

Erin Welsh

Hi, I'm Erin Welsh and this is This Podcast Will Kill You. Welcome everyone to the latest bonus episode in our miniseries of bonus content that we've been putting out over the past few months. If this is your first time tuning into one of these bonus episodes, I'll give a brief rundown of what I'm doing with them so you know what you're getting yourself into. In each of these bonus episodes I'm following up our last week's regular season episode by interviewing an expert about some aspect of the topic that we covered last week and getting to explore it in much more depth than we did in the regular episode. And also I'm asking these experts about their careers, what they like about them, how to get started, and any advice they may have for people who are interested. I've had so much fun putting these episodes together so far and I've learned an incredible amount about fascinating topics ranging from deadly rabbit viruses to how electricity actually works and beyond.

I am super pumped for this week's episode because it combines two things that I didn't expect I would ever get to talk about at the same time: koalas and sexually transmitted infections. In last week's episode Erin and I covered chlamydia, specifically the different ways that different strains of the bacterium *Chlamydia trachomatis* can cause disease in humans, diseases such as the classic chlamydia STI, the eye infection trachoma, and lymphogranuloma venereum. We discussed how these obligately intracellular pathogens complete their life cycle, how they cause the signs and symptoms they're associated with, the long history of their involvement as human pathogens, and where we stand today in terms of the global prevalence of these diseases. If you haven't listened to that episode yet, I'm going to recommend that you pause this, go listen to it, and then come back because that episode will give you some good background on these bacteria in general that will probably help in terms of providing more context for this interview today.

Okay so what are we going to be talking about today? Even though we covered quite a bit of ground in our regular season chlamydia episode, more ground than we expected to cover, in many ways we only scratched the surface of chlamydia because the world of these bacteria is much bigger than just what the human perspective shows. Chlamydiae are found in all kinds of animals from birds to free living amoebae, from sheep to salmon, from cats to koalas, and across all continents. They're everywhere. And while some species of chlamydia or chlamydia-like organisms don't seem to have a very strong impact on their hosts, others absolutely do. For instance *Chlamydia pecorum*, a species nearly ubiquitous and livestock around the world, has had devastating impacts on koala populations in Australia. And maybe you're familiar with this topic from headlines a few years back talking about One Direction and koalas and chlamydia.

In any case these population declines have generated a substantial amount of research into understanding how chlamydia is spreading among koalas and in creating tools that might be able to help us slow or stop transmission, with one of these tools being vaccines. From this research we have learned an incredible amount about *Chlamydia pecorum* and not just as it relates to koalas. While the koala-chlamydia relationship might be the one most likely to be splashed across headlines, *Chlamydia pecorum* can infect many animals and several other chlamydia species can carry great importance for other wildlife, for domestic livestock, or as zoonotic pathogens of public health importance. But we don't yet know quite as much about those host pathogen relationships. So it seems like what we need is a complementary approach, conducting more in depth studies on koalas, chlamydia, and vaccines, while also performing more exploratory research on how *Chlamydia pecorum* and other chlamydia species impact other wildlife and domestic livestock.

In this bonus episode I am beyond thrilled to talk with two scientists, Dr. Martina Jelocnik and Dr. Sam Phillips, both at the University of the Sunshine Coast in Australia whose research aims to do exactly that. These two super cool chlamydiologists have been examining questions of chlamydia in Australia from these different but complementary angles and I can't wait to hear what they have found. So let's get to it. We'll take a quick break here and then I'll let them introduce themselves.

TPWKY

(transition theme)

Martina Jelocnik

Hi, I'm Martina Jelocnik and I am a veterinary chlamydian, veterinary molecular microbiologist from University of the Sunshine Coast here in Queensland, Australia. And I work on veterinary chlamydia, livestock, birds, koalas, and all the other animals.

Sam Phillips

I'm Sam Phillips and I also work at the University of the Sunshine Coast at the Center of Bioinnovation. I worked with Martina for the last five years and I'm a molecular microbiologist working on the koala chlamydia vaccine as well as some other human chlamydia projects as well.

Erin Welsh

Wonderful! Thank you both so much for agreeing to chat with me today. I am very excited to talk so much more about chlamydia than I ever thought I ever would. So let's dive in. I was wondering if you could start off by telling me a bit about your educational journeys. Did you always know that you wanted to be a scientist or is that something you discovered later on?

