Serosurveillance of Feral Swine to Map Risk for Anthrax Exposure, Texas

[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 Rachel Maison, a graduate student at Colorado State University in Fort Collins, Colorado. We'll be discussing the potential use for serosurveillance of feral swine to map risk for anthrax exposure in Texas.

Welcome, Rachel.

[Rachel Maison] Hi Sarah. Thank you for having me.

[Sarah Gregory] Tell us about anthrax, in general—who and what it affects, where it's found, and how dangerous it is. It's listed as a bioterrorism agent, I believe, right?

[Rachel Maison] Yes. So anthrax is basically a term that describes the infectious disease caused by the bacterium *Bacillus anthracis*, which is a bacterial species that is most naturally found in the soil but can become dangerous if it gets into the body of an appropriate host. So this bacteria affects most mammals, including humans, and can infect hosts multiple ways, depending on the host species and route of infection, and depending on the resulting anthrax disease that is caused could be fatal.

So you are correct. It is listed as a bioterrorism agent (or an agent of potential bioterrorism) by the CDC. So one route of the infection that is particularly dangerous and is kind of why it's listed as such is the inhalational form of anthrax. And this form of infection is highly fatal, it can cause high fatality in humans and is, again, partly why it's considered to be very dangerous. So by the CDC it's listed as a Tier-1 select agent because of the high risk that it poses for deliberate misuse and the potential for being aerosolized and subsequently causing massive casualties and the significant threat to public health and safety.

I guess one example that many people might be familiar with and that kind of demonstrates the potential danger of this pathogen and how it might be deliberately used for bioterrorism is the placement of anthrax spores in letters that were sent to members of Congress and some people in the media back in 2001 right after the events of 9/11. So during this attack, spores were actually produced in a powder form and placed in envelopes where the recipients were kind of in a prime position to aerosolize those materials and inhale those bacteria after opening their mail. And this actually resulted in thousands of people being exposed and at least five people actually dying.

[Sarah Gregory] Give us a brief history of anthrax. When was it first discovered and first named by symptoms, and where?

[Rachel Maison] Interestingly, anthrax is actually considered to be an "ancient disease" because it appears as though humans have been dealing with it for quite some time. So it's actually suggested that symptomatic descriptions of the disease may have occurred as early as 1,000 BCE, some of these descriptions matching that of anthrax making appearances in some of the works of Homer and Aristotle. And it's also actually been speculated that what's described as the 5th and 6th plagues of Egypt in the Bible are identifiable with anthrax.

More officially, anthrax was first described clinically in the 1700s in Europe by a group of French scientists. But it wasn't until the 1800s that true study of the illness began, and where the

disease was actually linked to a microbial agent, which as we know now as *Bacillus anthracis* bacteria, by Robert Koch. But present-day genetic studies, even more officially though, have indicated that the origin of the bacteria was likely Sub-Saharan Africa, with subsequent global spread of the organism occurring after the introduction of domestic animals to the region and with the increased movement of both animals and humans afterwards.

[Sarah Gregory] How specifically does it affect a person or any mammal? Who or what is mostly likely to get it, and once gotten, what are the symptoms—obviously, additional to being highly fatal?

[Rachel Maison] Because it has such a wide host range, how it affects a person or other mammalian species really depends on the route of infection or how the bacteria end up entering its host as well as the species that is being infected. There are three major forms of anthrax that we know of, and one fourth form of anthrax infection that has been described in the past 20 years or so, based on these infection routes.

So first you have cutaneous anthrax, which happens when anthrax spores get into the skin, usually through a cut or a scrape. For humans, this often happens if someone is handling contaminated animals or animal products like meat, hair, or animal hide. This is also the most common form of infection for people and also the least dangerous. So symptoms for this form, for humans at least, is mostly the appearance of a lesion at the site of where bacteria entered and infected the skin, usually dark in color, and looks almost like a big, large black scab. These are, I'm told, usually painless and in some cases can actually resolve by themselves. But in more severe cases, these lesions can cause significant scarring and damage to the surrounding tissues, or as we've mentioned, even cause death if the bacteria enter the blood stream and the infection becomes more systemic and serious.

