Tracking *Bordetella pertussis*, Austria, 2018–20

[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. Adriana Cabal Rosel. She's a public health microbiologist at the Austrian Agency for Health and Food Safety. We'll be discussing the use of a new surveillance system to track increased cases of *Bordetella pertussis* in Austria.

Welcome, Dr. Cabal Rosel.

[Adriana Cabal Rosel] Hi, thank you for inviting me. This is a great pleasure for me to be able to speak here with you.

[Sarah Gregory] Well, we are very happy to have you. Let's start with you telling us what *Bordetella pertussis* is?

[Adriana Cabal Rosel] Yes. So that's a really simple question. *Bordetella pertussis* is a gram-negative bacterium that is responsible for the highly contagious respiratory disease known as pertussis.

[Sarah Gregory] Okay. So, we get vaccines for our dogs against *Bordetella*. How is *Bordetella pertussis* different from that, or is it even?

[Adriana Cabal Rosel] Yes, you are right. We do get vaccines for our dogs against *Bordetella*. But the dog's *Bordetella* belongs to the species *Bordetella bronchiseptica*, which is not the same as the one that produces disease in humans. The *Bordetella* that's produced between humans is named as *Bordetella pertussis.*

[Sarah Gregory] I see, okay. So, where does the name "whooping cough" come from? You hear that a lot related to *Bordetella pertussis.*

[Adriana Cabal Rosel] Yes, exactly. So the term "whooping cough" comes essentially from the sound that a person that suffers from pertussis disease makes while coughing. So not every person that has pertussis presents this characteristic feature. Usually these patients with pertussis experience around the second week after the onset of the symptoms a fit of coughing, followed by a high-pitched whoop sound, and hence the name of whooping cough.

[Sarah Gregory] Your paper refers to pertussis as a reemerging disease. What exactly does that mean?

[Adriana Cabal Rosel] Well, I'm sure you know the term of reemerging and reemergence. So reemerging means, as you know, and can be of course applied to other diseases and not only to pertussis. It means that pertussis is reappearing again with increasing incidence rates despite the high vaccination coverage, of course, in most of the countries.

[Sarah Gregory] How is it spread?

[Adriana Cabal Rosel] So pertussis is spread from person to person, meaning that an infected person can infect another when coughing or when they're sneezing, or even when spending a lot of time near one another by sharing breathing space.

[Sarah Gregory] Are household members more vulnerable to it, than say, getting it from someone in line at the market?

[Adriana Cabal Rosel] Definitely. In order to get pertussis, we will need to spend much time near the infected case because in this way you would be sharing breathing space. If you are not vaccinated, then the chances of getting pertussis when sharing this same space could increase up to 90%.

[Sarah Gregory] So it's not highly contagious like, say, measles, which you can get very, very easily...

[Adriana Cabal Rosel] Exactly.

[Sarah Gregory] Okay, so let's call it whooping cough. Whooping cough mostly affects children. Why is that?

[Adriana Cabal Rosel] That would be because children are one of the risk groups for pertussis. Normally the babies younger than 3–6 months of age aren't protected by vaccination because they are too young to be vaccinated. And then we have the teenagers that were previously vaccinated (in principle, they should get the vaccine), but the immunity waned. So the immunity has started to fade, so therefore they are considered also a high-risk group.

[Sarah Gregory] I see, okay. Can you be asymptomatically infected, like with COVID?

[Adriana Cabal Rosel] Yes, for sure. And this is one of the big concerns of pertussis. There is evidence that individuals that vaccinated with acellular *Bordetella pertussis* vaccine, which are commonly used in developed countries since the middle 90s, can become asymptomatically infected and then transmit pertussis to susceptible individuals. Then this transmission goes unnoticed, and it is really difficult to perform contact tracing because you miss many links in the transmission chain.

[Sarah Gregory] We've heard a lot in the news recently about pathogens being able to mutate and evolve into different variants. Is this also the case with *Bordetella pertussis*?

[Adriana Cabal Rosel] Aha, this is a really interesting question. So, yes and no. The mutation rate of a virus is considerably higher than that of a bacterium, meaning that in this case, in our case, *Bordetella pertussis* does not evolve that fast except for the antigen genes that are used in the commercial vaccines. And that is worrisome, because by generating new antigen variants the circulating *Bordetella pertussis* can escape vaccine immunity. So to put this in other words, those emerging antigenic variants (or here in our study so-called genetic profile), are different from the ones we see in the vaccine strains.

