

# A Koala Bite Wound and *Lonepinella* Infection

*[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 to Dr. Holly Sinclair, in Australia. She works in the Department of Microbiology at Pathology Queensland, Royal Brisbane and Women's Hospital complex. We'll be discussing a new type of infection spread through the bite of koalas. Welcome, Dr. Sinclair.

[Holly Sinclair] Thank you, Sarah. It's great to be here.

[Sarah Gregory] So, koalas are really bears, right? They're not the cute, cuddly possible pets so many people often mistake them for, yes?

[Holly Sinclair] Yeah, well firstly, koalas are not bears—we often hear them being called “koala bears” but they are actually marsupials. They carry their young in a pouch. Koalas' genus name is *Phascolarctos*, which actually is derived from the Greek, meaning for “pouch” and “bear,” but they are not actually related to any bears. Their closest relative is actually the wombat. Yes, koalas are cute and cuddly, their fur is thick and soft, and they have gorgeous fluffy white ears. But behind this cute and cuddly façade is sharp teeth and very sharp long claws for climbing and holding on to branches. So, if ever anyone has held a koala, they will notice that really strong grip. So, yes, I guess maybe not so cute and cuddly after all.

[Sarah Gregory] Your article discusses three bite wounds in three different people. Do koalas often bite or is this a rare occurrence?

[Holly Sinclair] Yeah, so our article reports on three separate cases of koala bite wound infection. Each patient was a wildlife worker and they sustained bite injuries to their thumb, hand, or wrist. The first case was actually bottle-feeding a new koala through their sanctuary. So, koala bites are a rare occurrence, other than, really, wildlife workers or those that handle koalas. Koalas will only bite if they felt threatened or scared. So, and this is usually from, with biting and scratching. Koalas keep to themselves, usually, in the bushland, so no, you shouldn't feel threatened. The koalas feed exclusively on eucalyptus leaves and they have a low metabolic rate. So, if they are not eating, they're sleeping. They actually sleep most of the day.

[Sarah Gregory] Tell us a little bit about the people who were bitten. You mentioned they were all animal workers. But geographically, where did these bites take place?

[Holly Sinclair] Yeah, so geographically, all the cases were in Queensland, Australia. And that...the koalas' habitat in Australia is mainly around the eastern coastal areas of New South Wales, Queensland, Victoria, and a small area in South Australia. In Queensland, the greatest concentration of koalas is in southeast Queensland, which is around Brisbane. Each case, yeah, as I mentioned, presented with bites to the hand or wrist after handling a koala as a wildlife worker. And each presented with a significant skin or soft tissue infection and each required combination of intravenous antibiotics and surgical debridement for control of the infection.

3:30 [Sarah Gregory] Okay, so you just said that the infection was significant and spread by the koala bite. So, after being bitten, what symptoms did the people have and how serious are these bites?

[Holly Sinclair] Yeah, so as with any bite from an animal, or even a human, there's bacteria within that oral cavity or saliva of the biter can be transferred to the skin. And with a break in that skin defense barrier, that can then lead to infection. The bacteria that causes the infection will depend on what organisms are present within the animal's mouth. And this is really the fascinating part of these cases. So, initially, the koala bite would start off with a puncture site, and then develop erythema, or redness, and a purulent discharge within days, if not treated. And some of these patients did not improve with oral antibiotics and did require surgical drainage for the infection. Yes, each patient was seen in hospital for intravenous antibiotics and surgical management. Yep.

[Sarah Gregory] Are they okay now?

[Holly Sinclair] Yeah, so each of them did require an oral, as well as intravenous, antibiotics. The third case actually was found to have pus, at the operation, within the thenar muscle compartment, which is a muscle compartment in the hand, as well as the metacarpophalangeal joint, which is a thumb joint, during the surgery. Yes, each patient recovered, and they may or may not be wildlife workers now.

[Sarah Gregory] [laughing] Oh, no! Okay, you did a good bit of testing to find the exact types of genomes to isolate this infection. Without getting too technical here, can you explain this rather complicated process?