So secondly, we have the gastrointestinal form of anthrax. This form is very rarely reported in humans in the US but is actually the most common form of infection for wild and domestic mammals. This type of infection happens after you consume contaminated or undercooked meat or animal tissues, or for grazing animals, sometimes eating plant or soil materials off the ground that are contaminated. And symptoms for humans are typically nausea, vomiting, bloody diarrhea, and sometimes fever.

The third form of anthrax, which we've kind of touched on, is inhalational. This happens in instances where anthrax spores are aerosolized. People may be infected by breathing in those anthrax spores and those bacteria getting into the respiratory tract. Symptoms in humans are unfortunately pretty generalized or vague for this form. You might have fever, chills, a non-productive cough, sometimes chest pain. And this form, like I said, is also the most deadly form since from the airway it’s very easy for the infection to then become systemic. And usually after a few days after symptoms start, people start to experience severe respiratory distress and difficulty breathing. And like I said, this could lead to a fatal outcome.

And so lastly there's a fourth infection route that has been recently identified in the last 20 years called injection anthrax, which is caused by injection of *Bacillus anthracis* spores into the body. This form is extremely rare, and actually hasn't been documented in the US. It has only been documented in the UK in drug users using contaminated needles. Symptoms for this form are pretty similar to the cutaneous form, but because the bacteria are actually being injected, the infection site is a lot deeper than the surface of the skin and can therefore cause even more severe problems like necrosis or decaying of the soft tissue or muscle where the injection occurred.

But beside humans, wild and domestic mammals can also be infected with anthrax. But again, how deadly a typical exposure is depends on the species. So generally herbivorous or ruminant species—like deer, cows, sheep—tend to be more susceptible to developing disease after being exposed to the bacteria, and they often die within a couple days after symptoms start. And usually for those animals, symptoms appear rather suddenly often in otherwise apparently healthy animals. Symptoms appear very fast and often include staggering gait, labored breathing, and depressive behavior.

[Sarah Gregory] Okay. So in your article, you talk about something called sporulation. This is spooky stuff. Would you elaborate on that and tell us what happens once the host mammal dies? This is the kind of stuff sci-fi movies to me.

[Rachel Maison] Yeah, it is very spooky. So when an animal dies from anthrax, and let's say that that carcass is opened after they die, either by some scavenger or a break in the skin, that kind of exposes those internal parts of the animal to the atmosphere. Those vegetative or active *Bacillus anthracis* bacteria are programmed to recognize that their host has died once that carcass has been opened and it's now time to go dormant simply by exposure to the oxygen present in the atmosphere outside of their host.

Once bacteria recognize essentially that they're no longer safe within their host and exposed to this atmospheric oxygen, they initiate the sporulation process where they basically build several protective layers of proteins that are highly resistant to environmental degradation, and also kind of impermeable to anything getting inside of them.

So during this process, in addition to kind of forming those protective layers, bacteria will also compact their genetic material into these dense packages and just degrade all other materials of that vegetative cell that take up metabolic resources and energy since they're no longer needed since they're not in their host anymore. In the final product, you only have those genetic materials and basically the bare minimum that are left to ensure that when a bacteria finds a suitable host again, that they have everything they need to survive when they "wake-up" again, so to speak.

[Sarah Gregory] So people breathe it in? I mean, how does it sporulate into a person?

[Rachel Maison] Yeah, that's a good question. So that spore form of the bacteria is essentially like a dormant form just so that it can survive in the environment between their hosts. And so once a suitable host comes along and either, like we were talking about, brings in that bacteria through their skin or inhales it or eats it, they'll eat or kind of absorb those spore forms. And only then when the bacteria are inside of a suitable host again, do they kind of recognize that okay, conditions are now right for me to grow again, and so they'll kind of wake up from that spore form and become vegetative once again.