[Sarah Gregory] So you found that some of the isolates didn't have something called pertactin. What is pertactin, and why is it important?

[Adriana Cabal Rosel] So, pertactin is a protein produced by *Bordetella pertussis* and also other species in the genera, but yet in our case we're talking about pertussis. So, this is also an antigen of the acellular pertussis vaccine, which is mainly used nowadays in developed countries (I'm sorry, this acellular vaccine). So since pertactin is an antigen, it is recognized by our body after vaccination with an acellular vaccine following antipertactin antibody titers that correlate with immune protection. However, what happens is that the negative part of this is that these antibodies are driving the emergence of pertactin-deficient strains that escape the immune

response. So we can here confidently say that there is a selective pressure for *Bordetella pertussis* to inactivate the pertactin production.

[Sarah Gregory] Are these new strains any more transmissible or dangerous than other forms of pertussis?

[Adriana Cabal Rosel] Okay. So, there is controversy regarding this issue but we can say that regarding transmissibility, the pertactin-negative strains are known to have a greater growth advantage, at least in vitro, than the pertactin-positive strains. So this would allow a high level of transmissibility between hosts which would be consistent with increasing in the numbers of infection with pertactin-negative isolates. But also we should consider the age, because transmission is dependent on age.

And regarding the second part of your question (regarding pathogenicity), there is also increasing evidence of emergence of *Bordetella pertussis* strains that carry polymorphisms (this is a…polymorphisms are mutations), for instance those mutations in the pertussis toxin promoter gene that produce a greater amount of pertussis toxin. And therefore, they are able to produce more severe forms of the disease.

[Sarah Gregory] You touched on this a little bit already, but what's the problem with the vaccines? Why are they becoming ineffective? There's a growing segment of the population who are vaccine-hesitant, especially for their children. Has this helped allow pertussis to spread and create new variants?

[Adriana Cabal Rosel] Okay. So, to answer to your question we must explain a bit of history. So the cellular vaccines, the cellular pertussis vaccines (also called whole cell vaccines) were substituted in the late 90s (at least here in Austria, in 1997) by the acellular vaccines because of the side effects (the whole cell vaccines produced huge side effects). So the difference between both of these vaccines are that the acellular ones do not contain the whole bacterium, but few antigens, and the immunity induced by the vaccine wanes faster for the acellular vaccine.

Another disadvantage of the acellular vaccines is that their antigens trigger (as I said before, I guess) an immune response and some, like the pertactin antigen, experience frequent mutations in their DNA sequence resulting in lack of pertactin production. So this is how the pertactin-deficient strains infect humans, by escaping the immune response.

And regarding the second question, the emergence and spread of new variants is due to vaccine pressure, not to vaccine hesitancy. So vaccine hesitancy can contribute, of course, to a general increase in the pertussis incidence. Although vaccination coverage for pertussis is still high (at least here in Austria), vaccine hesitancy of course plays a huge role in this increase of the incidence.

And with this I will conclude the question, we have to take of course into account that part of this increase in pertussis incidence is not real. What I mean is that there is an increase in the awareness among the clinicians, so pertussis nowadays is more often diagnosed and reported. But also our surveillance systems and molecular methods are becoming more and more sensitive. So there are many other factors that can potentially contribute to the increase in the incidence.

[Sarah Gregory] So, what alerted you in the first place that something was going on that you started looking into this whole situation?

[Adriana Cabal Rosel] Yes. So like in any other outbreak, we were detecting like in other developed countries an increase in the number of cases since 2015. But what did raise the alarm was the detection of an unusually high amount of cases via our case-based surveillance system. And this unusually high amount of cases occurred in the same district. So this is what pointed towards a possible outbreak.

[Sarah Gregory] And what was the goal of your study?