Sam Phillips

So I always wanted to be a scientist ever since I was in high school in Australia. I had an interesting journey moving through a diploma in laboratory sciences to fine tune my laboratory techniques and then an undergraduate and honors degree. My honors degree was actually in looking for vaccine targets in chickens for a disease known as campylobacter. From there I actually worked in diagnostics, diagnostic pathology and human pathology for seven years and then moved over into research where I was a research assistant for five years working in human papillomavirus vaccine analysis within Australia as well as some chlamydia projects which got me interested in chlamydia and collaborating with my eventual PhD supervisor Peter Timms and came up to Sunshine Coast to work on the koala chlamydia vaccine for my PhD. And since then I've continued working with Peter on the vaccine and am now the lead postdoc research fellow running four different vaccine trials.

Martina Jelocnik

Well as you can see I have a little bit of accent. So I'm actually not originally from Australia, I came from Belgrade, Serbia. Since I was young I was influenced by my aunt who was a doctor. So I always wanted to be a medical doctor and I was just so fascinated when she was talking about disease and microbiology. So as such I did medical high school in Belgrade and I started medical uni but because we moved like a family to Australia, so then I had to postpone a little bit my educational journey. So I wasn't a citizen and the universities were then a little bit expensive. So I had to wait until I became an Aussie then take a loan and dived back into the study. So I did undergrad majoring in microbiology. So yep, I always stayed true to my micro, it was ride or die. And that continued with honors and continued with a PhD in microbiology. And in honors is where I first heard about this pesky chlamydia.

So everybody was talking koala chlamydia, koala chlamydia, but there was another host livestock. And I was thinking okay well nobody's talking about livestock, I'll do it, let's see what happens there. And with Sam, so we were actually within the same group, we had the same supervisors and I'm kind of like that child that never wants to leave home. So then I stayed with my PhD in chlamydia and I got my fellowship. I get on chlamydia but this time on a slightly different chlamydia. So we started looking at chlamydia in birds, some novel chlamydia that doesn't get much attention in Australia. And I'm still here and I think we will see what the future holds. But now we are also looking at chlamydia and other bugs because chlamydia is never there alone.

Erin Welsh

Interesting. Wow, what amazing journeys. So I have another question for you before we dive into chlamydia talk and that is advice. Do you have any advice for someone who might be interested in pursuing a career in STIs or wildlife disease or one health? Anything you wish someone had told you at the beginning of your career?

Sam Phillips

My journey to where I am now, I learned a lot along the way and I made some mistakes and then I moved back into it. And I think don't be afraid for people wanting to get into research in general but into STIs and vaccines. Just start off in something which it could open the door to something you've never thought of before. I started out in the HPV vaccine research which it's already developed and I thought it can't be that much research, we're already giving it to people. But there's so much more you can learn. So don't close your mind after thinking that if something's already well known, there's still lots more that we can learn. And STIs, they have commonalities between the different species and so there's different antibiotic treatment between say chlamydia and gonorrhoea and mycoplasma, it's all basically the same antibiotics and they share different mechanisms. So don't think you just pigeonholed down to the one organism, you can always move on.

Martina Jelocnik

So my advice to young scientists is collaborate, collaborate a lot because you need those connections, we need to connect with our vets, with our GPs, with our researchers, researchers who work on a slightly different aspect of the host that you may do. That is the only way that you could get the full picture. And then by collaborating, and I often say talking with way smarter people than me, I continuously learn a lot and then I pick up something that I didn't think of. So for me a big part of what I do, it's a whole plethora of collaboration from vets, from the producers because especially in livestock they are the ones who are feeling the effects of this disease on the farm. So we need to go from the producer to the vet to the diagnostic laboratory team to us in research to all of our colleagues around the world. So I would say collaborate early and collaborate with good people and with a good team. When the collaboration cannot be sustained, that's okay too.

Erin Welsh

Both excellent pieces of advice. All right, now let's get into some chlamydia talk specifically chlamydia talk, specifically *Chlamydia pecorum*. Who does this pathogen infect and how is it transmitted?

Martina Jelocnik

Chlamydia pecorum although globally it's probably known as the notorious koala pathogen actually is not. I often say it's a livestock pathogen more than anything. So in fact a wide range of livestock including cattle, sheep, goats, as well as wild ruminants such as let's say reindeer, water buffaloes, and recently we also worked on studies when we detect *Chlamydia pecorum* in birds. How is it transmitted? We think that it's most likely fecal-oral transmission but it can also be from direct contact which Sam can explain, like when you have two koala fighting they can kind of touch each other and maybe transmit or when the sheep or cattle, it's in the close contact, so they can transmit let's say ocular *Chlamydia pecorum* infections. But most likely it's fecal-oral.

Erin Welsh

And how is it different from *Chlamydia trachomatis*? I mean *Chlamydia trachomatis* is a human-specific pathogen, right. But are there any overall big picture differences between those two species?

