

This Week in Microbiology

With Vincent Racaniello, Elio Schaechter, Michael Schmidt, and Michele Swanson

Episode 170: Rats, lice, and nanoparticles

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Vincent: This is TWIM, This Week in Microbiology, episode 170, recorded on February 1st 2018. Hi everybody, I'm Vincent Racaniello, and you are listening to the podcast that explores unseen life on Earth. Joining me today from Small Things Considered, Elio Schaechter.

Elio: Well hello there, nice to be with you.

Vincent: Welcome to February.

Elio: (laughs)

Vincent: We already have one month of 2018--

Elio: The year is over.

Michael: The year is over.

Vincent: It's moving quickly. Also joining us from Charleston, South Carolina, Michael Schmidt.

Michael: Hello, everyone!

Vincent: How are you?

Michael: I'm well! My colleague in the office next to me just came back to work reporting he had had the flu, so I was glad he stayed home.

Vincent: That is a good thing to do when you are in fact, but unfortunately many people do not.

Michael: Yes.

Vincent: And that's how you spread infections.

Michele: Let's talk more about the flu today, shall we?

Vincent: We will.

Michael: (laughs)

Vincent: Also joining us from Ann Arbor, Michigan, Michele Swanson.

Michele: Hello.

Vincent: You've been to California.

Michele: Yeah, Elio and I got a chance to sit and just chat.

Vincent: That's nice.

Michele: It was really lovely. In vivo, as he likes to say.

Elio: So lovely.

Vincent: You got away from the cold weather, right?

Michele: I did and I got to visit my daughter who is clerking for a judge out there. It was great.

Vincent: Wow, nice. San Diego. Alright. Well we are all back at TWIM, beginning of February, the year is moving fluidly under the bridge, I guess. And I have a followup email that I would like to read. This concerns a link that Michael had provided in the last episode. This is from Steven who is an emeritus professor of microbiology at Virginia Tech, Steven Boyle. Does anyone know Steven Boyle?

Michele: The name is familiar.

Vincent: Steven writes:

The formula for conversion of OD to E. coli cells per ml is essentially correct but based on incorrect assumptions.

Michael: Uh oh.

Vincent: He quotes:

"This calculator uses the extinction coefficients for E.coli and Yeast cultures to calculate the cell concentrations from the Optical Density (OD600) reading taken with a spectrophotometer".

The majority of light generated by the spectrophotometer is not being absorbed by the yeast or bacteria BUT being scattered! It is incorrect to state that live or dead cells have an extinction coefficient when they are in the path of light with a wavelength of 600 nm. Extinction coefficients are generated as the result of light absorption according to Beer's law." Beer's Law states that molar absorptivity is constant (and the absorbance is proportional to concentration) for a given substance dissolved in a given solute and measured at a given wavelength."

The other assumption that the calculator uses incorrectly is that the size of the cells is not accounted for....if E. coli is grown in an enriched culture medium e.g. Trypticase soy broth versus a minimal medium e.g. MOPS, the sizes of the cell are very different. Those cells grown in enriched medium are much bigger than those in minimal medium. Thus there are distinct differences in the amount of light scattered I.e. depending on number and size of the cells. In addition the amount of light scattered does not distinguish between intact live cells vs intact dead cells.

The more accurate way to use the calculator is to be sure to teach that the data generated should be made for each type of cell under specific growth conditions including the type of culture medium. Moreover, the calculator is based on light scattering NOT extinction coefficients.

I thought Beer's Law was—well, nevermind. (laughs)

Michael: (laughs)

Elio: We better say he is right and let's apologize.

Michael: No no no, I provided that reference, it was one of those handy dandy Google calculators that is out on the internet, so his comment, it's one of those details I didn't want to go into but I agree wholeheartedly with him because I was always taught it is about mass and that is what he is getting to, it is about the mass of the microbe.

Vincent: He also points out that if you grow E. coli in an enriched medium versus a minimal medium the cell sizes are different and therefore the scattering is going to be different.

Michael: Absolutely.

Vincent: So he says the more accurate way to use the calculator is to be sure to teach that the data generated should be made for each type of cell under specific growth conditions including the type of culture medium and not use extinction coefficients.

Elio: I never have, by the way.

Michele: When we use it for legionella, legionella changes its shape during its growth curve, and it can get very elongated and filamentous and that also then gives you a different CFU per OD unit.

Vincent: So an easy thing to do if you want to know the state of your culture, you can measure an OD600 and say okay, it's ready, it is in log phase or whatever. That's fine, but when you want to know per mL that is where it gets tricky.

Michael: Yep.

Vincent: Okay. Thank you Steven, thanks for listening. So there you go. We have a retired microbiology professor listening to TWIM and keeping us honest.

Michael: That's right.

Michele: Yes. Thank you!

Vincent: Yes, thanks a lot. We have a snippet and a paper for you today and I must say Michael found them both. How did you find them, Michael?

