Steve Lewis 00:00
Welcome to Speaking of Mol Bio, a podcast series from Thermo Fisher Scientific about molecular biology and its trending applications in life sciences. I'm Steve Lewis, and it is so great to be back for season four of our show. Today I'm joined by Dr. Nina Pollack, a researcher at the University of the Sunshine Coast in Queensland, Australia. Nina has played a central role in advancing the single dose koala chlamydia vaccine, a groundbreaking conservation effort that blends molecular biology with real world impact. We begin by asking Nina to explain her current role in more detail and her team's overarching project goals.

Nina Pollak, PhD 00:54
Yes. So, I'm based at the University of the Sunshine Coast, which is located in beautiful Queensland, Australia, and I'm part of the Center for Bio Innovation. It's a translational research center focusing on moving molecular biology out of the lab and into real-world applications. At a high level, I'm interested, pretty much, in reducing disease burden in both humans and animals and particularly where conventional biomedical pipelines struggle. We work, usually with endangered species and projects that don't have a big commercial incentive behind it. Much of my current work focuses on species like koalas, which are an endangered species across Eastern Australia. But I also work with platypus, which are near-threatened species, and livestock species such as pigs.

Steve Lewis 01:59
So Nina, we're so excited to have you here today, because you're working on a very interesting project. Why don't you share that with us?

Nina Pollak, PhD 02:06
I have played a central role over the past, I would say, two and a half years, in advancing the University of the Sunshine Coast's single dose koala chlamydia vaccine. This program has been running for over a decade, and it involved multiple postdocs, PhDs, honor students, veterinarians, wildlife hospitals and industry partners. So as you maybe are not aware, koalas are an endangered species in Australia. Especially the Northern population is affected. And the endangered species status for the koalas came out in 2022 in Queensland, New South Wales and the ACT. So, my work has specifically focused over the last two and a half years on improving the production workflow of these vaccine antigens that we need for the vaccine. Optimizing a bit the quality and consistency is very important for batch-to-batch considerations. And of course, we always evaluate the safety and the immunogenicity in controls as well as field settings with the koalas, it was also very important to transfer the technology from us as a university to our commercial manufacturer, and then I was also running and coordinating some koala vaccine trials. And that all together has contributed directly to the APVMA minor use permit, and this is the exciting news. So this University of the Sunshine Coast single dose koala chlamydia vaccine has been granted this minor use permit by a regulatory body that's known as the APVMA, the Australian Pesticide and Veterinary Medicines Authority.

Steve Lewis 04:16
Well, congratulations. That's very exciting news, and definitely an update from the last time you and I spoke.

Nina Pollak, PhD 04:23

Yes, it is very exciting. And as we are going through the next phase, which is pretty much getting ready to produce and supply, also to fulfill all of the requirements, because this is a conditionally approved minor use permit.

Steve Lewis 04:44
How long ago was it that you found interest in this area of research?

Nina Pollak, PhD 04:52
I have been following, personally, the journey of the koala for a very long time, because it's just one of my hobbies, animals, nature, conservation. I've always been interested, pretty much since I was a little kid, into everything that had anything to do with bears, even though we sometimes say the koala is a bear; it's not a bear. So I've been following this story very long, but I only got a chance to join this group about two and a half years ago, and when I got the chance, I had to jump and say yes, because it was super exciting to be involved in this project. And I find the koala in itself is a super interesting, very distinct species that I just love talking about.

Steve Lewis 05:41
One thing I've been thinking about coming into this podcast is how, over the years, there seems to be a lot of opinions about koala bears. I think early on, probably a couple decades ago, there was a lot of feeling of, “Oh, they're adorable, they're wonderful.” And in recent years, I think that shifted a little bit.