[Sarah Gregory] I've done a couple of podcasts this last year on pathogens in corpses and in museum specimens (not necessarily anthrax). But can an old body, human or animal, if disturbed release these anthrax spores and cause disease in the accidental victim?

[Rachel Maison] Yes, but only up to a point. So there's a couple parts to this answer. Yes, an old body (human or animal) if disturbed could potentially infect someone else. But since exposure to atmospheric oxygen (like we were talking about) will kind of initiate that process of sporulation, if a carcass is (or a body) unopened, then the vegetative cells will actually die within

the body as it decomposes because those vegetative cells are not good at competing with the putrefactive or other organisms that kind of take over during the composition process.

So generally, if a body or an animal carcass is left unopened for three days or longer at temperatures 80 degrees Fahrenheit or higher, viable *Bacillus anthracis* will likely no longer be recovered from that body. Now, I will say that keeping a body or an animal carcass unopened or impermeable to environmental degradation or manipulation by scavengers is usually not only feasible or what ends up happening. In some cases, some sporulation may occur due to those vegetative cells being exposed to atmospheric oxygen.

And so in these cases, you may actually be able to recover viable anthrax bacteria in the spore form from old specimens. And actually, human exposures have been reported in the past in people handling the bones, meat, hair, and hides of past infected animals that could have happened years and years prior.

Interestingly, and as kind of a side note, actually historically inhalational anthrax has been referred to often as wool sorters disease, because it was a relatively common occupational hazard for people who sorted wool and kind of dealt with animal hides, which sometimes may have come from anthrax-infected animals.

[Sarah Gregory] Interesting.

What regions of the US is it mostly found in, that we know of so far?

[Rachel Maison] Yeah. So what we know of *Bacillus anthracis* distribution in the US unfortunately is really largely just based off where outbreaks have occurred. And outbreak frequency kind of differs widely across the country, with some of them happening as far as 40 or so years apart in time. And so, regular occurrences of anthrax have been documented in Western Texas, parts of North and South Dakota, and Minnesota. But other states that don't have regular occurrences of anthrax but have reported outbreaks in the past include Colorado, California, Nebraska, and some southern states like Arkansas and Louisiana. And so it's kind of mostly just assumed that it's a possibility to be isolated anywhere. But other than those places that have had documented outbreaks in the past, we don't know where it's located in the US, largely.

[Sarah Gregory] Alright. Your article is specifically about feral swine and anthrax (and invasive feral swine). What are invasive feral swine?

[Rachel Maison] They are essentially pigs that look like and are crosses between wild boars and domestic pig breeds. Feral swine are considered invasive because pigs as a species are a non-native species in the US and cause significant problems for people, domestic animals, and wildlife in the places that they occupy. And this is because mostly they can eat or displace native wildlife species from their habitats, destroy native ecosystems, as well as human property with some of their behaviors.

So just for a historical perspective for how they got here, European domestic swine were initially introduced in the Southeastern US by Spanish settlers in the 1500s to mostly provide an easy and familiar food source and source of protein for the people that were colonizing North America at the time. But later though in the 1900s, Eurasian wild boar and Russian wild boar were also brought over and released to parts of the US (also in the southeastern region of the states), specifically for the purposes of sport hunting. Since then, all of these pig breeds, which actually fall under the same species classification of *Sus scrofa*, have interbred with each other as well as other domestic pig breeds to produce what we call today as "feral pigs" in the US.

[Sarah Gregory] And what's their range now?

[Rachel Maison] Currently, feral swine have been documented in at least 35 states and can be found mostly in the Southeastern US—states like Texas, Louisiana, Alabama, Arkansas, Florida—those are some of the big feral swine states. But they have also been documented in western states like California, Nevada, Arizona, and Utah. And actually a few years back, a small population was eradicated from western Colorado after crossing the border between Colorado and Arizona. So invasive species management and eradication is a constantly moving target for these guys. Off the mainland US though, feral swine are also present in Hawaii and the US territories of Guam and Puerto Rico.