Sam Phillips

The differences between *pecorum* and *trachomatis* are that *pecorum* can infect a variety of different hosts, *trachomatis* is strictly a human pathogen, we don't find it anywhere else. We can't even get it really to infect mice whereas *pecorum* will infect, as Martina mentioned, a range of different host species. As far as disease and infection routes, they're fairly similar. We know that *trachomatis* can infect ocular, gastrointestinal, urogenital quite readily. So that's fairly similar and the disease presentations are fairly similar. You do get the LGV strain, the lymphogranuloma venereum strains of chlamydia which are slightly different to what we see in animals although that could just be that we're missing a link there with animals. More research.

And the tissue tropism is really interesting. We've tried many different studies with pecorum to identify tropisms based on usually we look at a single gene, the outer membrane protein, the gene responsible for that is ompA which we don't find with Chlamydia pecorum or other species of chlamydia. But with trachomatis it's fairly stringent, we find that there's a specific ocular subtypes and specific urogenital types. Things start to get muddled when you start looking at the gastrointestinal infections but traditionally you can have the ocular strains, specifically they cannot infect the urogenital tract. That's due to some specific gene mutations that have occurred throughout evolution of trachomatis. So yeah, there are similarities but then there's also some really distinct differences between the two.

Erin Welsh

Where did Chlamydia pecorum come from? What are its natural hosts and how did it get into Australia?

Martina Jelocnik

Erin, that's a \$5 million question. I think we moved from \$1 million, I'm gonna go now to \$5 million question. So up to a while we all hypothesized oh yes, Chlamydia pecorum most likely came with the European colonization and with bringing the livestock because here and there using molecular studies we have these snippets of information which says you have koala strain, that they are genetically similar to livestock, thereby tantalizingly we say, 'Oh yeah, that's the origin.' I was a believer but then I converted. The wider the event and of course beyond gene typing schemes, we started looking at whole genome sequences, again that's stealing infancy for pecorum, and we are seeing very distinct lineages between koala strains and livestock strains. So then that opens up new questions. It's kind of like a Pandora's box. So you wonder are we not sampling the intermediate lineage? Are we missing a host? Maybe. We don't know.

Especially in Australia we are kind of host-centric. So it's either koala is a host or it's a livestock. But what about everything in between? So we know from our colleagues from Europe and the US that chlamydia can infect pigs, reindeer, chamois, ibex, a variety of birds. So honestly Erin, we do not know. It is very tantalizing to think that we do have or had some kind of a spillover but at the moment we really truly don't have solid information to answer that question. So we need to work harder, we need to sample wider, we need to sequence way more than we do now in order to answer such questions.

Erin Welsh

Amazing. So as we've talked about one of the species most impacted by Chlamydia pecorum is of course the adorable and charismatic koala. When did people first start noticing that koalas were becoming infected with this bacterium?

Sam Phillips

As early as European settlement in Australia there's reports of indications that the koalas were suffering from chlamydial diseases back then. This is all kind of based on observations of disease presentation back then, there wasn't a lot of diagnostic analysis specifically for wildlife back then. It gets a little bit confusing. I'm sure your listeners will know that the chlamydia nomenclature has changed over the years and it's not that long ago that we only discovered that there was more than just Chlamydia psittaci and Chlamydia trachomatis around. So pecorum has only been around for the last 30 years with identification. So yeah, it's difficult to say how long pecorum has been infecting koalas but possibly for at least the last 200 years.

Erin Welsh

What does an infection with Chlamydia pecorum look like in koalas and how fatal can it be?

Sam Phillips

I'll start at the top. So in ocular disease, koalas get infection in their conjunctiva and this creates inflammation which normally conjunctiva is quite smooth as it goes over the eyelids. This inflammation causes scouring of the eyelids, you get these like nodules and it eventually causes blindness in the koalas in their eyes. In the urogenital tract chlamydia can ascend the urethra and can't go into the bladder. This causes cystitis which is inflammation of the bladder wall and koalas can carry this for huge amounts of time. Obviously they don't have a local GP that they can go and get some antibiotics from so they suffer in silence with this disease. And it can end up, I've seen koala- with golf ball sized necrotic masses in the bladders from sloughing off tissue just from these chronic infections. It can ascend the ureter up to the kidneys, it causes lesions in the ureter and then can cause nephritis in the kidneys.

Then we also have reproductive disease as well. So there's been some recent reports showing that male koalas can have inflammation in the testes and that can affect fertility in males. And in females we definitely know that there is reproductive tract infections. There are links to the development of reproductive cysts which can in turn lead to infertility. How fatal is it? Koalas coming into wildlife hospitals, it changes over the years depending on breeding season or not but on average is about 50% of koalas die from this disease. It's a horrible disease for them. They come in with these severe ureter infections and you can hear them like crying from the urine, they become incontinent, in severe cases it stains their fur on their rump so much that they get these extra urogenital abscesses from their constant wet staining of their rump and they can't sit down. And then yeah, just becomes terrible.

