



# WINN FELINE FOUNDATION

For the Health and Well-being of All Cats

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## **40<sup>th</sup> Annual Winn Symposium – June 28th, 2018** **“Perplexing Paradigms of Feline Medicine”**

### **Transcript of Audio: Introductions, Dr. Katie Tolbert on “Exploring new therapies for a common cause of feline infectious diarrhea”**

#### **Introduction**

#### **Mr. Steve Dale:**

I want to welcome you to the Winn Feline Foundation 40 – you heard me right; the 40 Annual Symposium. Most of you in this room aren't even that old, right? Right! Thank you, yes! I knew you would agree!

The Winn Feline Foundation I will talk about and tell you about in just a little bit. This symposium has I just said there has been four decades of symposiums that were an idea of someone in this room to educate breeders in particular but also veterinarians and cat enthusiasts in general about cats. Forty years ago cats were thought of very differently than they are today. We didn't know much, we still don't know enough. Our two speakers are going to educate all of us about a couple very important topics. The Winn Feline Foundation has been around for 50 years. This is our 50 Anniversary. [applause] I saw this one individual in the lobby yesterday and I was talking to her and then someone came up and gave her a hug. Then 20 seconds later someone else came up and gave her a hug. Then someone else came up and gave her a hug. The last time I saw her, I had the pleasure of giving her an award. She just received an award; I think it was about 10 days ago. I think now it gets to the point where she goes to shop at the food store and she turns into the aisle where the cantaloupe is and she gets an award. [laughter]

I can think of no one more deserving and I am talking slow and spending time on this because this person deserves our time, our attention and she has our love and now our applause. You know who I am talking about Joan Miller. Like royalty. In fact I do want to say I have met very few people with determination like Joan has who has made a difference for cats but that determination matters in her own life right now. Without Peter, I'm not sure it would all be the same. If Peter were not a member of your family, you are a member of our family. Thank you very much! Now for what we are about to do here today. First of all Winn Feline Foundation is yes indeed celebrating 50 years and we have some very special people right here in this room from the Winn Feline Foundation. I will start off with members of the board of directors. I will let you stand up and in fact I would encourage you to do so. [applause]



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Janet Wolf in the back of the room, stay standing. At lunch today (don't move Janet) we were talking about the history of the Winn Feline Foundation. It turns out this organization, this nonprofit organization that has been around for 50 years has only had three executive directors. Janet Wolf was one of those three executive directors who cares so much that she is currently, and has been for several years now, the secretary of the Winn Feline Foundation. Thank you very much Janet! [applause]

Our current executive director is in so many ways a very special person who has elevated the Winn Feline Foundation in many ways. The way which us board members care about the most is we are raising more money than ever before to help cats. A round of applause, please, for Dr. Vicki Thayer. She also serves as our videographer. That is on Facebook Live so I have an idea. On the count of two say hello to everyone on Facebook, okay? One, two – hello Facebook! You don't get that everyday Facebook.

Now I do want to thank one other person who is a board member but he is not listed as a board member. Whenever Dr. Thayer is at any event anywhere in any part of the country including today at this event setting up all the technical stuff is her husband Bob Thayer, thank you! [applause]

In particular, I've got only two more to go, I promise. This does matter to me and it matters, I hope, to all of you. The president of the Winn Feline Foundation who is someone I knew, I am speaking for the entire board. It is with gratitude that you are willing to do this, Dr. Glenn Olah. We asked Dr. Glenn to serve for another 78 years and he declined. I don't know why. We do have an incoming president and that is Dr. Drew Weigner. [applause] In fact if you are from the Atlanta area, he is Atlanta's own Dr. Drew Weigner. The Winn Feline Foundation indeed has been around for about 50 years and this is our 40<sup>th</sup> annual symposium. We have been making a difference for cats for a very, very long time. That is our mission statement, Winn Feline Foundation advances feline health by supporting research and education. Is your cat up there? We help all cats and it doesn't matter much whether it is a feral community cat, whether it is an owned cat, whether it is a homeless cat in an animal shelter. We are all about helping cats. The Winn Feline Foundation started with, can you believe, \$100. Then \$25 more dollars to build a fee to create the foundation. The first grant was for \$1000 back in 1971 for UC Davis to re-establish a cat research colony decimated by FIP. Our history as it turns out is correlated with FIP and as you heard, some of you, last year in Chicago we are on the verge to finding something pretty amazing for a disease once called incurable or untreatable.