Michael: I was doing reading for my course, I am teaching med micro this semester to the dental students and the graduate students. And so like everything you are looking for the most current information to talk with the students about and I was looking at the growth paper from the week before and that wonderful review article that Elio sent to us a few weeks ago on physiology, so that got me into the physiology mode and I stumbled into

this paper in PNAS, one of the normal titles I look at, and it had math in it just like the paper that Elio sent to us. Consequently I was intrigued and I always find plague fascinating.

Vincent: Alright, tell us about it.

Michael: So the paper is “Human ectoparasites and the spread of plague in Europe during the Second Pandemic” and as I said this was in the PNAS early edition, and it was authored by Dean, Krauer, Walløe, Lingjaerde, Bramanti, Stenseth, and Schmid and they are at the Center for Ecological and Evolutionary Synthesis and--

Elio: That is quite a title, isn't it?

Michael: It is. It is. Because I think if we ask the average individual what caused the great plague or the Black Death in Europe during the dark ages or the middle ages or however you want to refer to it, most people will say well, it was the rodents, the rats. The rats had fleas and the fleas gave the plague bacillus to the humans and that was then how it went from being the bubonic form of plague to the pneumonic form of plague and that was what was responsible for the bulk of the death. And as some of you know the great plague of medieval times started in China, followed the great trade routes to Constantinople, and then to the great capital cities of Europe. According to the CDC, it was called great as it claimed an estimated 60% of the European population at that time.

Michele: Amazing.

Michael: You know you really get chills when you think about a microbe that is able to do that, and this paper is fascinating because it brings to us the math that we all love to not think about. I really liked how the authors introduced us to some modeling and how they effectively got to their title, how human active parasites, principally the *Pulex irritans*, which is a human flea, and body lice, *Pediculus humanus humanus*, and most Americans who have kids in the primary school system know the most dreaded note your child can bring home from school is that there is head lice running in your child's classroom and then you spend the rest of the evening picking nits or checking your child's head for these *Pediculus humanus humanus*, and this is the essence of the paper. Human body lice and human fleas were actually responsible for the great plague that started in 1334 and was with us through the 19th century when the third pandemic started and that again started in China. But that one was likely due to rodents.

Michele: And for human fleas you are emphasizing that there are species of fleas that preferentially feed on humans and then there are other species that preferentially feed on rats or dogs.

Michael: Feed on rats or on dogs, and any of us that have pets know that the most dreaded thing that your dog can get into is fleas because then you have powder and drops and it's just a delight (laughs) so.

Michele: But there are some regions of the world where there are fleas that feed on humans, that the human population can support them.

Michael: And so that you don't think that we are going back to the 1300s here on TWIM, because after all this is This Week in Microbiology--

Elio: (laughs) Not *that* week.

Michael: Plague is still unfortunately with us and in fact, if you go to the WHO site you find out that Madagascar of all places is experiencing its own epidemic presently. From August through October of 2017 there was a total

of 1,800 confirmed probable or suspected cases of plague, including 127 deaths. Of these 1,100 were clinically classified as pneumonic plagues. So that is especially disturbing that it is actually person to person transmission and your risk factor of acquiring this form of plague is breathing as opposed to being bit by a flea, whether it be by a human or whether it be from a rat.

So the paper we are going to talk about today is not this historical curiosity, it actually can fall right into modern microbiology. The authors have developed a susceptible infectious recovered model and in the show notes I put a link to a paper by David Smith and Lang Moore who will, for those of you interested in the differential equations, it is a nice instructional link to the SIR models in general. It's from the Mathematical Association of America and it will provide those who are math beings the overview of how the concepts behind the math impact the biology. But briefly the authors use this SIR approach, susceptible infectious and recovered, to demonstrate that it was the human ectoparasites, the flea and the lice of humans, that were likely to have been the dominant mode of transmission of human plague during the great plague of the middle ages.

Elio: These are different fleas than the ones carried by the rats?

Michael: Yes, different fleas than the ones carried by the rats.

Elio: So the rats are exonerated?

Michele: (laughs)

Michael: The rats are exonerated. That's the take home message, Elio, in a nutshell. They actually take you through these beautiful differential equations and the magic of their paper is because death was the endpoint and the churches were taking censuses documenting when individuals died, they have good records of when people died. And so they have recorded mortality, they have the population census because most individuals in Europe at the time were baptized, and so you have baptismal records and you have death records. They use that in their model and those of you following along in the paper, the parameters for their SIR models are found in Table 2, and they literally tell you what is important in thinking about how this microbe moved from person to person and whether or not their was an intermediate involved.

So they have human parameters, they have lice parameters, they have rat parameters and they have flea parameters. And table 2 in this paper goes through, it is properly referenced so if you are curious what the transmission rate for bubonic plague from a mildly infectious human to body lice is, you can go and hunt that up and learn all about it. And for things with unobservable parameters, they used Bayesian inference. Now, Michele asked me when she got this paper, she asked if I would simplify Bayesian inference for those folks following along who may not want to look at the math.