Nina Pollak, PhD 06:03
Yes. So koala on the first look, looks truly adorable and cuddly, but they are not really cuddly marsupial. But they are extremely specialized. So, koalas feed almost exclusively on eucalyptus leaves, and they are very low in nutritional value and very high in secondary plant compounds that are toxic to most mammals. And now to cope with this, koalas have evolved a highly specialized digestive system, and this dietary specialization basically shapes almost every aspect of their biology. So they have very low metabolic rates, and therefore they are very well known to sleep almost all day. So they have long periods of inactivity and rest. And what's probably also important to note here is that they have, because they eat these low nutritional value eucalyptus leaves, they have very limited physiological reserves and that makes it hard for them to deal with external stresses. So koalas are solitary animals. They depend, as I said, heavily on the availability of the food source, the eucalyptus leaves around them, and from a population perspective, they reproduce very slowly. So, females typically raise one joey per year under favorable conditions. So if we now take the slow reproductive rate, it's very critical for the whole impact on species population. So it means if we have something that affects fertility or survival of the koala, it has a huge impact on the population stability. And koalas are having this endangered species status because of many different issues they face. So we have habitat loss as one of the biggest ones, vehicle strikes, dog attacks, and climate stress that all contribute. But on top of that, we have infectious disease, and particularly *Chlamydia pecorum* is a major and ongoing threat. And when you now combine this low reproductive output and the disease-driven infertility, that population becomes highly vulnerable to long term decline.

Steve Lewis 08:46
You mentioned that the project has been going on for about a decade. Where was it when you joined?

Nina Pollak, PhD 08:53
So when I joined a lot of the animal studies controlled as well as field studies were already completed. Field studies were still ongoing, but we were at a stage where we wanted to put together a dossier that we wanted to submit to the APVMA. And this is when all of the quality control and batch consistency comes in, as well as when we had to really very thoroughly have SOPs ready to transfer to our commercial partner. So there was a lot of back and forth in terms of technology transfer, because it was basically the first time when it was not us researchers at the University producing the vaccine, but the commercial partner that we have.

Steve Lewis 09:53
Vaccinology is a challenging field of study, and I'm curious what levels of molecular biology techniques did it take to get to this point?

Nina Pollak, PhD 10:06
It's actually a lot of standard molecular biology techniques that got us to a vaccine. So maybe we have to first talk a tiny bit about our target, *Chlamydia pecorum*, in these terms. So it's a gram-negative bacterium, and it's an obligate intracellular bacterium. So what that means it lives within the host cell, so it has also a biphasic life cycle, so it alternates between two different phases. In those terms, we have the elementary body phase, which is the infectious phase, and the reticulate body, which is the metabolically active form of *Chlamydia pecorum* which replicates inside the host cell. So *Chlamydia pecorum* is a very tricky pathogen to deal with. This is very important to consider when you make a vaccine. For this vaccine to be effective for chlamydia, we need both, we need the antibody-mediated and the cell-mediated immunity. Because antibodies alone are just simply not sufficient. If we think about vaccines and how they are made to get a really good immune response, you don't only need the antigen side, you also need adjuvants. So, our vaccine has in total six components. So we have three antigens and three adjuvants. Our vaccine targets what we call MOMP. This is the major outer membrane protein of *Chlamydia pecorum*. So it's a protein that sits within the membrane, and there are different genotypes of that circulating throughout Australia. We have had some studies in the past of which of those strains are the most dominant ones, and they are MOMP A, F and G. So we have MOMP A, MOMP F, and MOMP G, those three in our vaccine, and then on top, we need the adjuvant component to basically get a stronger immune response for the koalas to fight the disease after vaccination.

Steve Lewis 12:30
This is a protein-based vaccine, I'm assuming?

Nina Pollak, PhD 12:36
Exactly. This is a recombinant protein. So we have made the major outer membrane protein, and we use the workhorse of molecular biology to do that. So we used *E. coli* for expression. So one of the very first steps was to clone the major outer membrane protein, the OMP A gene for the three different types, AFG, into *E. coli* and express it. So the expression just happened after induction with IPTG, and then we grow substantial amount of this protein. After this, you want to isolate the protein out of E. coli and get rid of all the rest of the other proteins that *E. coli* produces. So we used a column purification for this. Our cloning technique in those terms involved having a His-tag. So we were using a His-tag for purification. And after we purified it, we then had to dialysis it to make it clean, to get rid of all of the wrong pH and buffer components that were involved. And after that, we had a really clean solution that

we had to quality check. Some of the quality checks we did were on those very simple checks you just run an SDS page and see if your bands are very clean and clear, so you don't want any other bands to be there after purification. You really only want the bands corresponding to our, in our case, MOMP protein. We did some western blotting using, for example, His-antibody to do so, to see if we have purified only our MOMP. We used MOMP specific antibodies to show the same. And in addition to that, we actually excised the bands and sent them off to an external laboratory for mass spectrometry analysis, and they have then also given us the confidence that we truly have MOMP A, F and G. You may wonder why this is so important, but they're very, very similar. So it is very hard to prove that they are actually these three distinct recombinant proteins, because on a gel they have the very much, very same size, and it's very hard to distinguish that, and also the antibodies that we used in western blotting, they have a lot of cross reactivity between those genotypes. So mass spectrometry was actually the one solution that clearly showed MOMP A is A, MOMP F is F, and MOMP G is G.