Erin Welsh

That sounds really horrible. And how is chlamydia transmitted among koalas?

Sam Phillips

A lot of it is still sexual transmission but there's also the fecal-oral route. We believe that they do get infected when climbing trees if there's a koala above them that say urinates with chlamydia infection, that could be a spillover to ocular sites and things. We know that some joeys do get preliminary infections from infected mothers, not necessarily through birthing, there's quite a lot of antibacterial properties within the pouch because a koala's obviously a marsupial but then once they become a joey and they live on the back, they're crawling all over the mother's back and they can get infected that way. Yeah, there's other speculations but mostly it's through fecal-oral, sexual, and then mother to joey.

Erin Welsh

And if a koala recovers from an infection with chlamydia, can it become reinfected or is there any lasting immunity?

Sam Phillips

We have some evidence to show that infection doesn't give the koalas long lasting immunity. They can up to maybe a month or so. But we see depending on the wildlife hospital and population densities you can have up to 80% of the koalas that have become infected will eventually come back with new infections. It does differ with the different populations as well.

Erin Welsh

There are effective antibiotics that exist for chlamydia and *Chlamydia pecorum* but they aren't recommended necessarily for use with koalas. Can you talk about why that is?

Sam Phillips

The koala is a really interesting species. Their diet is based on eucalyptus leaves which are highly toxic. So the koalas have developed a unique evolutionary trait where they have cytochrome P450 gene which detoxifies drugs and chemicals. And koalas have huge repeats in its region, up to 16 repeats in its region which means they are really good at detoxifying chemicals and drugs and antibiotics. So a lot of antibiotics that would be useful for chlamydia, they need to be used in such high doses in koalas that it ends up being fatal for the koalas. So that limits the number of antibiotics we can use. The first line of defense for at least the last 10-15 years was chloramphenicol, that had shady efficacy in treatment. So it does work but only 60%-70% of cases. But that was the only antibiotics that have been trialed and what we could use.

So we're utilizing that but it also causes gastrointestinal dysbiosis, it doesn't work all the time. And when koalas have their gut flora stripped they can't digest their leaves and they end up starving to death. So antibiotics are a terrible choice anyway but they do help in some cases. Recently there was a trial that showed that we can start using doxycycline which as you can imagine is a lot wider distribution, it's used in humans so access is no problem at all. Chloramphenicol isn't used in humans anymore so nobody wants to make it anymore. So it's kind of difficult to get hold of as well. Antibiotics are great for their use in clearing but they have a lot of side effects as well so we like to think a vaccine is your best bet.

Erin Welsh

What have been some of the population level impacts of *Chlamydia pecorum* on koalas so far? And can you also discuss any of the downstream effects that koala population losses have had on other members of the ecosystem.

Sam Phillips

Population level impacts of koalas due to chlamydia, you can't just say that the population is being affected solely by chlamydia as animals are affected by deforestation, population encroaching onto their habitat. So as far as directly comparable to *Chlamydia pecorum*, it's a difficult question. But overall koalas, some populations have been completely, they're extinct. So we don't have those populations in Queensland that have become extinct, local populations. And the koala populations in Queensland and southeast Queensland have decreased, they are being listed as endangered just recently. So they've been decreasing ever since. They were listed as threatened in 2018 and now they've been further downgraded to endangered. We're seeing this in New South Wales as well, there's a strong decline. This has been impacted by recent fires as well in 2020. So that's almost sped the decline of these populations. When you get further down into southern Australia into Victoria and south Australia, the populations aren't endangered, the disease presentation is a lot less, there's other factors.

So there's a retrovirus, koala retrovirus that infects koalas we believe has an impact on the chlamydia disease. So we do see a shift of disease prevalence increasing in a southerly direction. The ecosystem, that was a really interesting question, I had to go to school myself a little bit on this. So koalas obviously they live in eucalyptus forests and not a lot of animals eat eucalyptus leaves. So koalas actually help to control the growth of these eucalyptus forests, they allow for light to be able to come through to the forest floor to increase the biodiversity on the forest floor which helps the forest floor organisms, so microorganisms, insects, even small mammals. They also help to control bushfires surprisingly, so as they keep the growth eucalyptus growth under control it's less likely to have a lot of dry leaves and stuff lying around. So there's less tinder for our bushfires. And their feces also helps with biodiversity, when it rains it increases the nutrients in the soils and increases all the organisms.

So we always write grants saying we need to save the koalas because they're huge tourism pull and that's the last species of the Phascolarctidae family and we should do it for human benefit but it actually has benefits to the ecosystem which really gets downplayed I think. I mean we've been working with koalas five years and I've never heard people talk about this before. So I thought it was a hairy question to start with but it's really interesting. I'm looking into it more, I'm actually gonna go back and read some more about. It was quite interesting.

