

Julia Petras: Good morning, everybody. I'm going to ask you all that question one more time. What do you think? An aromatherapy spray, a pet raccoon, and the Gulf Coast of Mississippi have in common? I never would have imagined that it was a rare tropical disease called melioidosis, caused by this bacterium. *Burkholderia pseudomallei*. And now I'm realizing that you guys can't see this bacterium that I'm talking about on this screen. Can we show this grand bacterium on the screen for everybody? It's actually quite beautiful. Here's that raccoon. There's the Gulf Coast in Mississippi, and there is the bacteria, there we go. OK, so melioidosis is caused by this bacterium right here, *Burkholderia pseudomallei*. You usually find this bacterium in places in the soil and water and places that look like this in tropical and subtropical regions of Southeast Asia, northern Australia, and Central America. And let me tell you my story of how we found it in these unexpected places during the investigations of two different domestic melioidosis outbreaks. Now imagine it's my first day of EIS. It's July 1st, 2021. It's 9:00 AM. I'm sitting at my computer in my new apartment in Atlanta when I get a calendar invitation in my Outlook for a meeting with the FBI. We needed to brief the FBI on an ongoing, mysterious outbreak of melioidosis. I wondered, Why would the FBI be interested in a rare tropical disease? I learned that *B. pseudomallei* is actually a tier one select agent, and it can be aerosolized and used potentially as a biological weapon. And we needed to brief the FBI because there were four patients who didn't know each other over a span of four months who lived in four different states, who were all infected by this same clonal strain, a South Asian strain of *B. pseudomallei* and we didn't yet know the common source of their infections. And they had never traveled outside of the United States. The most recent patient was the 2nd fatal case A 5 year old boy from the state of Georgia. Now at this point we felt the pressure mounting. We were wondering when and where would the next case show up. Our leading hypothesis was that it was an imported product from South Asia. But what product exactly? This question would continue to haunt us for another few months. The product was still out there, we were just waiting for the next phone call notifying us of another death. After two EPI AIDS patient families interviewed, reinterviewed, serological testing done, medical charts, reviewed and reviewed over and over again hundreds of household products tested from patients homes, nothing was testing positive and nothing was exactly adding up. So we decided to go back to the home of that five year old patient in Mississippi in in Georgia and collected one last round of product samples making sure to get any last remaining liquid containing products, especially ones that could be inhaled. At this point, it was 3 1/2 months after that boy had died. We had felt that we had missed our window. But two weeks later, I get a call from my colleague Mindy Elrod in the lab. A Better Homes and Gardens aromatherapy spray collected from that Georgia patient's home tested positive for *Burkholderia pseudomallei*, and the strain was genetically identical to the strain that infected all four patients. We thought the story was over when the nationwide recall was issued by Walmart and thousands of products were removed from the shelves. But then we learned an unexpected piece of information from the Texas State Health Department and here enters the raccoon stage, left. The family of the Texas patient reported that they had a pet raccoon that they said knocked over the later implicated aromatherapy spray, walked all over its liquid contents, then two weeks later developed acute neurological symptoms, trouble walking and then died of an unknown cause. And then they buried it in their backyard in Texas. So this all happened seven months earlier? What's the big deal? Well, if the raccoon actually died of melioidosis and was buried in the yard, then it could potentially contaminate the environment around it. And the last thing that we wanted was for this rare bacterium to be established in the state of Texas. So what does one do with this type of information? Well. You team up with the EPA. You put on a hazmat suit with your fellow LLS colleague, Maureen T.