For those of you in the audience who follow baseball, when we saw the movie Moneyball with Brad Pitt and Jonah Hill, which was based on Michael Lewis's 2000 book about the champion Oakland A's, or if you are like my friends who have spare time, it is how you win at fantasy football. Briefly in Bayesian, it's the process of reducing properties of an underlying probability. Will I hit a home run, will I hit singles, and then you add that to your analysis parameter of the data. So this is based on Bayes' law or Bayes' rule, which simply states that the probability of an event based on prior knowledge of conditions. So if you have a baseball fan in your family you know they are obsessed with batting averages, home run averages, and similarly in football it is all about tackles and pass yardage and sacks, so that is effectively the underlying metaphor you need to understand in order to take the differential equations apart.

Elio: I must say, though, when I look at table 2, I'm stunned that you can handle this many parameters and equations.

Michael: Yes! Well, you know, for the--

Elio: This is very stunning.

Vincent: Yeah.

Michael: For the three models they have the ectoparasite model, they have the pneumonic model, and they have the rat model. The ectoparasite model requires more differential equations than the pneumonic model. The pneumonic model only requires 3 differential equations in order to effectively fit the data to the curve and ask do the observed data meet their model criteria?

Michele: And is the reason, Michael, because pneumonic plague goes from person to person?

Michael: Yes.

Michele: So there are fewer actors that you have to take into effect?

Michael: There are fewer variables that you have to consider, so that is effectively the math distilled in a nutshell so that you can actually make beautiful sense of these things. Now the rat that Elio was talking about in the beginning, they have 10 differential equations that you need to do in order to fit the model.

Elio: I almost found a single differential equation, let alone ten.

All: (laugh)

Michael: So it's really elegant how they walk you through this, and in general the human ectoparasite model fits the pattern of the observed data, namely the number of people who died, and the number of people who died were enough. So they have these two small cities, Eyam and Givry, where it was difficult to distinguish between the models simply because they didn't have enough dead bodies, because the towns were too small. And then Malta and Moscow kind of screwed up their models, too, because they had two peaks and their models didn't consider that.

But when you look at the data it is beautiful because it effectively confirms that it was indeed the human lice and the fleas of humans that were responsible. And they throw another variable in here, they compared their three competing models using a process called BIC, and BIC is again, Bayesian Information Criterion, and here you need to know that the model with the lowest BIC is preferred. So a low number is good and is effectively saying this model is in agreement. So they fit their models and again, their data from the subsequent BIC analysis shows that the human ectoparasite had the lowest BIC value for all the outbreaks except the two towns that had the low populations, and for the remaining outbreaks the difference in BIC for the human ectoparasite and other candidate models was greater than 10.

Michele: And if I could just put a point on that, what is really impressive about this computational approach is that they were able to go back in time and look at what three different types of transmission and they looked over nine plague outbreaks across hundreds of years.

Michael: Centuries!

Michele: Yes. Over several centuries, and with that huge amount of data they could see which model best explains what is observed. So it's very different from bench science (laughs)

Michael: It is very different from bench science, but I think the other reason I wanted to bring this to the attention of our listeners is more and more we are seeing in the primary microbiology literature the use of this Bayesian inference where you have some data, you have some other data, you have these disparate data sets, and people are trying to bring things together. This is especially apparent in things like the microbiome. And so I think as you begin to read papers for which we have real data and we know it does indeed hold, it is important that you begin to think about these things and take it apart. The last piece of data that I would like to share with you in this snippet is the basic reproduction number, because many of you have probably been listening to the news about the flu and even the reporters are now talking about basic reproduction number or R_0 . And what they've learned here is the R_0 for their model is--

Michele: What is R_0 ?

Michael: R_0 is if I have an infection, the number associated with it is the number of other individuals I am likely to infect. So their R_0 suggests that if I am infected with plague, I am likely to infect between 1.5 and 1.9 other individuals, considering it on a population.

Elio: Do we know what the number is for the flu?

Vincent: It's similar.

Michael: It's similar, and in fact, if you are interested, measles, which is a nice delightful virus which also has airborne transmission has an R_0 where one case of measles will infect between 12 and 18 other individuals.

Michele: Wow.

Elio: A big R, my God.

Vincent: There's one of the most contagious human viruses but I would like to remind everyone that we do have a vaccine.

Michael: That was my next point.

Elio: Unlike some other viruses.

Michael: Well, the 1918 pandemic of influenza had an R_0 that depending on the city was between 2 and 3. So that is the last piece of historical data I would like to share with you. Summing up, these authors did an outstanding piece of work, their study supports human ectoparasite transmission of plague during the second pandemic including the great pandemic or the black death, and using the recent experimental data on human fleas and body lice as plague vectors, they have developed this compartmental model concept that captures the dynamics of the human ectoparasite transmission. In other words, fantasy football for lice and disease. I really found that this paper from a snippet was compelling. It had many important learning objectives for, I think our listeners will find interesting and it teaches a bit of history.

Vincent: Michael, if you were testifying in front of congress and the honorable senator from South Carolina said, Dr. Schmidt, do these data prove that human ectoparasites transmitted the plague, what would you say?

