral aerosols to understand the replication competence that is residual in those aerosols. And so, if you do that enough times at enough time points, you can draw a line. And you can draw a line to show the degradation of those particles and the loss of replication competence in that. And the reason that I keep referring to it as replication competence is that we also have a way of measuring by PCR, most people know what PCR is, that is measuring the genome copies, the viral RNA, that's in that chamber. And that's kind of our standard to show that our concentrations never change, no matter how long we suspend it. It's pretty much flat. And that's a good kind of validation, that we have the same, or similar concentrations in that chamber.

But when we do the replication competent-dependent assay, which is a plaque assay or TCID50 assay, we see a reduction of replication competence, or kind of the infected residual that's left there. Now, what we were expecting is that, in 8 to 12 hours, we would see a significant drop off of that…that replication competence. And we didn't see that. We did our experiment up to 16 hours of suspension, and it was a flat line as well. So there were some residual infectivity after being suspended for…for nearly 16 hours in that chamber. And so that was…that was a, you know, remarkable finding on our part.

And, you know…and there's some limitations with that. So, we only—because, you know, everything was urgent—we did that experiment once. And one can imagine how long that takes if we're doing that, and then having to redo it and redo it. So, we…we published this with the limitations stated clearly that, "Hey look, this is a single iteration. Future experimentation will include multiple iteration," and that sort of thing. So, but it was still a…a remarkable finding and it can be added to the collective data of…that's out there. And I should mention also we did this in the absence of sunlight, so it was in a completely black—you know, pitch black—it's pitch black inside of the chamber. So there was no integration of that factor.

[Sarah Gregory] So, the bottom line is, I mean…real life is not usually in a total black room like that or space, but…but potentially this lasts, the aerosols in the air—somebody coughing,

sneezing, whatever they're doing—can hang around for quite some time. Much longer than originally thought, right?

[Chad Roy] Well, yeah. I mean, because we didn't have an idea of that. And so, you know, whenever we're assessing this, because this is brand new, we wanted to make sure that we had the full constellation of effect, right? We wouldn't want to just start, you know…you know, it's always a…I always think of it this way, you know, if you see if you're in a port and you see a big cruise ship pass by, and you get to look into one port hole of that cruise ship and you see people in that one port hole and they're, you know, eating dinner, does that define that cruise ship? Well, obviously not. You'd want to know more about what's going on in that cruise ship because there's multiple port holes. You know, when we approached this, we wanted to get a baseline, absence of sunlight, you know, let's see how long it does last and then from there we can start adding our factors in, like sunlight, like the folks at NBACC did that showed, "Wow, it's got such a dramatic effect," at least on that particle size on this virus. So that's…you know, that's a way that we all you know, observe change and watch a phenomena in front of us.

[Sarah Gregory] So, why is this disease so very contagious? I mean, SARS wasn't this contagious. MERS is, you know, contagious but not nearly this contagious. Why is this so contagious?

[Chad Roy] The biggest distinction that I can see between the two is, and I think others would agree, is that both SARS—and just to contextualize it, SARS and SARS-2 were both airborne transmissible. We know that now. I don't think we need the WHO to come out and proclaim that, we know it. There's an overwhelming amount of evidence. The difference between the two is that the pathogenicity of SARS-CoV-2 is much lower than SARS-1, meaning SARS-1 got into a human being and you know, there was a greater percentage of individuals that succumbed to disease that was not necessarily reliant on the presence of a comorbidity that was on board with that person.

SARS-CoV-2 is an asymptomatic disease in young adults, for the most part. I mean, if we're talking generally here, for young adults and children, whereas there's more consequence with folks with comorbidities or elderly or you know, that sort of thing. So, we have morbidity and mortality you know, in a certain segment of the population, but with that younger cohort there is more efficient transmission. So we see this just infecting enough, just getting enough people sick

to have efficient transmission, especially with an airborne acute viral disease such as SARS-CoV-2 and COVID, compared to SARS-1. Which kind of almost burned out, right? It killed so many people that it wasn't an efficient transmission. So, some people have heard of the R-naught and its efficiency of transmission. SARS-CoV-2 continues to creep up and creep up and creep up, because of that fact. So, that efficiency of transmission I think is—and the lack of mortality in a large percentage of those infected—is what is prolonging this disease.