[Sarah Gregory] Because pigs can basically live off of almost anything, so I would imagine that they can sort of just keep going, right?

[Rachel Maison] Yes. So another part of what makes them such a good invasive species is that they're very opportunistic. They are omnivores, meaning that they eat both animal and plant material, and so in that way they're very adaptable to kind of any climate that they find themselves in. Additionally, they are capable of reproducing anytime of the year. They can have anywhere between five and ten piglets in a litter, and so in that way their populations can just explode.

[Sarah Gregory] Wow. Too bad they don't eat cockroaches.

[Rachel Maison] Yup.

[Sarah Gregory] Are they biologically different once they become mixed up like that than regular farm pigs?

[Rachel Maison] Most of the differences between what we call feral pigs and domestic pigs (like those you would see on a farm) are mostly in appearance and behavior. But physiologically speaking, they are the same. Like I said, they are actually classified as the same species (*Sus scrofa*) and are still capable of interbreeding with each other. So any feral pig out on the landscape could presumably breed with a domestic farm pig. And actually, the working definition of the term “feral swine” in the US (used by the United States Department of Agriculture) include pigs of Russian or Eurasian wild boar descent, or escaped domestic pigs, or hybrids of the two. And so, in this way you might think of the differences between farm pigs and feral swine as the same as if you were talking about domestic versus feral cats, for example, where they mostly just kind of differ between their behavior and some of their appearance.

So in appearance, feral pigs have typically very course hair and thick skin. Some may also have noticeable or long tusks. Their piglets are often striped or spotted (kind of like deer offspring), but they grow out of this coat pattern as they get older. And behaviorally speaking, feral pigs are often much more aggressive than your typical farm pig, and they can actually run up to 30 miles per hour. So they're very fast. Other than that, though, just like farm pigs they really like rolling around in the dirt and mud, just the same as a typical farm pig.

[Sarah Gregory] Aw. Can people eat them?

[Rachel Maison] Yes. There are actually parts of the United States where hunting feral pigs is kind of a big thing, and people will do that either for sport hunting or for sustenance. So people are known to eat them.

[Sarah Gregory] Okay, let's go back to anthrax for a minute. How is it managed and contained considering it could be found in so many different ways and places?

[Rachel Maison] That's a good question. So typically, in areas known to be endemic for anthrax or in areas where outbreaks have occurred in the past, preventative management for anthrax mostly consists of people with livestock vaccinating their herds at least once a year with the livestock vaccine, basically just to prevent any animals from succumbing to anthrax if they are ever exposed. And that vaccine is pretty efficacious. It's been known for people to vaccinate their herds, that if exposed, will likely survive from succumbing to disease.

Otherwise, management is unfortunately pretty reactionary, in that you just kind of watch clinically for any animals behaving weirdly or, unfortunately, any suspicious carcasses on the landscape. So if a carcass is found and suspected to be infected with anthrax, it is usually incinerated because it's the most effective way to kill all those vegetative and sometimes sporulated cells. But in cases where incineration is not a practical option though, burying the carcass at least six feet in the ground is also considered acceptable. But I will say that it’s not uncommon for those sporulated cells to survive this process, and spores could potentially resurface if the soil is disturbed later on. And sometimes if an outbreak of anthrax is observed in a herd of animals that have not been vaccinated, vaccination can also be used as kind of a reactionary way to prevent any other animals from succumbing to disease, should they be exposed during an outbreak.

[Sarah Gregory] Is there a vaccination for people? I've never heard of one.

[Rachel Maison] Yes. So actually there is a vaccination for people. The vaccine that's licensed for use in the US is called Anthrax Vaccine Adsorbed or AVA for short, but it's actually only administered to people who could potentially be exposed to the bacteria occupationally. So it's not given to the general public because anthrax infection is typically rare in humans. So it's only really given to either military personnel or laboratory workers who might actually be working with the bacteria or samples that could contain it.