Steve Lewis 15:33
What did it take to identify that there were three different MOMP proteins that were needed?

Nina Pollak, PhD 15:42
Yeah, so there has been a lot of surveillance studies going on in the past, and we have also a study that we recently published in 2025 that looked at the *Chlamydia pecorum* genotypes that are out there. And so it was important to show that MOMP A, F and G are truly the dominant circulating genotypes out there, and this is why the vaccine is actually using those three particular recombinant MOMPs in the final formulation. There are definitely other strains out there, and at the moment, we don't exactly know the impact of those different strains. It's also very hard to say if there is some cross protection by having these three antigens. You know, you could still have cross protection to other variants out there, but it's difficult to prove that. One thing that was very interesting, though, is when you start to vaccinate animals for particular genotypes A, F, and G, you could potentially shift *Chlamydia pecorum* towards the other genotypes, kind of it's trying to evade the protective effect from vaccination in those terms. But we have not seen any shift of genotypes, which is very good for us, and this means we are targeting the correct antigens within vaccine.

Steve Lewis 17:22
We're excited to share a new season of Speaking of Mol Bio. And we know that we have you, our loyal listeners, to thank for the growing success of our podcast series. As a thank you, we're offering a free portable wireless speaker so you can listen to the podcast or your music anywhere. I have one at my desk, and I love how easily it connects to my phone. It's nice when I want a break from my headphones or I want to share what I'm listening to with others. I hope you'll visit thermofisher.com/molbiopodcast to request yours today. Please note this item is only available in some regions and only while supplies last. Again, visit thermo fisher.com/ M O L B I O podcast to request yours. And now back to our interview.

Steve Lewis 18:13
What makes vaccines, in this case for a bacteria target, more effective than, say, antibiotics?

Nina Pollak, PhD 18:23
We have to probably talk a tiny bit more at what level koalas are actually affected by *Chlamydia pecorum*. So if you think about koala presenting with chlamydiosis, there are two major forms that you

can observe. So koalas can have ocular disease. So that includes conjunctivitis, keratitis, and in some very severe cases, koalas can turn blind. On the other hand, you have the urogenital disease. So this leads to cystitis, so like the infection of the bladder, urinary incontinence. We call that in the field, “wet bottom” because you can easily spot it. If you see a koala in a tree with this wet bottom syndrome, and sometimes the disease can ascend up the reproductive tract and then cause infertility. So we have these two things that we can easily spot. It's either the ocular disease or it's this wet bottom. The disease can be also very fatal. So longitudinal studies have shown that a substantial proportion of infected koalas progress to clinical disease over time, although the exact proportions are varying very much between populations and studies. So why this matters overall is we can observe the disease very easily, so we have practical field endpoints and we have relevant outcomes, like the infertility in those terms. So it's very rare for intracellular pathogen to have all these. We use PCR and quantitative PCR. So we are genotype as well. And we just simply, after amplification of our major outer membrane protein via the OMP A gene, we can sequence it. And this is how we study what strains are out there, and also basically prove that the koala has an infection going on. You can have infection without disease, and sometimes infection can also just naturally clear up, basically. So not every koala that's infected will progress to disease. So overall, molecular tools are pretty much essential, at every stage.

Steve Lewis 20:59
You mentioned that the symptoms for *Chlamydia pecorum* in koalas are pretty devastating, and then the breadth and scale of this is at epidemic levels.