Erin Welsh

So for koalas, is there any individual or population level variation in resistance or susceptibility to chlamydia infection?

Sam Phillips

So for chlamydia infection, it's similar to trachomatis that we see in humans, so probably we estimate about 80% of koalas that become infected. Don't actually develop the disease. We don't know why that is though. We do think that the koala retrovirus has an effect on immune responses to chlamydia which then allows for chronic infections and development of disease. The population level is difficult, the different populations have different interactions. So in mountainous areas there's some geographical barriers that stop the spread of chlamydia into populations that you would assume that interact. Whereas in areas near the coastal areas where there's not huge geographical barriers, the koalas can interact between populations. So they're quiet, they can be territorial in their population groups and don't have a lot of exchange between the two populations. So it's a complicated question. But we do know that once it gets in there and infects enough koalas, those populations decline and eventually they'll become extinct if we don't get in and do something, especially with the other pressures of increased bushfires, decreased forestation, flooding, wild dogs. We had a population that was almost completely wiped out by a single domestic dog.

Martina Jelocnik

And also Sam actually mentioned koala retrovirus which is a huge viral infection but let's not forget that there is a koala herpesvirus that the research is now starting to emerge to suggest that that virus contributes to decreased immunity or can now exacerbate chlamydial infection maybe. And plus there are other bacteria also in fact in koalas, that's why I would say chlamydia, it's never there alone exactly and you never know whether that's antagonistic, whether that's synergistic, mutualistic, we honestly don't know. There is actually so much that that we need to look in terms of the co-infections.

Erin Welsh

Yeah it's such a complicated story. And so what about Chlamydia pecorum, are there different strains across the landscape that are associated with disease severity or different host species?

Martina Jelocnik

Okay. So when we look at genetic diversity of chlamydia pecorum, so what we see, let's look at koala strains. So koala strains are diverse. They are very genetically diverse but closely related to each other. We have evidence that showed that koala strains infecting koalas in the northern parts of Australia like Queensland, New South Wales are genetically distinct than those infecting koalas in south Australia or Victoria. There is a bit of anecdotal evidence that there is less disease. However when you look at the genomes of either of these strains, all genomes contain the same virulence factors, they're highly conserved. So I would then say that any strain would have a pathogenic potential and any strain of this can cause any level of disease.

So now that we looked at koalas and we know that there are diverse, we look at livestock. So in livestock actually we see a bit of a more disease association. So we have this very interesting clonal lineage, so that's my flavor lineage of the month. It's called sequence type 23 it's associated with polyarthritis in sheep and cattle, sporadic bovine encephalomyelitis in cattle, as well as fetal loss in both sheep and cattle. So these strains are highly clonal, genetically identical, minor differences and we never see them in koalas. So that's a very good thing. So these guys, these sequence type 23, they are specifically contained to livestock at the moment as best as to our knowledge and as best as to our breadth of sampling. So we do see a bit of association with the disease severity in the host species but within koala we do see differences between strains infecting northern versus southern koalas. But honestly I really don't think that we can say that any koala strain is less or more severe. In a nutshell they all cause some level of of clinical disease.

Erin Welsh

Interesting. All right, we're going to take a quick break here and when we get back I want to shift our attention to some other chlamydia species of public health or veterinary interest.

TPWKY

(transition theme)

Erin Welsh

Welcome back everyone. So far we've mostly chatted about *Chlamydia pecorum* and in our regular season episode *Chlamydia trachomatis*. But these aren't the only two chlamydia species of public health, veterinary, or wildlife importance. What are some other chlamydia species that we should be paying closer attention to and why?

Martina Jelocnik

Besides *Chlamydia pecorum*, whether it's koalas or livestock, I would say that a species that is widely researched and going farther back than *Chlamydia pecorum*, it's your good old *Chlamydia psittaci*. So *Chlamydia psittaci*, it's an avian pathogen traditionally and it has zoonotic potential, quite easily can spill to humans and cause disease in humans, respiratory disease which can be mild, which can be very severe, very severe pneumonia. And also *Chlamydia psittaci*, I love that species. I'm blown away by *psittaci* and its potential. It actually infects an extremely wide range of hosts, birds, and there is more than 500 different species of birds: water birds, pigeons, psittacine birds, chickens, poultry, ducks, everything as well as livestock. You can easily find it in livestock. And we recently acquired a tiny piece of evidence that *Chlamydia psittaci* can also infect marsupials, so we did find it in kangaroos. And of course the zoonotic potential and human infections, they are being constantly reported throughout Europe, USA, as well as in Australia.