Michael: I would say they are highly suggestive.

Michele: And to that point I really appreciated that the authors end their discussion and then drew from the current epidemiology literature and pointed out human studies that are consistent with their historical interpretation. So, Michael, you mentioned Madagascar, because they have ongoing plague epidemics, there is a great research site. So a group from the Pasteur Institute of Madagascar working with colleagues from the World Health Organization did a study where they collected fleas, 319 fleas, from houses in Madagascar, and then speciated those fleas and then looked within each species of fleas using PCR for evidence they are carrying pestis, the bacterium Yersinia pestis. And the only flea that they found that contained Yersinia pestis was indeed this irritans, what was the first name?

Michael: The human one.

Michele: Yeah, the human flea.

Vincent: Pulex irritans.

Michael: Pulex irritans.

Michele: So again, and they found some of those infected fleas in homes where there had been a case. Again, they couldn't say with certainty that the flea was the source of the disease in that particular person but the way the evidence, I agree it is consistent with the human flea vector.

Michael: Yes.

Elio: It's a great story.

Vincent: Michael, is this useful for helping to control current epidemics of the plague?

Michael: I think as Michele just highlighted that the WHO is indeed looking in Madagascar to address whether or not it's rats or human transmission, the disturbing thing in Madagascar is the number of pneumonic cases because they are unlike, you can protect yourself by cleaning up your house and getting rid of the fleas with a pesticide or bathing or washing your clothes. But the principal risk factor with pneumonic is breathing and so if you are living with an infected person or you are the caregiver for the infected person it is really, your risk is much greater. That's why it is the paper that has everything.

It is reacquainting us with important concepts like the basic reproduction number and again driving home the importance of vaccination, if you know you are not and all of these things, it really is a good exercise. And the math was fun when you looked at it, I showed it to one of my colleagues whose husband is a math professor at the college of Charleston and he found it absolutely fascinating as well.

Vincent: Nice. Alright, thank you, Michael.

Michael: You're welcome.

Vincent: And now we have our second influenza paper on TWIM of this flu season, so it is appropriate as everyone knows it is in the news, everyone is talking about what a serious influenza season it is. So we should talk about influenza vaccines, and this is a paper published in Nature Communications, it's called "Double-layered protein nanoparticles induce broad protection against divergent influenza A viruses." First author is Lei Deng and last author is Bao-Zhong Wang and this comes from Georgia State University, Georgia Institute of Technology, and Emory University School of Medicine. This is a Georgia product.

Michael: (laughs)

Vincent: Now as everyone knows, influenza is a respiratory disease that here in the US in a temperate climate it is seasonal, it comes up in the fall and lasts through the winter. It goes away in the spring. It is here every year, it can be serious. In the US, it can kill anywhere from 5,000 to 40,000 people in the flu season. Those are based on historical numbers, so you have to take it seriously. We do have a vaccine, we have several vaccines against influenza virus but the problem is that the virus can change from year to year. It can change in a small way, which we call antigenic drift, and that would make it necessary to make a new flu vaccine every year. And so the World Health Organization actually has an extensive global network which samples influenza viruses all the time and then twice a year says, okay, should we change the vaccine? And they make decisions. So it has to be re-manufactured and retested in those years. And sometimes it changes in a big way which we call antigenic shift and that leads to pandemics like the 1918 pandemic, 1957, 1968, 1977, and 2009. I know them all because I did my PhD on influenza.

Michael: Oh.

Vincent: Knowing the years of the pandemics is sort of like initiation rites. So we have a few influenza vaccines, many of them are produced in embryonated chicken eggs. I just read a paper, by the way, that says if you are allergic to birds or feathers, doesn't matter, you can still get that vaccine. We also used to always say that if you are allergic to feathers don't get it but you can still take it, apparently it is not an issue. And then there are flu vaccines grown in cell cultures, and these are all inactivated non infectious vaccines, and then there is also a flu mist which is injected into your nose with a syringe without a needle on it but just sprayed into your nose and it replicates in your respiratory tract. So we have a bunch of them but they are not that great. What do I mean by not that great? If you immunize a hundred people you are lucky if sixty of them will be protected against influenza after immunization. Which is not to say that those forty are not protected, they make it a less serious disease, but we clearly need a better vaccine. And more importantly, we need a vaccine that we wouldn't need to change every so often.

Michael: And not as expensive because in order to get one dose of vaccine you require one egg.

Vincent: One egg per dose, that's right.

Elio: It's almost an embarrassment because we have been so successful with so many vaccines and microbiology has done more than anything else to allow modern civilization in terms of health and here is one where we are sort of behind.

Vincent: Nevertheless, I do get it every year.

Michael: I do, too.

Michele: The vaccine, not the flu. (laughs)

Vincent: The vaccine, yes. I think it is important to get it because even if it is not a hundred percent it will protect against serious disease and it will reduce transmission, which means you are helping other people.