[Music Video]

I wear the Winn Feline Foundation on the sleeve of my shirt because every day I demonstrate how much I care for cat health. If you have support of the Winn Feline Foundation you have demonstrated the same.

We have funded so many amazing studies and there is so much exciting research yet to be done.

Your gift is extremely meaningful to the work that we do. It makes a huge difference to the lives of cats now and in the future.



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My personal thanks to all of those wonderful crazy cat people out there, part of our crazy cat family that have a lot of faith in Winn over the years and have supported Winn with their dollars and encouragement.

I so appreciate all the donors that donate to fund important research through the Winn Feline Foundation.

It is all immaterial without the dedication of our donors. Our donors truly are the ones that provide this type of cutting edge medicine for felines.

In particular all of the donors for allowing us to do the work that we do in support of every cat every day, our greatest thanks goes to the donors who support every effort that we have been able to make over the last 50 years.

I am truly honored to be a part of Winn to be able to serve our donor base and have a common dream of having healthy cats in the world.

I want to personally thank all the donors out there for all that you do to support the Winn Feline Foundation. Without their help we could not provide the help to felines that we do today. You have made a difference to pedigree cats, you made a difference to cats in animal shelters, you have made a difference for community cats. The Winn Feline Foundation is about all cats every day. [applause]

Can you hear me? Yes. It is just easier for me if I do it this way. Maybe it will pick me up a little bit, I don't think we need it.

This very complicated graph essentially shows this, in 2006 we were over here as far as fund raising. Today we are up here. Translated, here is what that means. That is the amount of money (woohoo!) that the last funding period the Winn Feline Foundation was able to grant, that amount of dollars. Hopefully, that is a number that is halfway to where we want to be in a couple years. We are determined to get there to where we could gift a million dollars. That is our goal to help cats during one funding cycle. I think we are going to be able to do it. Of course we can't do it without your help. We can't do it without the help of your friends, relative and neighbors. This is the Winn board of directors. By the end of 2018 Winn Feline Foundation will have funded over six and a half million dollars of research grants in its 50 year history. It started with, how much did I say? It was like a hundred bucks. That is not bad is it? I am very proud of the fact that every day, every cat somewhere in the world, I don't care where that cat is on the planet, has benefitted from studies that we have funded. That is a fact and for me to be a part of that board, I'm very proud of that. These are the speakers that I will introduce you to today and we are celebrating after this! This is our educational event, but you must go to the after party. [laughter] The after party and none of this would happen without our sponsors of which we have many and which we are grateful for. While we are grateful to all of you who I know give dollars to the Winn Feline Foundation we are always looking for time. If you want to volunteer in some way we have lots of committees which you can indeed volunteer. That after party I mentioned, that would not happen without the graciousness and the generosity of IDEXX. I want to thank IDEXX for doing that. [applause]



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IDEXX is just one of the companies in veterinary medicine that has been supporting the Winn Feline Foundation now for many, many years. With gratitude we are able to drink because of IDEXX. [laughter] You know, on second thought I am not sure that is the message they want me to convey, I don't believe.

This in fact was my cat Ricky and we began because Ricky died of heart disease, the Ricky fund to raise money for feline hypertrophic cardiomyopathy. Similarly there is a fund called the Bria Fund and that raises money specifically for funding FIP studies. I don't know, Joan Miller, you have been around just a decade or two, could you have envisioned one day on day a speaker standing here from Winn saying, "You know we are on the verge of finding a treatment that works for FIP." That is pretty impressive. I know, I see some tears there that are going to make me teary. [applause]

I don't know your password Glen, and you are not going to tell me and tell all of them. I don't know why. [laughter]

I believe my first job is to introduce a new mom, I hear. Dr. Katie Tolbert completed her veterinary degree in small animal medicine and surgery, internship at the University of Georgia. She then completed a small animal internal medicine residency at North Carolina State University. Following her residency she pursued a PhD in comparative biomedical sciences at North Carolina State University. She is currently on faculty as an assistant professor at the University of Tennessee. [applause] Actually, now I don't even think she needs to come up and talk. [laughter] Her clinical research program is focused on small animal gastroenterology with a special interest in the investigation of the efficacy of antacids, drugs that help with intestinal problems. She has been looking into GI therapy for cats that have runny stool problems for many, many years and we have funded you before and we are so happy you are here. Please help me welcome Dr. Katie Tolbert. [applause]

## **Dr. Katie Tolbert:**

Thanks very much Steve. Can you guys hear me okay in the back? Yes.