So in Australia psittacosis is a notifiable disease. So the human cases, they are reportable and notifiable. So that's why *Chlamydia psittaci*, it's definitely a species that we need to be very much aware about. And the hosts that are infected with *psittaci*, they mix with a koala, they mix with your livestock, they all mix with us humans. So when we go to the beach, all the lovely psittacine birds, they sit with us, they eat our crumbs, but there is always a danger of spillover. And very recently, well not that recently, in the past decade we had a very interesting what we call the Australian *psittaci* horse story. So we had *Chlamydia psittaci* causing outbreak in thoroughbred horses and causing fetal foal loss. So as such there was a huge economic impact to the stud owners but also we also had a novel zoonotic transmission where the veterinary practitioners or students unknowing that there is a chlamydia, they handled placental material and became infected with *Chlamydia psittaci* and developed pneumonia and a respiratory infection.

Erin Welsh

With several of these chlamydia species we've got wildlife, domestic livestock, and sometimes humans that can all be involved which brings to mind of course one health. Can you talk about why it is so important to consider these pathogens from a one health perspective?

Martina Jelocnik

Well for example I'll go back to the *Chlamydia psittaci* in horses example, because that example I think really threw a spinner in it, we were all like whoa, that's very unique. So to the best of my knowledge chlamydia in horses is not very common in Europe nor let's say in the USA. So we really have a unique one health story in Australia. So horses wildly interact with our wildlife birds, with our wildlife parrots. Parrots are infected with *psittaci*, we believe and we have evidence that spillover from bird to horse was the cause of equine infections. Then we have humans who both interact directly and indirectly with birds and with horses. So in research, we knew *Chlamydia psittaci*'s zoonotic potential, all the strains that are clonal belonging to a known virulent lineage.

But for example, the GPs, the general practitioners in human medicine, they may have not been fully aware how common is the potential for spillover. So that's why that one health collaboration is very important. And literally every chlamydia should be considered as a one health pathogen rather than it is solely contained to human like *trachomatis* and/or to koala. So it's very important that we broaden that collaboration, that we broaden our communication because especially in Australia, wildlife, domesticated animals and humans, they are very interactive. They are not isolated systems.

Erin Welsh

Next I was wondering if we could talk about things like land use change and climate change and how these things are affecting the prevalence and distribution of chlamydia species. Kind of a big question but what do we know so far?

Sam Phillips

I can comment on especially during droughts koalas, all of their fluids they get from eucalyptus leaves and during droughts the trees don't hold as much water and the koalas don't get as much fluid. So they have to come down out of their trees and they find water from water holes or from troughs that farmers have used which is encroaching on eucalyptus forests. So there's an increase in the interaction between livestock and koalas which then droughts are pulling the koalas down out of the trees and they're moving around between them. So there's a high risk of spillover for chlamydia species between koalas, livestock, even birds as well. Birds come down and eat the feed that's for the livestock. So then they're interacting more with koalas. So all these climate change effects being drought or even floods and things like that is segregating different populations. They are all affecting the way that the animals are interacting and then there's a one health potential for spillover and cross transmission of different species is increased exponentially. And obviously land use for humans is affecting koalas and devastating the populations.

Martina Jelocnik

Yeah. The habitat loss I think it's always the biggest problem because if you remove the natural habitat of your wildlife they become stressed, they go search for food, they encroach the human residential areas where as Sam pointed there are dogs, there are domestic. For example cats in Australia can cause devastation to the natural wildlife. So I think it's a such a complex area to have idea what is happening with the land change, land use change as well as climate change.

Erin Welsh

What do you see as some of our biggest gaps in knowledge regarding other perhaps lesser known chlamydia or maybe just less talked about chlamydia across the landscape and why is doing exploratory work investigating these host pathogen relationships, why is that so important?

Martina Jelocnik

Well honestly I think we really have a lot of knowledge gaps ahead of us. So as I said we are very centric in species and in host. And recently we did one of the bigger bird surveillance studies where we looked at the population of wildlife birds for chlamydial species for prevalence and diversity. And that is where we discovered that besides psittaci and pecorum, we also have this novel emerging avian chlamydia in two strains in our crows. Then we also saw strains that they are described globally overseas in waterbirds, some novel species. So that is when we were like okay, there are more hosts, most species. So who is now the next spillover host? So we have a big surveillance work that we genuinely need to do.

And it's also interesting that in Australia in particular besides sheep and cattle, eventually one goat here and there, we still haven't looked at the chicken nor pigs that they are also one of the primary hosts for chlamydial species. And I think even with our wildlife and Sam would probably say that besides koalas we still haven't looked at other marsupials in more depth. So koala shares habitat with other smaller marsupials like possum, bandicoot, petaurus, little wallabies. But we still haven't done any proper and in depth surveillance for all these hosts. And that is very important because if we note an emerging pathogenic or zoonotic species in this host, that is very important for control of this infection. So we still need to do exploratory and investigative work beyond our everyday research.