[Sarah Gregory] Okay. So, sort of reiterating here, basically SARS just killed a whole bunch of people and died out because everyone that got it kind of died. And this just keeps just going on and on and on because there's all these people walking around with it that don't even know they have it and then they're giving it to people that when they do get it, it's much more serious.

[Chad Roy] Right. So, it's got to be the perpetuation of transmission that we're dealing with. Now, the small aerosol studies that we did to try to understand the basic, basic stuff of that…that generation and transport part of the equation, right—so we have three parts of that: we have the generation, we have transport, and we have deposition, or into a human host—showed us at least on the…for a baseline, "Wow, this is an efficient transmitter you know, of disease in this form," And in fact, when we compare SARS and MERS with SARS-CoV-2, it's a little bit, at least in those dynamic efficiencies, a little bit heartier. Not much, but a little bit heartier. And that's what we show in the first part of that article, in that it has the potential to at least, survive in aerosols as well. And these are aerosols that will travel for a long, long, long period of time and distance—you know, of course, with the limitation that it was done in pitch black. So, you know, that gives us the evidence that, you know, public health leaders can say "Oh, okay. This is what we need to do in terms of…"

[Sarah Gregory] These findings have huge public health implications. Tell us how they should influence public health information and guidance.

[Chad Roy] So the way that I look at it, Sarah, you know, is this is one piece of the puzzle in the context of airborne transmission that is, we hope, extremely helpful to public health leadership to make those decisions on what is best practices to keep everyone not infected and safe, and also with a dose of, kind of, reality and reasonableness to the equation. And, you know, it's always better to have a constellation of data to make decisions, rather than the absence of data. Because in the latter, there's a dose of, you know, people that can be unreasonable about expectations of

how human beings interact and, you know, how we protect ourselves. And nobody wants to do that, everybody wants to protect, you know…you know, themselves and each other and their families. And when there's an absence of data, it's very difficult to not just default on worst-case scenario. And with this emerging disease, I think we've been…kind of been under the gun, so to speak, on protecting ourselves.

A perfect example of that is our kids going back to school, you know. I have kids and we went through that calculus, and we listened very, very closely, you know, to our public health leadership on what to do in that situation. Because ultimately it is our decision, right? And I'm heartened that we do have the data that we have in front of us, even though this disease has only been around for…this virus has only been really around and we've studied it for the last six months.

[Sarah Gregory] Right.

[Chad Roy] You know, it's so quick to try and make these decisions. But I believe that people are being very thoughtful, and if they consider the context of this disease. And…so, we were really excited when we had these results, and even though the data, you're right, is very sobering and, you know, it's not necessarily fun, it's just…data is data. And, you know, it's…we're happy that we made a contribution in that way so public health leadership can do their job and figure out what we need to do.

[Sarah Gregory] So, you said you were an aerobiologist. But frankly, I have to say I've never heard that term before, and it sounds really interesting. So, tell us about your job and what you find most interesting about it.

[Chad Roy] No, you're 100 percent right, Sarah. You know, it's a kind of a little corner of science that I got involved in a real long time ago, actually in my graduate work, and I've been plugging away ever since. And it's very interesting, always learning new things. I, you know, I first trained in it at, of all places, the University of Iowa, which is a great, great place and my alma mater, and from my PhD work…and introduced to bioaerosols and aerobiology and the study of kind of this ecology of infectious disease in the air. And I was working then with agricultural bioaerosols—which are polymicrobial and very complex—and host response to inhalation of those. And really my formative training in infectious disease aerobiology was kind of bootstrapped at the U.S. Army in USAMRIID, which was just an absolutely wonderful training ground for that, and I

honed my skills there with a handful of other aerobiology folks that are there, so, kind of a small group, and then here at Tulane where I've been at it for the last…nearly 14 years.