I asked Vicki what the crowd in the room was like, what kind of experience has she had. She said well you are going to have some veterinarians who have been practicing for a long time, you are going to have some young veterinarians, you are going to have veterinary technicians and then you are going to have people who just really love cats and want to make sure cats are as healthy as possible. I was like okay perfect. Let's see how I can address this room together. There may be some times where if you are a veterinarian, I simplify things too much for you or if you are not a veterinarian or a veterinary technician maybe you won't know what I have said and so at that point it would be totally fine to say what the heck are you talking about, please explain more. I think we are supposed to hold questions until the end but I would rather if you have a question that is related to "I don't understand what you are talking about" feel free to say that because probably 20 other people in the room also don't know what I'm talking about so I don't want that to go on for too long.

Today what I am going to predominantly talk about is some of the research that Winn has funded which is basically to look into treatments for what we call a common cause of feline infectious diarrhea and



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research which is looking for effective gastroprotectants for cats. For cats that have GI diseases, how can we make them feel better, can we stop them from vomiting? Can we get them to eat better? We are going to talk about a drug that we have investigated for that, specifically we are looking at cats that have kidney disease for that purpose. Then if we have time, which I don't think we will, Vicki asked that I highlight a couple cool studies. New stuff that is going on in just feline research which might be of interest to the group, I have a long list. My guess is that I won't get through all of it but we will do our best.

Why even care about trichomonads which is what we are going to talk about today?

Well trichomonads are these really cool predacious lumen dwelling protozoa. When I say lumen dwelling, I mean basically they get into the GI tracts, the lumen of the GI tract and then also the reproductive tract. The reason why they like to be there is because they like really warm, anaerobic regions and those are basically the warm anaerobic regions. When trichomonads first were discovered it was actually these venereal trichomonads that were the most widely studied because people got this. People get an STD, it is actually the most frequent non-viral sexually transmitted disease. Don't worry we're not going to talk about it for too long. It is called *Trichomonas vaginalis*. The reason why that is really important and I'm sure you guys understand, when we study things in animals we need the ability to say "Well how does that relate to humans?" Because if we want to try to make a huge difference on a global scale what we need to try to do is get y'all's support to start investigating some of these things and then attract people in industry who are like "That might be something we are interested in to, because it might not only help cats but also humans." Trying to show that cats also have some diseases that are important for humans is really good because that means help in the order of millions of dollars.

*Trichomonas vaginalis* is the most frequent non-viral sexually transmitted disease and it is a really important one because it causes infertility, abortions and it also increases the risk of HIV and can cause cancer. Along the way they also discovered that a protozoa parasite called *Trichomonas foetus* was a venereal pathogen of cattle and did similar things as *Trichomonas vaginalis* in people. It also caused abortions, infertility and that lead to a really considerable economic loss in the feed lot industry. It wasn't until about 1999 that an investigator at NC State named Jody Gookin, whom many of you may be aware of actually identified that there were trichomonads in the intestinal tracts of cats. They weren't really sure what these things were doing there. Are they just hanging out? Are the commensal or are they actually causing a problem? They did find that they were highly similar so when you looked at them under a microscope they looked like the Trichomonads that you could also find in cattle. But, they had this unique tropism, so this unique niche where they would go into the intestinal tract rather than the reproductive tract like in cows. Then subsequently Dr. Gookin showed that not only can you find this in cats but you can find it in a lot of cats. She did some work looking at sort of pure breed cat shows and what she found was that up 30% of cats actually had this protozoa kind of hanging out in their intestines. That wasn't a very good thing because then she discovered that this pathogen that is kind of hanging out in these cats that are going to these cat shows... How many of you have been to cat shows? [laughter] Up to 30% of cats are infected with this parasite that is highly contagious, that is not a very good thing.



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What she was able to show was that unfortunately in some of these cats, and we don't know why some cats show signs of infection and some don't. It could cause this chronic foul waxing and waning diarrhea that could last for the cat's life. These cats could become subclinical meaning they didn't have diarrhea and they looked completely healthy but they still had this bug in them and then years later they could break with diarrhea again. Not only was this in catteries but it was also in shelters. Why do you think this was a big problem to find that this was a highly prevalent, highly contagious bug in shelters? Why was that problematic?