Sam Phillips

It goes back to the other questions, the other answers as well. We're pushing all of these animals into confined environmental spaces where they're having to share populations that are overlapping between farm animals and birds and koalas and these increased risks of spillover between different species. And currently we don't know what species are even there to identify if there is spillover. Some of these pathogens can have devastating effects. We look at say chlamydia abortus in livestock can decimate farmed populations of sheep and we've already identified that there's changes in that species to be able to infect non placental mammals.

So we can see alien strains of Chlamydia abortus. We still don't know how that happened and what effect it's having on the avian species. And you get into migratory birds and you can get transmission across different continents. And we just have no way of being able to identify if these things are happening and if they're having a significant effect on different areas. We saw with coronavirus it's so easy for a pandemic to get out of control and then the spread is impossible to control. And we were looking for these things. Some of these chlamydia species, we're not even looking at.

Erin Welsh

So we try on the podcast sometimes when we can't end on a hopeful note, we don't always get to do that. But in this one I would like to try. So let's turn towards vaccines. For decades people have been working on a vaccine for chlamydia and success finally seems just around the corner with this new Chlamydia pecorum vaccine that's currently in trials with koalas. Can you talk a bit about this vaccine, like what kind of vaccine it is and what have the trial's shown us so far?

Sam Phillips

Yeah, definitely. So as you mentioned we've been working on the koala pecorum vaccine for 10, I think it's 15 years now. There's been many different... Sorry, I've got a half hour presentation on this so I'll try to keep it short. So the current vaccine that we use which has come through a lot of development is a recombinant protein vaccine. This is using the major outer membrane protein of Chlamydia pecorum. There's three Chlamydia pecorum strains that are used in the vaccine and we chose these strains because these are the strains that are widely spread throughout Australia. So we can use this vaccine in populations right across the country. The specific protein that we use is actually not an easy protein to use, there's not a lot of trials in other vaccines as well in human trial vaccines using this protein because it's quite difficult to isolate, it forms hydrophobic regions which makes it difficult to purify. So we've spent quite a lot of time working on being able to get this specific protein to a level that we can utilize in vaccines.

The reason we've done that is highly immunogenic, we believe at least in trachomatis this protein is definitive of different tropisms, it's quite an important protein. And it's surface exposed so it's more likely to attract a stronger immune response during immunization. The other part to a vaccine when you're using recombinant proteins is an adjuvant. We've trialed several different adjuvants and the adjuvant we use is a three part adjuvant which we believe is really important with our vaccine. So with koalas we need to have a single dose vaccine, we can't have double dose boosters, koalas don't come back for appointments when you tell them to. So we need to have a single dose vaccine.

So the adjuvant that we utilize allows us to have the single dose by forming particles within the vaccine mix. So it forms these biodegradable particles to allow the vaccine to disseminate across the koala's body. And then as these particles degrade, it exposes the antigens to the koala immune response, you get a systemic response which is much longer lasting. We believe it's at least 2 years protection in these koalas, remembering koalas only live in the wild between 5 and 8 years and reproductive between the ages of 2 and 5 possibly. So we don't need to have a vaccine that lasts the extent of the human life span of 80 years. We only need 5 years. We've tested this out for 2 years. We've got some anecdotal evidence to show that there is protection from infection down to 3 years. And we're planning on doing a new trial which will go out for 4 years. And we believe this is all down to the adjuvant that was chosen. We get a great immune response due to the antigen but a systemic response that's long lasting we believe is due to the adjuvant that we utilize.

Erin Welsh

In general what are some of the biggest challenges in creating an effective and durable vaccine for *Chlamydia pecorum* as well as other species of chlamydia?

Sam Phillips

Yeah. In a nutshell, the infection site. So chlamydia is majority a mucosal infection, mucosal vaccines are notoriously difficult to produce. You look at influenza vaccines in humans, they only last six months maximum. The only mucosal infection vaccine that has proven widely effective is the HPV vaccine. Chlamydia can transmit around the body. The range of tissues and presentations that chlamydia has with *pecorum* but with *trachomatis*, *psittaci*, *abortus*, it's so varied, it can affect so many different sites and have different traits that one vaccine to clear all chlamydia infections is highly unlikely. I think trials have shown with *trachomatis* that the vaccine that works for ocular infections but not for urogenital infections and who knows what it's doing to the gastrointestinal side where you have severe complex immune interactions between bacteria and post immune responses. I wouldn't say that there's a vaccine that's going to fix everything around the corner if at all but that specific vaccines to fix specific problems is more likely.