And I can say with some confidence…there's no other aerobiologists at Tulane. Even though it's a great school, you know…its maybe one or two aerosol science folks, but…that are into it as much as I am. So I've found it's been just a fantastic and constantly learning, constantly learning place to be in science. And you know, I do a lot of work with animals as well and looking at host response to aerosol models of disease and the…kind of the pathophysiology of aerosol infection. And so, that's a big, big part of what I do as well. Yeah…and so, that's why I'm in the school of medicine and, you know, I've had great experiences along the way with, you know, getting to spend time at CDC, for instance, and working there for a short period of time on collaborative projects. And all of this in biocontainment…so I've done a significant amount of work in DSL3, but also DSL4, lab safety level 4, with different agents as well. So it has been an incredible, you know, constellation of different experiences that…that are fascinating.

[Sarah Gregory] So okay, now you've mentioned animals, so I have to ask….maybe this isn't part of anything you've looked at or studies, but there's been a couple of reports of COVID in cats and dogs. And I have two little dogs, and I'm afraid to take them for a walk. What do you think?

[Chad Roy] What I think is you should go take those dogs for a walk without fear, and remember that anytime we are outside—and I don't want to, you know, give public health edicts here—but when you are outside, you are surrounded by millions and millions of cubic meters of air. And the dilution effect that one experiences when they are in the outdoor environment, even in an completely still outdoor environment, is so great as to minimize your risk, so low, and your dogs' risk, so low, that we need to start thinking about this virus and viral infection as not on the "one-hit" theory, right? So, one infectious particle gets into your dog or gets into you and it causes disease. Infectious disease does not work that way. You know, it's, it's just…it doesn't work that way. And so, please, please, please go take your dogs for a walk.

[Sarah Gregory] Okay, well thank you. That's actually very comforting, yes. Now if I could just overcome the heat! No, seriously, that is very comforting, thank you.

Okay, and a final question here. What do you do for fun and relaxation? Tulane is in Louisiana, which has been sort of a COVID-19 hot spot at one time. How are things going for you there?

[Chad Roy] Yeah, so you know, Tulane's great. I'm so happy. I'm also an alum of Tulane, so kind of coming back home, as it were. It's been such a big part of my life for so long. The last six months have been, for many of us, an absolute blur. Like, I try to think back on April or March and what I was actually doing, and I can't. I tell my wife many times I can't even remember what I was doing in March and April just because it's such a blur. The…we usually have our research activity here is normally at a fairly high tempo, but this has been…this has been overwhelming.

And the people that work in the laboratory, you know, have kind of moved out of the laboratory in my position now, but the people that are in my lab, for instance, they've worked every weekend for the last several weekends, 14 hour days, and every day during the week just working on…on animal modeling, on these questions, on protection studies, and everything else that we're engaged with. And, you know, I'm almost embarrassed when I sneak out of here at 6:30 at night and they're still here working. And so, I mean the frontline work that these individuals are doing, you know…and these are not doctors and nurses, these are laboratory personnel, veterinarians, that are…and others that are just so incredible dedicated that…oh gosh, it brings tears to my eyes thinking about how hard people are working right now to find answers, and that kind of thing. So, it's been a, I guess, weird time to be in a…you know, where we are right now. But normally, we have a pretty good time down here.

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

[Chad Roy] Well, thank you Sarah. It was nice talking to you as well.

[Sarah Gregory] And thanks for joining me out there. You can read the September 2020 article, Persistence of Severe Acute Respiratory Syndrome Coronavirus 2 in Aerosol Suspensions, online at cdc.gov/eid.

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

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