People who do receive the vaccine to prevent this occupational exposure get a series of five shots over the course of 18 months, and then receive annual boosters thereafter.

[Sarah Gregory] Goodness, five shots.

[Rachel Maison] Yup.

[Sarah Gregory] Okay, back to these feral swine. Apparently they do not succumb to anthrax, and why is this?

[Rachel Maison] This is actually somewhat unclear to us still, but likely has to do with differences in their physiology, their behavior, probably also the doses that they're normally exposed to on the landscape through their normal activities, as well as the typical exposure route—that being either, like we were talking about, gastrointestinal, inhalational, or cutaneous.

So I think I've mentioned before that typically those herbivorous or ruminant species appear to be most susceptible to developing fatal forms of the disease—and these are species like cows, goats, deer—whose digestive system is kind of characterized by the four-compartment stomach and fermentation by those gut microbes, in contrast to species like pigs or carnivores or humans where we only have a one-compartment stomach that's pretty acidic. So it’s been speculated by some that the pH level of the stomach and digestive tract of these animals is—at least for

herbivores—just not low enough to kill *Bacillus anthracis* spores or vegetative cells, and so these animals are more likely to get sick from an exposure.

In contrast, true carnivores, scavengers, or omnivores (like pigs and humans) that kind of regularly consume animal materials that require a very acidic stomach could serve as a means to kind of protect them from at least gastrointestinal anthrax, should they be exposed that way.

[Sarah Gregory] And how does this anthrax-resistant wildlife contribute to the anthrax epidemiology?

[Rachel Maison] Depending on the species, anthrax-resistant wildlife can contribute by either helping to perpetuate the infectious forms of anthrax (so that dormant spore form that we've been talking about) by manipulating or opening infected carcasses while they are feeding on them. So that, again, will kind of expose those vegetative cells to atmospheric oxygen and initiate that sporulation process and kind of perpetuate the infectious form of the bacteria on a landscape. Or they could even carry spores to other places, either on their feathers, fur, or there have been documents of some scavengers kind of carrying spores through their gastrointestinal tract and depositing them elsewhere after feeding on infected animals. Less actively, though, a species could contribute to epidemiology simply just by being one of the incidental hosts being exposed to these bacteria. And so for those resistant species, this could mean just potential exposure and developing some sort of immune response to the pathogen.

[Sarah Gregory] Explain how anthrax status in these swine, coupled with location data, would help map bacterial presence?

[Rachel Maison] Sure. So since feral swine are one of the more resistant species to developing anthrax after being exposed to the bacteria that cause it, we believe that looking at the antibody status of these pigs would kind of indirectly indicate that an animal has at some point during their activities have been exposed to the bacteria. Because looking at antibody levels would indicate that they at some point have developed an immune response against that bacteria, suggesting that they've been exposed.

So taking this alongside the location of where an animal was sampled or the home range of that individual, you could then presumably begin to conclude that a particular area is likely harboring anthrax-causing bacteria. And interestingly, since an individual pig is only known to have a home range between one and five km^2, you could kind of use these home range estimates to kind of map potential regions where the bacteria might be present.

[Sarah Gregory] Where did you conduct your study and why did you choose there?

[Rachel Maison] We conducted our study in Texas because anthrax is a disease that is very consistently reported in select regions of the state, but not others. So in fact, there's a particular region on the western side of the states that's known to locals actually as the Anthrax Triangle, where anthrax is very often reported in domestic livestock and farmed deer populations. And in contrast, areas outside of this region very rarely (or sometimes never) have experienced outbreaks of anthrax.

So we have this very interesting situation where you have outbreaks in one region of a state and not another. And we also chose Texas because concurrently, feral swine are actually present in pretty much all but one county of the state. So that allows us to kind of compare anthrax exposures in animals both inside and outside of this Triangle region where you have high incidence of anthrax.