Nina Pollak, PhD 21:12
We usually say, in general, we see infection levels, especially up here in the Northern regions, of 70%. And again, I want to stress 70% infection does not mean that all of these animals have disease. But depending on the age structure and all the environmental stresses going on in their habitat there is, it's more likely for them to progress to disease over time. And one of the big parts when we were applying for our permit was a large-scale field trial. There was over 500 koalas that were vaccinated over a 10-year period with approximately 20% of coverage, vaccine coverage in this particular population. And I have to also say what the limitation here was, there were a few different formulations used over this 10-year period, but most of them were using the recombinant MOMP protein, just a few little variations. But overall, what this study showed is a reduction in progression to the disease. And so what it means a koala gets the disease later. As an example, instead of getting it at five years of age, it gets it at eight years of age. And that is very important, because what it does, it prolongs the window while this koala is breeding and basically contributes to population growth.

Steve Lewis 22:56
What does it look like to administer a vaccine in the field?

Nina Pollak, PhD 23:02
So as you can imagine, the koala is not the animal that will come to you and see you, even though it looks so cuddly, as we said in the beginning, it's a solitary animal. It's a wild animal, and it is not easily yet caught. It lives up in trees, and that makes it very complicated. Most of the koalas that we have vaccinated, and with we, I mean the veterinarians in the wildlife hospitals and in the sanctuaries are animals that basically had a misadventure, we can call it that. Too close to streets, were either hit by vehicles or attacked by dogs or kind of had some form of trauma, were spotted by the public and therefore were collected by some, mostly volunteers, that then bring them to the wildlife hospitals and

sanctuaries. And this is where usually they will get their care and as soon as they are healthy, and prior to release, they will get vaccinated. The vaccine is a single dose vaccine, because as you can imagine, a booster vaccination will not work because the koalas just tend to not collaborate with us. They just don't want to come back to us for the booster shot. It's a subcutaneous vaccination, just one shot, half a milliliter and the animal is fine. It gets monitored before it's released, so there are no adverse effects. Further to that, we have had some studies where we, on purpose, went into some regions and attempted, for example, to vaccinate 50% of the population. So this includes drones to even identify how many animals are in this area, then it involves a whole team going into that region and trying to catch these animals. So we have a fantastic other project at our university that uses detection dogs, and they will actually find the koalas, and then the team will put field traps around these trees and try to catch these koalas. But the koalas are actually quite smart, and sometimes they waste the traps. So it's not that easy to basically say, “Hey, let's go out there catch some koalas and vaccinate them.”

Steve Lewis 25:29
It's pretty rare that we get to speak to someone representing a group who is so deep in vaccine development. So I wanted to ask, and for the listeners, I'm not going to miss this opportunity. What did it look like for a technology transfer perspective, and I imagine there's probably unique "koala-ity" control measures as well.

Nina Pollak, PhD 25:57
I love the "koala-ity" control measures. I must admit, since I only joined late in this whole project of a decade of research, I'm probably not the right person to answer this question. But there has been so much effort in trying to get this vaccine approved because, I guess we are a bunch of very stubborn people that when we get told this is not possible, we just will simply make it possible and try to achieve it. There's so many different stages in making a veterinary vaccine, especially for an endangered species, that I think in the beginning when this project started nobody has really thought of, so it has been a steep learning curve and the pathway to this approval was definitely not a straight line. So it was, there was there were lots of ups and down in terms of episodic funding and also the commercial incentive behind it. We were very glad that we found a commercial partner that actually was taking this project on, because there is no money in it. We really depend on the government to fund this. In reality, we're not making enough doses to make this commercially viable, and our clients are wildlife hospitals and sanctuaries that are also struggling to get enough money for their own cause in those terms. So there have been, like lots of challenges, and we are talking to different groups about our experience on getting this vaccine approved, and basically everyone wants to know what would be a straightforward kind of plan, but I must say, there is no straightforward plan with an endangered species. Everything is complicated. There are no challenge studies to be done that everyone wants, but it's an endangered species. Nobody will do that. Nobody will infect on purpose an animal to then test if the vaccine has an effect or not. There are just lots of hurdles that we had to jump through, but we were very glad that we have this minor use permit pathway that actually enabled that. This pathway made it possible to look at this real-world evidence from our controlled but also field trials and approve it with conditions that we still have to fulfill over the next years before it gets a truly registered product.

Steve Lewis 28:56
I think it was very insightful that you shared some of the challenges along the way. But also throughout the conversation, you've really highlighted the importance of conservation and being good stewards in general for animal populations.