Elio: There have been a whole bunch of deaths, there have been a whole bunch of deaths, tracking all over where people are not vaccinated.

Vincent: Yeah, that's right.

Michele: I think, Vincent, you mentioned in an earlier discussion that another shortcoming of the vaccines is the immunity is not durable.

Vincent: That's right, it doesn't last a long time. It fades.

Michele: For reasons that are not understood or?

Vincent: No, it's not that it's not understood, we know it. Basically what we are saying is your memory of immunization is not very good. We don't really know why that is. So that is another thing that we would like, we would like a durable vaccine that lasts and that can protect you against any kind of influenza virus that nature would come up with.

Elio: Another thing which is a mystery about flu is why it is seasonal. There is no good explanation for it, is there?

Vincent: Some work done a number of years ago suggested that it has to do with humidity, so in the winter where the humidity is low and the temperatures are low, this favors persistence of infection in the environment, so there have been some transmission experiments that support that. I think that the data are pretty good. The problem is, Elio, that there is also flu in the tropics. It can also be seasonal there where they have lots of humidity, so it is not the only answer to that one. So we need a universal influenza vaccine that will induce broad cross protection against lots of different influenza viruses and gives good long lasting immunity. And that is what this paper is trying to do. It looks pretty promising.

Now a lot of people are working on this. We are throwing a lot of money at it. People are trying to make universal vaccines. There are two approaches that have been used by others and they have been somewhat successful, at least in the lab, and those two they are going to pick up on in this paper. One of the proteins on the surface of the influenza virus is a glycoprotein called hemagglutinin. This is an important protein because when you vaccinate people with an influenza vaccine the immune response against that hemagglutinin is protective, and people have found that this protein, it looks sort of like a lollipop, a lollipop head and a long stalk that attaches it to the virus. The stalk is highly conserved among different influenza virus strains.

So people are trying to figure out how they could immunize people with the stalk and get a vaccine that would protect against many different strains. It has been somewhat successful in various experiments. The other approach, there is another protein in the influenza virus membrane, it is called the M2 protein. This is also conserved among different influenza viruses and people have tried to immunize animals with that protein and some good immunity has been developed but it is not durable. So one of the problems is when you immunize people with proteins, individual proteins are not really great at making strong long lasting immune responses.

Think of a virus particle. It is small particle, flu is about 30 or 40 nanometers in diameter, it is a particle that is presenting the proteins of this particulate and that appears to be really good for making an immune response. Whereas if you put proteins in they are small and they may not last a long time. So what they do in this paper is they make what are called protein nanoparticles. So nanotechnology. You have all heard of it. Small particles which carry drugs or therapeutics of various kinds. The particles can be made of various things. They can be made of plastics or metals, but here in this protein they are making nanoparticles which means they are small. They are making them out of influenza virus proteins. They are going to use those to immunize mice and see what happens.

Michele: It's real bioengineering.

Vincent: Bioengineering, yes.

Elio: You are going to probably tell us how they make the particles, but I thought it was really wild. All they do is they add ethanol.

Vincent: (laughs) That's right.

Michael: (laughs)

Elio: They coagulate them.

Vincent: That's right, that's absolutely right.

Elio: They desolvate them.

Vincent: They desolvate them. So what they do, they take these two proteins, the stalk of the HA and the M2 protein, and first they make a tetramer of multiple copies of the M2 protein which they have taken from human influenza viruses, swine influenza viruses, bird influenza viruses, and others. So they put four in a row and that makes a tetramer and they make four copies of that.

Michele: And that makes sense, right, because this virus does hop from species to species and we get this mixing of the different viral segments so we get these chimeras. I thought that was brilliant that they just cover all of it.

Vincent: They just cover all of it. So you have a vaccine which maybe you get once or twice in your lifetime and that would cover everything. So they start with this M2 protein from various influenza viruses. They make a tetramer out of it. Then as Elio said, they make a particle out of that by adding ethanol which takes out the solvent and it causes these things to form a particle, a nanoparticle, which you can't see it in a regular microscope, you need an electron microscope. So that is part 1. And then they say okay, we want to add the stalk of the hemagglutinin to this, so they take these nanoparticles made of M2 protein. They add the soluble version of the hemagglutinin stalk which they have produced. And then they crosslink it with a chemical crosslinker to the M2 nanoparticles.

Michael: It is not anything wildtype, it is completely an artificial construct.

Vincent: Totally artificial, there is nothing like this. But it turns out that it looks sort of like a virus particle, it is about the right size and so you basically have a core made of M2 protein and then little HA stalks decorating it. That is their protein nanoparticle. There is two that they make, so all the HAS of the influenza viruses that are out there you could divide them into two groups, group 1 and 2, and if you immunize with those from one group they do not really cross react with the other so any vaccine that is universal has to have both in it. And then they show that these nanoparticles have the right proteins in them, they seem to be folded properly, they are recognized by monoclonal antibodies, and then they inject them into mice. By the way, they can control the size of these particles by how much they desolvate them with ethanol, by how much they crosslink. They say that small ones are better, this is something I learned, they say small nanoparticles induce cytokines which give you a better immune response. I didn't know that.