[Sarah Gregory] Okay. So sort of along the same lines, you said in your article that these feral swine might serve as biosentinels in Texas. Explain to us what that means, exactly.

[Rachel Maison] Yeah. So, again, pigs are one of those species that are known to be resistant to developing anthrax after being exposed to bacteria that cause it. Pigs and feral swine though are known for their propensity to root and wallow in soil. So we were talking about how farm pigs and feral pigs earlier like to roll around in the dirt. And so, in that way just kind of knowing their behavior and knowing that *Bacillus anthracis* is a bacteria that resides in soil typically, it seems that feral pigs are probably a species that are very likely to be exposed to this pathogen on a regular basis just through how they're interacting with their environment. And so in contaminated regions, they're probably one of the most likely species that come across the bacteria compared to other wildlife or domestic animals that aren't manipulating that soil as much.

Additionally, feral pigs are a very opportunistic species that eat almost anything, and they have been known to scavenge on the carcasses of other animals. So taking these things together, we are suggesting that looking at whether or not a pig has been exposed to anthrax by examining their blood for anti-anthrax antibodies. We might use this as an early warning sign for anthrax bacteria in an area, and therefore indirect indicators of bacterial presence. And so this would be more ideal than taking soil samples across a large region or chunk of land and processing them to see if it contains *Bacillus anthracis* bacteria.

[Sarah Gregory] So you analyzed serum samples from these swine. Where did you get them?

[Rachel Maison] We got all of our samples from the feral swine serum archives collected and managed by the United States Department of Agriculture at the National Wildlife Research Center office in Fort Collins, Colorado. So in addition to collecting and using serum from feral swine removed by the USDA for routine disease surveillance, the program actually keeps a subset of those samples and archives them for future use or retrospective analysis as needs arise. And so actually anyone who's interested in utilizing these samples present in the archives and collaborating with USDA can inquire to do this. And this is especially a great resource for retrospective studies, since these archives go as far back as 2007.

As far as where these samples originated physically, they came, again, from feral pigs that were residing in Texas. These animals were removed off the landscape by USDA for invasive species management, or most often damage management, as the agency works a lot with private landowners and ranchers or agricultural folks whose crops might be experiencing damage from feral swine.

And so as I mentioned before, since Texas has such well-documented and predictable occurrences of anthrax in its western region (in that Anthrax Triangle), we selected the seven counties present in that triangle region that are likely contaminated with anthrax and kind of called those counties “endemic” for the disease, and then selected another seven counties that weren't in that region and called them “non-endemic” since they don’t experience those regular, predictable occurrences of anthrax like those documented in the Triangle.

[Sarah Gregory] So you used serum from this bank. You weren't out in the field in Texas taking blood samples from swine, right?

[Rachel Maison] Yeah. We got these from the bank. We actually went as far back as 2007 within the archives and selected samples retrospectively from each—what we were calling the endemic region of Texas and the non-endemic region.

[Sarah Gregory] And were there only certain samples that you looked at, say based on age or gender, or something like that?

[Rachel Maison] Yeah. So obviously for any study, you want to try to get a representative sample that might allow you to infer most accurately about your target population, which for us was the feral swine population of Texas. And so this often means that in addition to a proper sample size, you have adequate or equal representation of kind of the subgroups within your population of interest. And so for us, this meant that we wanted a good mix of samples that came from female and male pigs, as well as younger and older pigs.

Importantly for the age-distribution analysis, since our research question and hypothesis for this study was based on whether measuring pig exposure to anthrax might be indicative of actual anthrax bacteria being present in the soils that those pigs are utilizing, we wanted to eliminate the possibility of getting false positive results from the presence of maternal antibodies in really young animals. So to take a step back, in mammals (including humans and pigs), before a child or an offspring is born, antibodies from the mother are transferred to offspring across the placenta and kind of serves as a mechanism of protection for that offspring until their immune system has developed enough and can more or less fight for itself. These antibodies that are passed include those against things that the mother was exposed to and can be present in an offspring's blood up to six months after being born in humans, or one to two months for pigs.