Nina Pollak, PhD 29:15
Yeah, I'm glad we were able to talk about the impact of vaccination on koalas. And I think it's important to us as a group, because we publish all of our findings. We're very transparent and we want to really make it clear that chlamydia as a disease has an impact on koalas, but it's still habitat loss that actually affects this species the most. So we always say, “If there is no tree, there is no koala.” Vaccination won't do a thing. But to all of the people that have questions of about why vaccinate in the first place, this is one tool that can be used for koala and something that we, I think, wanted to talk about earlier on was the use of antibiotics. And because koalas have this very specialized digestive system, antibiotics get very quickly out of the system, so it's very hard to sometimes treat a koala with antibiotics, and it needs prolonged time and sometimes also much higher doses. And now these antibiotics destroy their gut microbiome, and it's a new problem that you then face. So veterinarians are really trying to balance it, if they want to use antibiotic in the first place, and that will help the koala in that particular case, or if it makes everything worse, because they're going to destroy the gut microbiome, and then the koala can't digest the eucalyptus leaves anymore, and it's actually worse off. So this is why vaccination as a tool is so effective. It's just a new tool that can be used on top of the tools that were available. And we are still working on second generations of this vaccine, because, of course, we want to improve it from a perspective of production we want to make it less complicated, easier to produce, which makes it cheaper and cheaper means we can just reach more koalas, because it's more sustainable for bigger deployment.

Steve Lewis 31:36
It's a really noble and thoughtful thing you and your team are doing. And I think it's a really interesting perspective that we don't hear very much about, especially when you're so close to widespread administration in this generation and then future generations of the vaccine. So thank you. We'll go ahead and wrap the episode with our two final questions. For a young scientist who might want to follow in your footsteps, what advice would you give them?

Nina Pollak, PhD 32:12
To not be afraid to take on difficult projects when people tell you it's impossible. There is always a way. You just need to find the right team to do the job. So, I think one of the biggest advices is just have a look around and try to join groups where people have a can-do attitude.

Steve Lewis 32:36
I love that message of resilience and passion. Over the next five years, what do you see as the technology that you're most excited about?

Nina Pollak, PhD 32:59
In terms of the vaccine space, I think there is not really new technology. Like I'm excited about new technology, but I think it's technology that we have already used plenty that will actually help us in this space. Because people are more likely to accept things that they already know about. So this is why we, for example, chose, I mean, even though this happened about 10 years ago, but this is why this vaccine is a vaccine that uses recombinant proteins as antigens. Because, as you know, there are lots of new technologies out there, like mRNA-based vaccines. However, I don't see an mRNA-based vaccine has a future in an endangered species such as the koala. I don't think that people will be

accepting those technologies in such a vulnerable species, they want to use technology that has proven over the years that there are no side effects.

Steve Lewis 34:04
So it's too new and not established, and you're worried public perception may not support it?

Nina Pollak, PhD 34:11
That, I would say that is one of the things that you have to consider in that particular space.

Steve Lewis 34:18
What would you say to challenge that perspective?

Nina Pollak, PhD 34:22
As a scientist, I mean, I'm definitely always kind of convinced by data. So maybe one of the challenges that we all face is that we have to better communicate our results to the public and engage the public early on, so that we have just a better understanding and probably are not too scared about new technologies. But I understand probably the hesitancy of the public, because it gets more and more overwhelming reading through all these countless, sometimes AI-generated, posts online and trying to filter out what is truly correct and science-based and not just made up because somebody wants to push their own agenda.

Steve Lewis 35:17
Well, I think the aspect of scientific communication and the importance of putting yourself out there like you've done today is a really admirable thing, and we're so lucky to have had you today. So thank you for joining us, Nina.

Nina Pollak, PhD 35:31
Thank you. It was a pleasure to talk to you, Steve.

Steve Lewis 35:36
That was Dr. Nina Pollak, a researcher at the University of the Sunshine Coast's Koala Chlamydia Vaccine Project in Queensland, Australia. Speaking of Mol Bio is produced by Matt Ferris, Sarah Briganti, and Matthew Stock. Join us next time for more fascinating discussion about the wide world of molecular biology. Until then, cheers and good science.