Michele: Is that in a tissue culture dish or in a mouse?

Vincent: It's in an animal.

Michele: Because it might be just how if you can imagine if it was a big hunkin protein it might just sit at the site of injection, but if they are smaller they might diffuse a little better.

Vincent: That's right. I think they have looked, I am not sure if it is in an animal but the paper is talking about dendritic cell processing and it is better if they are smaller. The other thing about these nanoparticles which they've mentioned is, you know, they have crosslinked the hemagglutinin protein to the core and they said, they probably come off very slowly in cells that pick up the particles, the antigen presenting cells. And they think that a slow release of the antigen is really good. The real key here is they take these particles, which by the way they look at in the electron microscope, they can see that they resemble virus particles and that they are spherical. They immunize mice by injecting them into the muscle. They give two injections and they include adjuvants. An adjuvant is a chemical that you mix with a vaccine that will give you a more robust immune response. If you have ever had a vaccine and your arm hurts within the next few hours, that is because you are having a good immune response at the site of injection, and adjuvants make it even hurt more.

Michael: They do indeed.

Michele: A little inflammation will call the white blood cells to the site.

Vincent: Inflammation is good. A lot of people have the human papilloma virus vaccine, for example, has a very high adverse event rate which is mostly pain at the injection site because there is adjuvant in that vaccine, but that means it is working. Anyway, they get really good immune responses against these two different kinds of nanoparticles, and they find that antibodies are produced that will interact with the original particles and they react, remember they have two different kinds of particles, one against group one, one against group 2HA, and they both react with the right HA. And if they make a cocktail of the two, they mix the two different kinds together, they get a really broadly reacting immunogen, which when injected into mice makes antibodies that will react with a whole range of hemagglutinins, bird, human, etc.

Michele: I loved the way they displayed that result, too, I believe in figure 2B they used what is called a radar diagram.

Vincent: Yeah, it is pretty nice.

Michele: Which schematically can show the profile of the antibody response and compare directly multiple antigens, so I really appreciate whenever I read a paper when somebody has got a great visual to display a lot of complicated data.

Vincent: So they look at not only antibody responses, they look at cellular responses, they find that immunization with these nanoparticles induces lymphocyte populations, they have an assay for that. And they also show that these are virus-specific, so they look at both antibody and cellular responses, and both of them are probably important for resolving influenza virus infection. But now the key is, what happens if you challenge these mice? You've immunized them, now you infect them with influenza viruses. Because it is a mouse, you have to use a mouse adapted influenza virus, so you can't use every strain.

Michael: But this goes importantly to show that should the virus have a dramatic change in its makeup, this vaccine cocktail will likely work.

Vincent: It should, it should, because we don't know when it will happen in people, because people are not mice, they can process the antigen differently. So this will have to be eventually studied in people. But with the mice, these immunizations with these particles confers complete protections against death and weight loss with the same type of hemagglutinin. If you mix them, then it will protect against either one.

Now the cool thing that they can do here, you need a mouse adapted virus, but you can give that mouse adapted virus different genes on the surface you can give them a different hemagglutinin. So they could test, for example, different avian influenza virus hemagglutinins and they see that they get protection against these as well in mice immunized with a mixture of these two different nanoparticles. So they are protected against death, they are protected against weight loss, they have less virus in the lungs, and they have less pathology in the lungs as well.

If they take serum from these immunized mice they can transfer it to another mouse and protect them against infection. They show that this protection requires a certain type of immune cell which is known to be involved in protection against infection. In fact, the last infection is really interesting, if you can deplete in your lungs, you have what are called alveolar macrophages. These are cells that are important in defenses against virus and bacterial and other infections. If they deplete the macrophages, even if they give the mice serum from immunized animals they will not be protected. So not only are antibodies involved in protecting but macrophages as well. They find that this protection lasts up to four months after immunization in mice. Mice only live about two years, so that's pretty good.

Michael: That is pretty good. Do mice undergo the same sort of senescence to flu vaccine that humans do?

Vincent: That's a good question. I don't know the answer to that.

Michele: What do you mean by senescence there, Michael?

Michael: We don't get lifelong immunity to a flu vaccine and our immunity wanes.

Vincent: Yeah, that's the thing we were talking about earlier.

Michele: The durability, I see.

Michael: Yes, the durability of the vaccine.

Vincent: Yeah, I don't know the answer to that, I know that many experiments have been done in mice with the human type vaccines, I don't know the answer to that. So that's the paper. Basically they have developed this very interesting protein nanoparticle which looks like it could be a universal vaccine, it seems to protect against lots of different isolates of influenza virus and I didn't mention it but the H5N1 is one that scares many people, the avian virus, the H7N9 which is currently causing outbreaks in China, protects against all of those. So here you have a very interesting formulation, it is not hard to make, you don't have to grow it in eggs, these are just proteins that you produce. So this is a proof of concept, and of course now they will do some more animal experiments, but eventually this could in theory go into clinical trials in people and maybe some day be used.