[Adriana Cabal Rosel] So for the first time in Austria, we wanted to establish an isolate-based surveillance system for pertussis (this was never done before) that could allow us to do genomic surveillance of pertussis strains that were circulating in the country and in this way we wanted to complement the information collected by our surveillance case-based system. And following this objective, we wanted to investigate if this increase in the incidence corresponded or not to a specific genetic profile, maybe pertactin-deficient, in line to what had been already discovered in other countries.

[Sarah Gregory] So your study analyzed isolates from 123 whooping cough patients in Austria during 2018–2020. Were these mostly children or adults, and were they vaccinated or unvaccinated?

[Adriana Cabal Rosel] So, it was quite a heterogeneous group but most cases belonged to the age groups between 1 and 15 years old (meaning the risk groups). But overall, less than half of the cases (so around 43%) were vaccinated and 31% were unvaccinated. Unfortunately, as is always the case in this kind of study, 25% of the cases had an immune status unknown. So, vaccination status was unknown. And this is, of course, a limitation of the study that yeah, we cannot conclude many other things that we would like to conclude with this.

[Sarah Gregory] How did you go about tracking these variants down? I believe you created a new type of surveillance system, right?

[Adriana Cabal Rosel] Yes, exactly. We created an isolate-based surveillance system. As I said before, this isolate-based surveillance system was complementary to the case-based surveillance system in which all incoming isolates were whole-genome sequenced (so I mean in the isolate-based surveillance system were whole-genome sequenced), their genetic relatedness was assessed with our new core-genome MLST scheme that we developed back in 2018 when we started this project. And later on in the later stage, what we did was to extract the antigen genes (so the sequences of the antigen genes) from our whole-genome sequencing data and compare it with the circulating strains also in other countries (like in the U.S., UK, etc.) in order to detect what we call in our study genetic profiles.

[Sarah Gregory] In what way is your system different from any other one in Austria?

[Adriana Cabal Rosel] Okay. So as I said before, we have a pertussis case-based surveillance system in place, because pertussis is mandatorily reported in Austria. And this type of surveillance is based (it was based before) on the electronic notifications from clinicians. So this surveillance (the case-based surveillance) does not need of isolates in principle because pertussis is usually diagnosed based on the symptoms and based on PCR confirmation. Of course, some laboratories perform...still perform serotyping and isolation, but that's less common. So this is one of the advantages of our surveillance system. Our isolate-based surveillance system allows us to type this *Bordetella pertussis* isolates that are circulating among the population.

Then another, well, our isolate-based surveillance system complements the case-based surveillance system. And its mission was (and still is) to track also the genetic variants that circulate in Austria in order to detect outbreaks that maybe could go unnoticed only with the case-based epidemiological data.

[Sarah Gregory] So how did you structure this study?

[Adriana Cabal Rosel] So the first thing we did was to set up the isolate-based surveillance system by getting everybody on board, and this was not an easy task. We first checked with the laboratories that were interested in participating in the study if they had the appropriate media to participate in pertussis. And to set this up, we coordinated all the sample collection, we shipped suitable nasopharyngeal swabs, etc., etc.

Afterwards in the second part of the study, we generated our core-genome MLST scheme. Because as I said before, by then in the early...in the year 2018 (at least 2018) there was no core-genome MLST available. Afterwards, we got to know that the Institut Pasteur had developed another core-genome MLST. But, yes, there wasn't any and we typed our isolates with this core-genome MLST that we created. And last, what we aimed to was to perform a genetic analysis on the recovered isolates to elucidate which genetic profile we had.

[Sarah Gregory] Give us the highlights of your study now.

[Adriana Cabal Rosel] Sure. So, in our study we describe a novel pertussis isolate-based surveillance system in Austria but also the main novelty is that we generated this new core-genome MLST (multilocus sequence typing scheme). And this was really successful for assessing the diversity of *Bordetella pertussis* strain in Austria and also investigating these genetic variants.

Another highlight of our study was that during this three years of the study, we obtained (we can say) the amount of pertussis isolates (123 isolates) and we typed them with the core-genome MLST. And as we…as I said before, we assessed the genetic profiles. So these would be the highlights of our study.

[Sarah Gregory] Okay. So tell us about your findings now. Anything different than you've, I mean, anything more than you've told us already?