To kind of avoid seeing positive antibody responses due to the passing on of this maternal antibody and instead be more indicative of a true environmental exposure to bacteria we were looking at, we decided to exclude any serum samples that were collected from juvenile pigs, which the USDA conveniently classifies as less than two months of age when collecting their samples in the field.

[Sarah Gregory] So this is all about serologic surveillance. Tell us how that works in relation to anthrax management.

[Rachel Maison] Serologic surveillance is essentially surveying or looking out for pathogens or exposures to pathogens by examining the serum component of the blood taken from individuals. So in the case of anthrax where not all species are affected by the bacteria the same, measuring the immune response in individuals that are more resistant to developing and succumbing to disease, but that are most likely exposed to those bacteria might help to indirectly identify areas that might be of high risk either to more susceptible species or to humans that could potentially be exposed.

Right now, part of the difficulty in identifying anthrax risk zones is that unless you actively sample the soil or know of an outbreak that occurred in an area in the past, you likely won't know that anthrax bacteria are present until it’s too late and you see dead or dying animals. So separately in areas where there are no livestock being cared for and observed regularly or in more remote areas, you might also never come across a suspicious animal carcass. And because wild animal populations are pretty difficult to observe, you might never come across anything suspicious on the landscape that would indicate to you that *Bacillus anthracis* might be present.

And so actually past studies done in the Serengeti, where anthrax is considered an endemic disease, have found that the presence of anthrax antibodies in the serum of those species that are more resistant to anthrax—like dogs, lions, and vultures—have been found to be closely associated with areas contaminated with anthrax bacteria, as well as associated with anthrax-related livestock deaths. So this indicates that looking at the serology in these resistant species might be a good predictive tool to help identify outbreak areas before they happen. And in doing so, you can then warn people with livestock so they might decide to vaccinate their animals or to be suspicious of any animal deaths and carcasses found so that this disease could be better managed and not so reactionary.

[Sarah Gregory] Okay. So going back to your study, what did you find?

[Rachel Maison] We ended up getting 478 serum samples across Texas—half of those coming, of course, from that Anthrax Triangle region, half from outside. And in examining all of those samples, we actually found relatively high seropositivity in all of the animals sampled, meaning we found a high proportion of antibody-positive animals (about 44%), indicating exposure occurring in feral swine throughout Texas to *Bacillus anthracis* or anthrax-causing bacteria.

We found higher prevalence of antibody positive individuals in the endemic region (so that Anthrax Triangle region) versus the non-endemic region. But we did find about 37% of antibody positives coming from that non-endemic region, indicating perhaps that animals are being exposed to these bacteria outside of that high outbreak zone, if you will. And so interestingly, looking at the subgroups of animals that we had in our sample set, we also found seroprevalence to be higher in adult pigs than in sub-adult pigs by about 10%. However, we did end up having a great deal more adult swine represented in our study sample pool, so this trend will likely need further study to confirm since that sampling distribution was not equal.

So we also found seroprevalence to be higher in female swine than in males, also approximately by 10%, which was kind of interesting and something that we didn't necessarily anticipate. That will likely need to be teased out a little more. Additionally, our statistical models suggested that all of the variables that we were interested in—so region (endemic versus non-endemic), age-class, and sex and the GPS location of the sample that was collected) were all informative for predicting whether or not a pig was antibody positive or not. However, when we examined the statistics further individually for each of those variables, only latitude was statistically significant, indicating that using continuous location data as opposed to just binarily defining whole counties as either endemic or non-endemic might be useful for documenting this exposure in pigs.

[Sarah Gregory] Are there any further studies or next steps that you'd like to see?