Michael: The exciting experiments are going to be in ferrets.

Vincent: Ferrets will be next, yeah.

Michael: Whether or not this protects ferrets from a live challenge of normal virus.

Vincent: By aerosol transmission too, yeah.

Michael: Yes, aerosol transmission.

Michele: So they actually start with a couple tubes of pure protein of each of these flu proteins, so listeners might wonder well how do you get that protein? The method they are using is an insect cell expression system, so they can infect these insect cells with a baculovirus which is designed to expressed the flu protein and then they harvest the proteins from cells. I don't know, do the cells then secrete the recombinant protein?

Vincent: They do, it's made so it is secreted.

Michele: So you can imagine having--

Michele: Having large vats, then, of these insect cells?

Michael: Just continuously making it.

Vincent: So in fact, one of the flu vaccines is called Flublok, it is made in insect cells, it is the complete HA including the lollipop head, and it is secreted, it forms virus like particles and those are purified and injected. Unfortunately they are not any better than the conventional vaccine but the production method is very cool.

Michele: So you think that the production of this will be cheaper than eggs?

Vincent: It should be.

Elio: You're the expert on any viruses, what's your guess? Is this going to be the answer?

Vincent: It looks promising, but the problem is these are mice and things can be very different in people. As with any medical product, you test them in animals, it looks good. You go into people, it can be completely different. So you can't predict. So far this looks really good, it looks like it is cross reactive, and so if these are antigenic in people then it would work. But we will know in maybe about five years or so. By the way, they also say that because these are protein nanoparticles, they don't need refrigeration or freezing to keep them. They will last for three months at room temperature.

Elio: Oh boy.

Michael: Wow.

Michele: That's great.

Vincent: So that's good. You can give them to a country that doesn't have a lot of refrigerators--

Michael: Or infrastructure.

Vincent: And you can store them for three months until you use them.

Michele: And it should make it cheaper in developed countries, as well.

Vincent: Yes. So this is very cool.

Michele: I also thought it was neat that they mentioned in addition to this vaccine application, these same bioengineering principles could be used for slow release delivery of protein therapeutics.

Vincent: Therapeutics, drugs of various kinds, yeah. It's very interesting, it has a lot of potential. I think this is a

very cool discovery. Other people have worked on these for other purposes, but this is the first I know for flu. It looks great, it made the news, lots of news outlets have covered it.

Michele: I was disappointed they didn't have a name like Flink. (laughs)

Michael: (laughs)

Vincent: Yeah, right. (laughs) I have one word for you, Flink.

Michele: Double layered protein nanoparticles just doesn't roll of the tongue quite as well.

Vincent: No, it doesn't.

Elio: So Vincent, are you going to discuss this paper over at This Week in Virology?

Vincent: No, this is a TWIM paper. Just one, yeah.

Michael: I beat 'em to it, Elio! I got it for us.

Elio: Yay, yay, yay.

Michael: I got it for us.

Vincent: I don't like to double dip because there is so much out there that you don't have to do that. But Michael got it, I said okay, and I think--

Elio: It's okay to discuss a bacteria paper at TWIV.

Vincent: Sometimes we do bacteriophages on TWIV, right, but we don't do pure bacteria because that's really TWIM. But as you'll see, we have a letter. I don't know if we will get to it today, from someone who said they love the cross discussion, we had Despommier on TWIM a while ago, we talked about a virus on TWIM. They said they really liked that. So I'll read a couple of emails, this one is from Anthony, we actually have two from Anthony. He writes:

Late last night in that little interval between exhaustion and sleep after showering, I take a cat for a little walk in the hallway, I then sit on the steps with her for a few minutes. My thoughts strayed to Chimpanzees, wondering how they bear a tropical climate without bathing. Might there be something in their skin microbiome that naturally cleanses? Might here be the means to wage war against MRSA, analogous to the Merck's goldmine found just under the green of a Japanese golf course?

And he is referring to ivermectin.

I'd forgotten my conversation with myself until you mentioned in TWiM 168 about the dairy farmers showering less than once a day. Might the richness of their superficial flora extend past the nose? Might that make less frequent bathing possible?

Michele: Ooh, I like that idea. (laughs)

Vincent: I don't know, it's a good thought. I don't know if chimps care how they smell, right?

Michael: Probably not.

Vincent: It's probably a human thing. Someone pasted in about that--

Michael: That was me.

Vincent: Tell us what that is, Michael.

Michael: Elio, do you know a Richard Gallo at the University of California San Diego? He is chief of dermatology.

Elio: Yes, I do.

Michael: He said good bacteria are educating your own skin cells to make your own antibiotics and it has only been in the last hundred years that we have made bathing a daily practice. Are we overdoing it? So Gallo believes showering not only removes lipids and oils that keep your skin from drying out, showering also removes some of the good microbes and I know that you will actually change your flora and you will get more nitrogen consumers. The ammonia oxidizers will actually begin to come up on your skin. In fact, that should have been one of the questions we asked our astronaut on TWIV when she joined you at ASM. How do they deal with the whole issue of not showering daily?