[Adriana Cabal Rosel] Yes. So, well as we expected, all these 123 isolates from Austrian cases differed genetically from the vaccine strain. And I said expected because we were seeing in other countries that this was happening, so that's why we were expecting that they would differ from the vaccine strain. They differed both in their core genomes but also their vaccine antigen genes. It was really interesting to see that more than 30% of the isolates were pertactin-deficient, which is in agreement with other findings in other countries, in particular with those countries that introduced the acellular vaccines at the same time as Austria did (so by the year 1997). In addition, we detected 8 core-genome MLST–based clusters when applying a preliminary cluster threshold of 6 alleles or less. And one of these clusters was pertactin-deficient and possibly part of a local outbreak in one of the districts in the province of Salzburg. This was one of the main findings.

[Sarah Gregory] So, you mentioned vaccination status being unknown as one of the challenges of doing this study. Were there any others?

[Adriana Cabal Rosel] Well, as I said before, getting everybody on board was quite a task because you have to take into account that not every laboratory is prepared or has the knowledge to cultivate pertussis. Pertussis is difficult to be cultivated, you need a specific media, expertise, and not all the pertussis-positive patients have a positive culture afterward. So this is one of the challenges.

[Sarah Gregory] Let's go back to the vaccines for a minute here. Are they effective at all anymore?

[Adriana Cabal Rosel] Okay. So this is a really interesting question, thank you. So, we cannot say right now that they are no longer effective, but what we can say is that there is evidence that indicates that vaccine efficacy for infection and transmission with pertactin-negative strains would be (according to recent mathematical modeling) as low as 6%. But for disease prevention, that is different to infection and transmission. Especially prevention of serious symptoms in children, the efficacy of these vaccines is still high. So that's why vaccines are still recommended.

[Sarah Gregory] Okay. So people should still get vaccinated against it?

[Adriana Cabal Rosel] Definitely. People should get vaccinated against whooping cough because acellular vaccines are effective at preventing disease as I said before. And of course while we wait for improved vaccines that limit infection and transmission, we need to get vaccinated, and this is particularly relevant for the risk groups. Secondly, the efficacy of these pertussis vaccines against disease is higher than against infection as I said before. So at least we need to protect these risk groups against disease.

[Sarah Gregory] Okay. So you mentioned just now waiting for new vaccines. Are there any new or updated vaccines in development?

[Adriana Cabal Rosel] So this topic is under discussion for already many years. I've seen that there is a consensus that new vaccines will be developed and as far as I know, only one group in France in collaboration with a biotech company in New York is working on that. So, I think it's a whole cell vaccine that tries to compensate in a way the vaccine escape induced by the acellular vaccines and therefore, it would aim at reducing the transmission. Last September when I checked, they were already in phase one of the clinical trials and it appears to be safe and effective. So I think this is a good news, definitely.

[Sarah Gregory] What about people who have already had whooping cough? Can they get one of these new variants and should they get vaccinated?

[Adriana Cabal Rosel] So, neither natural immunity due to previous infection nor vaccination provides life-long protection, meaning that reinfections are not uncommon. And therefore, vaccination is more than recommended in all age groups, also including adults. So yes, you can get a new variant and still you should get vaccinated.

[Sarah Gregory] Is there something that people, especially parents and grandparents of young children, should be concerned about?

[Adriana Cabal Rosel] Well pertussis, maybe you know, it can be life-threatening in small children. So that´s why in particular the risk groups should be vaccinated. So these are usually infants between 1 and 4 years old and kids at school age. However, it is also recommended for

pregnant women and adults also to receive some boosters. So yes, my concern would be that, yes, we have to get our children vaccinated. Otherwise this can pose serious problems.

[Sarah Gregory] Okay. I just want to be clear, here. Did you say it's not recommended for pregnant women?

[Adriana Cabal Rosel] No, it is. Sorry, it is recommended for pregnant women also, and adults also. But this varies between countries. So, for instance, here it's recommended to vaccinate adults. But in other countries I know that this is not in place.

[Sarah Gregory] What can we do to protect ourselves against this disease besides vaccines?

[Adriana Cabal Rosel] So besides vaccines that are the first line, of course you can apply general hygiene measures like for COVID—reduce your contacts, etc., wash your hands frequently. You know, exactly like for COVID that is also airborne.

[Sarah Gregory] How will your isolate-based surveillance system for pertussis in Austria be helpful?