[Rachel Maison] The next steps of the study in my mind are more controlled laboratory-based studies where the immune response of animals with known exposure to anthrax bacteria are measured over time and compared to the serological response that we've seen in samples that were collected in the field. Because one of the limitations of this study that we did is that we're just kind of passively observing exposure of animals in the field. And so, having a more controlled environment where we know how much bacteria those animals are being exposed to and exactly when they're being exposed to it will kind of help us to tease out that immune response and kind of get more biological information from those animals, not just the binary positive-negative.

We're actually just wrapping-up such a study in Colorado State University's BSL-3 facilities, where we actually exposed naïve, seronegative pigs to various levels of a *Bacillus anthracis* strain and measured their antibody responses over time. So hopefully there's more to follow on this in the future.

In addition to serology, however, I think it would also be good to know if these feral pigs might be playing a more active role in anthrax epidemiology, particularly if they have a role in spreading that infectious spore form to other areas or if they play a significant role in perhaps exposing spores that might otherwise be buried in soil so that they're more accessible to come into contact with other more susceptible species or even humans. Other studies, for example, have been able to recover viable spores from the feathers of scavengers (like vultures) after they've predated on infected carcasses. I'm also kind of interested in if spores could be isolated from fur of pigs that have been wallowing in contaminated soils, or potentially carried in their nasal passages from rooting or sniffing around in contaminated dirt.

[Sarah Gregory] That would be really interesting. Well, all of it would be interesting, but yes, that would be something completely different.

So Rachel, tell us about yourself, your graduate work, and how you became involved in this study.

[Rachel Maison] Yeah. So I am currently a graduate student at Colorado State University in Fort Collins, Colorado. I'm looking to get my PhD in Biomedical Sciences here, and my project currently is looking at studying the pathogens that might be carried by feral swine in the United States, or pathogens whose epidemiology may potentially be influenced by the presence of invasive feral swine. I have a Bachelor of Science (also from Colorado State University) in Fish, Wildlife, and Conservation Biology. So I've kind of always had this interest in invasive species and how they might contribute to the health and wellness of native wildlife populations.

After I graduated with my bachelor's back in 2016, I actually got a job with the USDA, actually at the National Wildlife Research Center where we got our samples for this study, working as a laboratory technician with the National Wildlife Disease Program and the National Feral Swine Program, helping them with national disease surveillance in wildlife and feral swine populations and actually kind of helping to manage that feral swine archive, interestingly enough. And so while working with the USDA, I was introduced to my advisors now, Dr. Richard Bowen and Dr. Angela Bosco-Lauth at CSU, and kind of their work with infectious diseases and wildlife. And just in conversation with them it was just kind of serendipitous that they were receptive to having a graduate student, and I was becoming more and more interested in wildlife disease as a field of study and kind of the overlap between how invasive species might contribute to the epidemiology of some diseases, and how that might affect either native wildlife populations or human health.

And so, I applied and was brought on as a graduate student and after doing so, continued to work with the USDA and identified anthrax as a potential pathogen of interest to study in relation to feral swine populations. It appeared in the literature that there were no studies that have been done in the US, although other results in the Ukraine done on anthrax exposure in wild boar kind of held promise for that species as being a potential biosentinel and just through looking at serology. Additionally, despite evidence of exposure, no one has also looked into whether feral swine might play a more active role in anthrax epidemiology, such as (like I was saying before) perhaps helping to disseminate those spores on the landscape, or if they play a role in kind of

resurrecting those spores that are buried in soil profiles. And so, that's currently what we're working on right now and what my dissertation is kind of centered around.

[Sarah Gregory] Well, I wish you the best of luck in pursuit of that. That's a very interesting and obviously needed topic.

And thank you for taking the time to talk with me today.

[Rachel Maison] Yeah. Thank you so much for having me and giving us the opportunity to talk about our research and what we're doing with feral pigs. I really appreciate it.

[Sarah Gregory] And thanks for joining me out there. You can read the December 2021 article, Potential Use for Serosurveillance of Feral Swine to Map Risk for Anthrax Exposure, Texas, USA, online at cdc.gov/eid.

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

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