[Holly Sinclair] Yeah, well, the first step is to culture bacteria from the samples, so we cultured bacteria from either tissue or swabs from the human wound. The bacteria grew best on chocolate agar at 37 degrees in a microaerophilic condition. There was a small gram-negative bacilla and we were initially having trouble identifying this bacteria in the laboratory. And this was mainly because it wasn't present within the usual databases commonly used for human pathogens. And this is a veterinary pathogen, or a pathogen made by animal bites, so it wasn't within the system. So, in each case, we weren't able to identify it with the routine phenotypic methods or identification systems.

So, another method of identifying bacteria is by genotypic methods. And this one method is by sequencing specific genes. Our laboratory usually uses the 16S ribosomal RNA gene for bacterial identification. However, even this method was unable to assign a species with accuracy, so, therefore, we sequenced three other housekeeping genes, *infB*, *rpoB*, and *recN*, for more accurate identification. And we discovered novel species within the genus. So, I guess, getting back to basics, so bacteria is made up of genetic material in the form of DNA; genes are made up of strands of nucleotide bases, which form amino acids and then proteins. So, different genes make up the genome of an organism. Each gene has a double-stranded DNA and each strand is made up of nucleotides.

So, we can sequence specific genes with the primers that start the chain sequence along. DNA sequencing determines the exact order of nucleotides along chromosomes or genomes. So, a nucleotide sequence can then be matched with a known nucleotide sequence in databases. So, the steps involved would first be to extract DNA from bacteria, perform DNA amplification, and then sequencing the PCR product. We then have a sequence or...of a complete or partial gene for a specific isolate. And with that sequence, we can obtain information about the relatedness of an unknown isolate to a reference strain. We look at how similar the sequences are, depending on how many nucleotides match...nucleotides match and give a percent similarity. For example, 100

percent means that all the nucleotide bases match perfectly. So, we determined the similarity of the four koala bite isolates with each other, as well to a reference strain.

[Sarah Gregory] So, what organisms were ultimately found?

[Holly Sinclair] Yeah, so with the gene sequencing, we identified four isolates as belonging to *Lonepinella* species. So, *Lonepinella* belongs to the Pasteurellaceae family, which is a group of bacteria that usually resides within animals' gastrointestinal tracts. There is currently only one species to this genus, *Lonepinella koalarum*. And also, anyone has visited Brisbane and wanted to see koalas or even hold a koala, they have perhaps visited the Lone Pine Koala Sanctuary. And this is where the bacteria was first discovered, in the feces of koalas in the Lone Pine Sanctuary, hence, the name, *Lonepinella koalarum*.

So, the first time it was described was in 1992 and it was assigned the genus *Lonepinella* in 1995 by Osawa and colleagues, who discovered a gram-negative bacteria that could degrade tannin-protein complexes. It had an enzyme tannase. So, tannase has the ability to break down the tough tannin-protein complexes that is present in eucalyptus leaves. *Lonepinella* has been found in the koala digestive system and is thought to make up over 60 percent of fecal microflora colonizing both the cecum and proximal colon. So, therefore, it probably plays an important part in the digestion of the diet of koalas. We now know that *Lonepinella* is present in all four of koalas and can be transmitted to humans through a bite or a break in that skin barrier. We now know that *Lonepinella* can cause human infection after a koala bite. So, we know that *Lonepinella*, based on this study, does behave similarly to other Pasteurellaceae members after animal bites. So, certain bacteria can give us a clue to the etiology of the, the animal. So, for example, *Pasteurella canis* from dog bites and *Pasteurella multocida* from cat wounds, *Lonepinella* does seem to behave similarly, producing virulent skin infections. And our cases required combined surgical debridement and antibiotics for source control. And we found that the basic 16S ribosomal RNA gene sequencing, which is a common method of organism identification, was unable to identify the bacteria. We therefore sequenced a number of genes to better understand their relationship to each another and the reference strain, *Lonepinella koalarum*. These genes have been studied in other Pasteurellaceae and together can give a good indication of the genome. So, these genes are conserved within species and can be used for identification. So, our analysis, our phylogenetic analysis, revealed that one of the isolates was likely belonging to *Lonepinella koalarum*, but the other three were more likely novel species. So, this is being explored further with whole genome sequencing analysis, as we speak.

[Sarah Gregory] Okay, so you also tested the samples for antimicrobial resistance. And what did you find there?