[Adriana Cabal Rosel] So mostly our work adds knowledge, again, about the circulating *Bordetella pertussis* genetic profiles and will facilitate in case of an outbreak rapid identification of new strains, also because we have developed this core-genome MLST scheme. And of course because we can compare internationally nowadays (with core-genome and other tools) these strains once we have sequence data.

[Sarah Gregory] And as you just said, other countries. So your study was based in Austria, so what implications does it have for other countries?

[Adriana Cabal Rosel] So this question is in line with the previous one, so yes, definitely for genomic surveillance at a global scale our study has an impact. We have seen that our genetic profiles and also in general our strains (the genome of our strains) resemble those currently circulating in other countries. Moreover, our core-genome MLST scheme has been applied successfully and also towards other strains that they were not coming from Austria. So, definitely our core-genome MLST and in general our study can be an example for the future for other studies based on genomic surveillance and can be also applied for other strains.

[Sarah Gregory] But along with that same theme, what do you see that needs to be done in the future in the way of more studies or more action?

[Adriana Cabal Rosel] So, apart from developing new vaccines that protect against infection (not only against disease), genomic surveillance is a must. So we need to standardize our methods in order to be comparable between laboratories. Especially in pertussis' case, this is important. So we have right now this analysis, core-genome MLST, whole-genome MLST, they are really, really helpful in assessing genetic relatedness of pertussis. But of course we need to gather complete epidata and also to study the genome rearrangements in the pertussis strains to further differentiate isolates that may seem genetically the same but they are actually not related geographically and time-wise.

[Sarah Gregory] It’s been a little more than a year since the start of COVID-19 pandemic. Since last March, flu season pretty much didn't happen this year. Have cases of whooping cough gone down also at all because of interventions like mask wearing, handwashing, and social distancing?

[Adriana Cabal Rosel] Definitely. They have definitely gone much lower and the same applies to other respiratory diseases like the seasonal influenza. This is definitely due to the containment measures.

[Sarah Gregory] And since the cases have gone down, will that help slow the spread of these new variants in strains?

[Adriana Cabal Rosel] My personal opinion is that it can be slowed down, but as soon as we go back to our normal lives, we will be seeing again an increase in the incidence. However, this might not be a constant increase, but in form of epidemics that might last 2 years and then we see a decrease in the number of cases, and then an increase, etc. It's like exactly the case of the U.S., that you have an epidemic in 2010 that lasted until 2012, and then again in 2015.

[Sarah Gregory] Tell us about your work and what you like most about your job.

[Adriana Cabal Rosel] Well, so I am a postdoc researcher and I work as a public health microbiologist in AGES, the Austrian Agency for Health and Food Safety. And well, I can say that I am a bit of a multitasker. So, I do outbreak investigation and surveillance of communicable diseases mainly using bioinformatics tools. And I am also involved in a One Health project that's basically aimed at assessing antimicrobial resistance genes in environmental sources. So, I do a bit of everything as well. I am really passionate about my job, it's really challenging but it's quite diverse.

[Sarah Gregory] And what do you like to do in your free time, if you have any, of course? Do you stream shows? And what shows do you like and what do you like to munch on while you're watching them?

[Adriana Cabal Rosel] Well, right now we have no free time. My free time consist now on laying on the sofa and watching Netflix while eating popcorn. But of course, before COVID I used to travel a lot, so I am really eager to have the—how is it called—the international green pass for COVID, since I have recently gotten my vaccination. And yes, I'm looking forward to this international pass so that I can be already traveling.

[Sarah Gregory] I think that's a EU pass, I don't think we have that here or in Canada. Yeah, so what Netflix show is your favorite?

[Adriana Cabal Rosel] My current show, well, all kinds of thrillers I like, specifically. I'm not a huge fan of reality shows though, so mostly like serious I like to watch, like Stranger Things, for sure.

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

[Adriana Cabal Rosel] Thank you, thank you very much. It was really nice. Thank you, and have a good day.

[Sarah Gregory] You too.

And thanks for joining me out there. You can read the March 2021 article, Isolate-Based Surveillance of *Bordetella pertussis*, Austria, 2018–2020, online at cdc.gov/eid.

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

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