And you dig up a raccoon and you decontaminate the burial site. And that's exactly what we did. And what did we find? We found the unexpected. Not only did we find it completely intact, raccoon entombed under a foot of soil that had been there for over a year. But a swab that we had purposely took from behind the eye of that raccoon that we were hoping was brain tissue turned out to be PCR positive for *B pseudomallei*. Another unexpected finding that nobody saw coming. And there was no evidence of environmental contamination, by the way. So after several media interviews and publications in the New England Journal of Medicine and MMWR, I thought I was ready to retire as a first year EIS officer. But I was far from done because shortly after we get a call from the Mississippi State Health department. Another confirmed case of melioidosis with no travel history outside of the US. Now at this point it's May 2nd, 2022 and you might be thinking, could this be another aromatherapy associated case? I could tell you, but before we left for Mississippi, we knew this was something different. This was a completely different outbreak. We knew that this patient was infected by a distinctly different strain of *B pseudomallei* than the one that we had found in that aromatherapy spray. It was of Western Hemisphere origin. And it was genetically identical to the strain that infected another Mississippi patient in 2020. Now these two patients live just 7 miles apart in the same Mississippi county on the Gulf Coast. We wondered, could these infections be linked to another imported product, maybe from South America? Or could they have acquired it from the local environment in Mississippi? For years, there had been this growing hypothesis that this bacterium could be endemic to parts of the southern United States, despite several negative environmental sampling investigations. Could this be our chance to finally prove this hypothesis? We would need to investigate this from all angles. And the next thing you know, Maureen Tea and I are back again traveling, this time in the government van down to Mississippi. When we arrived in Mississippi, Maureen and I teamed up with Doctor Byers, the state epidemiologist, and his team. We all decided that the best chance for us to figure out how these two patients acquired their melioidosis infections were to take a literal immersive approach to the investigation. The health department was so invested in this. Investigation that the public health nurse Gail even lent us her kayak so that we could get into some hard to reach spots to sample. The state epidemiologist, Dr. Byers, even attempted to wear the same pair of waders that fit me in order to get the samples that we needed. So we went to the home of that most recent case, and we spent a day with him and his family in their home. We sampled various household products that he used in the month prior to his illness. We followed him around his yard where he showed us exactly where he would do yard work, and we sampled there. We went to all of these different secret fishing spots that he showed us around the county that he would go to, and we sampled there too. Now, Maureen and I felt pretty hopeful at this point that we had collected the samples that we thought were going to be positive. We were in swamp Bayou territory. It just looked like the perfect place for this bacterium to hide out. But we knew there was one more key place that we wanted to visit and sample and on the night. Before we were set back to Atlanta. I get a phone call, actually, I've been trying to reach this patient for weeks, and I finally got him on the phone, the first patient that was infected back in 2020, and he gave us permission on that final day to sample his yard. He was as intrigued and eager to find out the source of his infection as much as we were. And because of the good report that the health department had established with him and his family, we were able to make that critical connection and collect. Those samples on his property. When we arrived to his property, we were surprised to see that he lived in a subdivision much like the one shown here. There was no swamp or Bayou that we could see. We never would have expected that the three samples we had collected from the subdivision property shown here would have given us our positive results. A few weeks later, two soil and one water

sample tested positive for *B. pseudomallei*. Not only were they positive, but the environmental strains were genetically identical to the strains that infected the two patients. I cannot emphasize enough how huge of a moment this was. This was the first time that this bacterium has ever been isolated in the environment in the continental United States. It's not every day that you get to discover a newly endemic disease. We had found the needle in the haystack again. So while it may seem like these investigations came together so seamlessly, I want to emphasize that finding this bacterium in these unusual places required a team effort and a high level of persistence. For us, the stars aligned with amazing collaborators, state and local health departments, epidemiologists, laboratory scientists, clinicians, medical examiners, patients and their families, and the bacterial special pathogens branch. We would not have been able to identify the unusual sources of these cases if it wasn't for that collaborative collective effort and dedication from everybody involved. I've learned that a boots on the ground approach is sometimes what's needed to solve these types of investigations. While we all love our data, our computers, our comfy sweatpants at home, it cannot always replace shoe leather epidemiology. It was critical for us to keep an open mind and not cross anything off the list of hypothesis because it seemed just too unlikely. So I challenge you the next time you feel stuck in an investigation. And it's nothing's really making sense. That's when you know it might be time to cast a wider net. Explore those crazy ideas, ideas. Dig a little deeper. What you're looking for might very well be the most unexpected thing. Don't get easily discouraged. Trust in the process. Listen to the patient and their family and those that have paved the way before you. Sometimes you just need to tuck in your sleeves. And dig up a raccoon. Thank you very much.