Vincent: That's right, nothing they can do about that. I think it smells on the space station.

Michael: Yeah.

Vincent: Of a variety of things, yeah. So Anthony also sent a Times article of a few weeks ago, it was an article about E. coli deaths from contaminated romaine and Anthony asks, would rinsing help? Michael, I think you put this information in here.

Michael: I did, from the USDA of washing produce, and the United States Food and Drug administration strongly encourages us to indeed wash our fruits and vegetables before eating.

Vincent: So if they had washed the lettuce would it have helped, or, it seems to me you can't get everything

Michael: You can't, you can't scrape it all the way off, and it really depends on the initial dose that has been associated with that contaminated romaine.

Vincent: So if you could get it down in numbers you would probably help yourself out, right?

Michael: Yeah, because remember for normal E. coli you need to ingest about a hundred thousand in order for it to manifest disease. Some of the E. coli with the hemorrhagic toxins that are in them, like O157H7 you only actually need between 1,000 to 10,000 to ingest in order to manifest disease.

Michele: And that has more to do with the bacteria's ability to tolerate the acid bath in our stomach than it does the toxin.

Michael: That is true.

Michele: So acid resistance is a serious virulence trait for these--

Michael: Enteropathogens.

Michele: Enteropathogens, that's the word I wanted, thank you.

Vincent: I also recommend that even if the bag of lettuce says pre washed, just wash it.

Michele: I do.

Vincent: Because you just don't know. Michael and I were at a talk at ASM one year, someone from the USDA I think showed a picture of a bag of pre washed lettuce and one of the leaves was just covered with dirt inside the bag, so don't trust them. I recently went to an indoor farm in Brooklyn, we did a podcast there a couple of weeks ago with Dickson Despommier and they grow lettuce in hydroponics, there's no dirt, and they gave us a box of lettuce to take home and I still washed it because who knows who is touching it, if they washed their hands. We have an email from Shinichiro Enomoto who was one of the co authors on a paper we did a couple of weeks ago about the path to endosymbiosis. He writes:

Thank you for discussing our paper. I used to listen to TWIV and TWIM regularly when I had a long commute. I would have loved to hear Elio's thoughts on our work. I am his fan and do not mean any disrespect towards Dickson. As per Dickson, we would love to know where *Sodalis praecaptivus* lives after injection.

Elio was out for that episode.

However, injection into the thoracic cavity is artificial and we don't know the true lifestyle of the *S. praecaptivus*. With these caveats, I imagine that the bacteria live throughout the hemolymph, as I have recovered colonies from an amputated leg. We don't know if they are intracellular, though we have preliminary data that suggests that the bacteria can survive for few days in mouse lymphocytes.

We regard the *S. praecaptivus* as a "protosymbiont" and consider it ancestral to many of the insect symbionts. The two relatively "young" symbionts, *S. glossinidius* (of tsetse fly) and *Candidatus Sodalis peirantonius* (of rice weevil) have different homes and lifestyles. *Ca. S. peirantonius* lives in bacteriomes, near the gut and near the ovaries, it is suspected that the one near the ovaries gets transmitted and one near the gut provides amino acids.

In contrast *S. glossinidius*, lives in the hemolymph and bacteriomes have not been detected. And this bacterium is facultative and has been cultured without the host. Moreover it was recently shown that even paternal transmission can occur.

Given enough time, *S. praecaptivus* appears capable of evolving into different lifestyles. But it is also possible that there are many protosymbionts that are already specialized towards different insect hosts.

Shin Enomoto. That's cool. I love when the authors write in.

Michele: Yeah, and really elevate the level of discussion for us, that's great.

Vincent: Elio, I don't know if you caught it, but he said he is your fan.

Elio: I'm glad to hear that, I'm becoming his fan. It's mutual. This is lovely stuff that he shared with us.

Vincent: Alright, let's end it there. We have a few more, but send it in, you can send us emails, twim@microbe.tv, and you should subscribe to the show so you get every episode as it is released, and if you like us a lot consider supporting us financially. You can go to microbe.tv/contribute for different ways that you

can do that. Today we have been blessed to have on our show Michele Swanson from the University of Michigan, thank you Michele.

Michele: Thank you!

Vincent: Elio Schaechter from Small Things Considered, thanks Elio.

Elio: My pleasure. Thank you.

Vincent: Michael Schmidt is at the Medical University of South Carolina, thank you, Michael.

Michael: Thanks, everyone.

Vincent: I'm Vincent Racaniello, you can find me at virology.ws. I'd like to thank the American Society for Microbiology for their support of TWIM and Ray Ortega for his technical help and Ronald Jenkees for the music you hear at the beginning and end. He is at ronaldjenkees.com. Thanks for listening everybody, we will see you next time on This Week in Microbiology.

(music)

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Transcribed by Sarah Morgan.