[Holly Sinclair] Yeah, we did. We did antimicrobial susceptibility testing by Etest MIC method, which was used with interpretation break points to *Pasteurella multocida*. We found two isolates appeared to be resistant to penicillin. Otherwise, all isolates appeared to be susceptible to other antimicrobials, including third-generation cephalosporin, beta-lactam/beta-lactamase inhibitor combinations, and ciprofloxacin. Therefore, we do...we would encourage testing antimicrobial susceptibilities if one did culture this *Lonepinella koalarum*.

[Sarah Gregory] You mention in your article the nucleotide sequences were deposited in GenBank. Tell us what GenBank is and what's the reason to deposit anything in it.

[Holly Sinclair] Yeah. GenBank, it's free, an open-access genetic sequence database. It contains a collection of all publically available DNA sequences. So this really allows researchers and scientists to access the most up-to-date sequences. It is important to deposit sequences in GenBank, I guess to continue this wealth of knowledge. So, GenBank can be used to identify specific sequences, for example, that we use to identify bacteria. Our nucleotide sequences are now in GenBank and could help further micro...future microbiologists to sequence and identify *Lonepinella* in their lab, or from cultures from koala bite wounds, or even from koalas themselves.

[Sarah Gregory] Is the infection contagious? Once somebody has a wound, can it spread to other people?

[Holly Sinclair] No, this is...it's a zoonotic infection by its coming from animals to humans. It has been transmitted from...so, it's been transmitted from the animal bite. The bacteria was within the oral flora, and was transmitted during that bite. We shouldn't be concerned about it being transmitted between people.

[Sarah Gregory] Okay, so how does it affect the koalas?

[Holly Sinclair] Yeah, well, as I mentioned before, *Lonepinella* actually can be really important to koalas. It makes up a part of their gut microbiome. It possesses tannase, which is an enzyme that breaks down tannin protein complexes. Eucalyptus leaves are rich in tannins and difficult to digest. *Lonepinella*, therefore, helps with the digestion of the koalas' diet. There's an ongoing project with Queensland Alliance for Agriculture and Food Innovation, Centre for Animal Science in UQ, who are...which is swabbing koalas, so they've swabbed, so far, 23 koalas, and all of those koalas have been positive for *Lonepinella*-like organisms. So there's more to come.

[Sarah Gregory] How's this study useful to clinicians? Why is it significant enough to be published in EID? I know you mentioned that now you can put the sequencing in GenBank, but there must be more than that.

[Holly Sinclair] Yeah. Well, now we know that *Lonepinella* can cause human infection. If a human is bitten by a koala, then we know the infection will likely be associated with *Lonepinella*. The infection is similar to that seen in *Pasteurella* after dog- and cat-bite wounds. It can be a purulent wound infection requiring combination antibiotics and surgical drainage. Our antibiotic susceptibilities showed that some isolates had resistance to benzylpenicillin. We have discovered likely novel species within the *Lonepinella* group that can cause human infection, and there will likely be more to come.

It is also really important to know that this is a difficult organism to identify in routine clinical microbiology laboratories. It is fastidious, requiring enriched media, such as chocolate agar, and grows best in microaerophilic atmospheric conditions at 37 degrees Celsius. It will not identify in the current commercial systems and, and at the moment, the gene sequencing with matching to sequences in the GenBank database will provide the best chance of identification.

[Sarah Gregory] So, what's your relationship, Dr. Sinclair, to koalas? Tell us about your job, and what you're interested in, and of course, how it relates to isolating genotypes.

[Holly Sinclair] Yeah, so I guess my relationship hasn't been directly dealing with the koalas themselves. I am a medical doctor in clinical microbiology and infectious diseases. So, my

interest is bacteriology, phylogenetics, and human infection. I do enjoy going to the Lone Pine Koala Sanctuary and I have been very lucky to work with a fantastic group of researchers. There is ongoing work with whole genome sequencing analysis and identification of *Lonopinella* from koalas. So, with this study, soon we will have more information about *Lonopinella*, its importance to koalas, and its effect as a pathogen in humans, as well as its place in the Pasteurellaceae group. I've really enjoyed working on...on *Lonopinella*.

[Sarah Gregory] Thank you so much for taking the time to talk with me today, Dr. Sinclair.

Listeners can read the January 2019 article, Identification of *Lonopinella* species in koala bite wound infections, Queensland, Australia, online at [cdc.gov/eid](http://cdc.gov/eid).

I'm Sarah Gregory for Emerging Infectious Diseases.

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