Martina Jelocnik

I guess we could also say and we were discussing actually this with Sam just the other day whether we call a better reply stock. So if you create a vaccine that will stop the disease, not necessarily infection but if it can stop that infection to develop into the full disease, I think that's also one aspect that you could say that the vaccine is effective. Of course the ultimate vaccine would be like your classic vaccine MO, *modus operandi*, stops infection. That's it, just blocks the pathogen. But even if we achieve no disease, I think that's also a great achievement for veterinary vaccines.

Erin Welsh

And so besides potentially protecting koala populations and helping koalas to recover, what are some other impacts that this vaccine might have in terms of public health or wildlife veterinary health? What will this be able to tell us about administering a vaccine in wildlife which is a little bit more different than administering it to livestock?

Sam Phillips

Definitely. We're breaking new ground with this. It's as you said, it's never been done before. As far as I can tell there's not a vaccine successfully administered in wildlife. So besides all the complexities and difficulties in actually running vaccine trials but getting a vaccine to a point where it's available for veterinarians and wildlife carers to be able to utilize is really difficult. And we're trying to induce new policy within the Australian federal governments to allow for funding of such a vaccine. We're not looking at a research project but we're also not looking at a commercially viable vaccine. So this is, yeah, it's new territory. We can't say that to a company, 'You make this and you'll make so much money,' because you won't make any money. And then how do you get to the koalas? And then how do you know which koalas have had the vaccine? They don't have a health card or anything to say that they've been vaccinated.

So it's incredibly difficult. We're trying to work our way through it. Our vaccine is getting registered with the AVTA which is the Australian Veterinary Therapeutics Administration, so that's TTA for animals. So we're putting together an application for that to have it registered and be available for people outside of a research project. But the funding and financing for such a vaccine is still complex and the number of doses we need is small in comparison. We need probably we estimate about 2000 doses a year. When you compare that to say COVID where we needed two billion doses a year for manufacturers it's really small and surprisingly it's difficult to be able to produce small levels of vaccine.

It's many different problems which we're trying to work through and we have solved quite a lot. We've got a manufacturing partner that's willing to come onboard and make the vaccine. We've got access to adjuvants. We've got plans to distribute this throughout wildlife hospitals. There is frameworks that have been developed to go out and vaccinate wild populations and use microchips to track which ones have been vaccinated and which ones haven't. So yeah, we are on our way, well down the track. There's been two years in the making so far and we're nearly ready to submit our application. So yeah, this is how you break new territory and get things done. You have to push people outside of their comfort zones.

Erin Welsh

Absolutely. Oh, it's so exciting. It seems very hopeful. So I've got one last question for you two before I let you go. And that is can you share some of your favorite pieces of chlamydia or koala trivia?

Martina Jelocnik

Okay, I'll start. So did you know that chlamydia infects flamingos?

Erin Welsh

(laughs) No, I did not.

Martina Jelocnik

Okay. And recently by our European colleagues, they discovered two new species belonging to a new genera within family Chlamydiaceae. There you go. And did you know, Erin, that another new species of chlamydia was isolated from crocodiles?

Erin Welsh

What?

Martina Jelocnik

Yes. And its name is Chlamydia crocodili. So it is remarkable, that is exactly what we said. There is a chlamydia for every host on every continent wherever you want it.

Erin Welsh

You just have to look.

Martina Jelocnik

Seek and you shall find. And you shall find a lot.

Sam Phillips

I think Martina stole mine, I was going to say something along the same lines. I could add that there are some theories that chlamydia is responsible for the mitochondria in multicellular organisms. Some links there. There's also some evolutionary biologists say that's phooey. Yeah.

TPWKY

(transition theme)

Erin Welsh

Thank you so much Dr. Jelocnik and Dr. Phillips for chatting with me today and for answering all of my many, many chlamydia questions. If you would like to learn more about any of the topics we touched on today, check out this episode's post on our website thispodcastwillkillyou.com where I'll link to a few papers. Also on our website are the sources for all of our episodes, transcripts, quarantini and placeborita recipes, our bookshop.org affiliate account, Goodreads list, links to music by Bloodmobile, links to merch and Patreon and so much more. Listen, follow, and leave us a review on Amazon Music, Apple Podcasts, or wherever you get your podcasts.

And don't forget you can listen to new episodes one week early on Amazon Music or early and ad free by subscribing to Wondery Plus in the Wondery app. Thanks again to Bloodmobile for providing the music for this and all of our episodes. And thank you to you, listeners. I hope you liked learning so much more about chlamydia than you probably ever thought you would. And a special thank you as always to our wonderful, generous patrons. We appreciate you so much. We have got a brand new episode on a brand new topic coming out next week. So until then, keep washing those hands.