Who wants to adopt a cat that has the most foul-smelling diarrhea that you have ever smelt? No one. It became a really big problem because a lot of these cats were being euthanized because of this parasite. The other thing that was a problem was not only was it highly prevalent and highly contagious is that there was only one treatment available. Currently there is only one treatment available and that is a drug called ronidazole. How many of you have heard of a drug called metronidazole. We're all like yep. [laughter] I have a stock of it in my cupboard, me too! Ronidazole is very similar to metronidazole but it actually can treat some *Tritrichomonas foetus* so this pathogen that we see in the cat. The unfortunate problem with ronidazole is that yes sometimes it is really effective but then in certain cats it actually can cause side effects, things like neurotoxicity so that is basically where it is causing a problem within the brain and spinal cord and these cats can be very uncoordinated and can have seizures and that sort of thing. The other problem is that we are recognizing more and more that cats now have this infection that is actually resistant to ronidazole. How many people want their cat to have an infection that can't be treated by anything that causes diarrhea that could last up to two years and then they can become what looks like healthy but then years later break with that same problem and have that diarrhea again?

No one, so it is kind of a bad deal. There is a need for more effective therapies but unfortunately the pathogenesis, essentially how this parasite causes disease has been relatively unknown. That was really sort of where I came across Jody Gookin. I was like, "Man, this is really cool, this seems like something that we need to study." Let's study this parasite and try to understand it better. In my PhD I studies feline *Tritrichomonas foetus*. What we discovered is that adherence, what I mean by adherence is basically this parasite we think is spread fecal orally. Do you guys know what I mean by that? You go in the litter box, you do your business, somebody else goes in the litter box and they had this infection and they are dancing around the litter box. Then you are like that is cool, okay. (Licking) then you have the infection.

What we discovered is that it gets into the intestine that way. It is hanging out in the intestinal lumen and then what we notice is it seems to infect the last part of the intestine so what we call the distal small intestine and the first part of the large intestine, we call it the colon. What you can see here is basically where we took a cat that was naturally infected. It had to have biopsies and so this is basically the intestinal lining that you can see in blue. The red bits here are all the feline *T. foetus* pathogen just kind of lining, coating basically this colon. What we discovered is that this parasite, this bug basically kind of intimately associates itself with that first part of the intestine. Here we can see on what is a transmission electron microscopy image so this is basically the colon. This is the intestine and these are those parasites. These parasites have these little flagella on their head and they use this flagella to move around and stick to the intestinal lining. That is basically what we see here, those parasites



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underneath the intestine. They kind of stay in the lumen and they adhere. When they adhere they cause inflammation and we think it is this process that actually contributes to their diarrhea. If adhesion is a critical first step in causing disease, if we could find a way to stop the adhesion potentially we could stop the disease. That was sort of what my PhD work was about. The additional part of my PhD work was not only understanding how does *T. foetus* cause disease but also how could we study *T. foetus* without having cats? It would be ideal that we kind of test some of these ideas and these hypotheses without using live animal models. The other part of my PhD was essentially to kind of create and validate a way to study this in the lab so that we can start looking for targets that might impact adhesion and then those would lead to therapies for these cats. What we did was we established this model so essentially we have this model system where we actually take these trichomonads, this bug from cats that were naturally infected, we take it right out of their feces and then we basically have a model system where we can kind of recreate the intestine and evaluate what is going on. Here what you can see is these trichomonads of feline *T. foetus* in green and the intestinal cells are in blue. Using this model system again we determined that adhesion is a critical first step in causing disease. We also determined that adhesions occur via specific receptors.

Do you guys know what I mean when I say the adhesion occurs via specific receptors? It's kind of like Lego. A Lego fits only in a certain way. It has the pointy out thing, I'm a mom you can tell. [laughter] The pointy out thing and then something that goes on top of it. You can think of Legos as essentially being a receptor ligand interaction. That is basically what *T. foetus* is doing. The intestine is expressing receptors and the *T. foetus* has the ligand. That suggests then that if adhesion is a critical step and this adhesion occurs via these specific receptor interactions potentially if you could find what receptors are important you could decrease clinical signs of disease. Our objective for helping cats is and has been to try to identify these targets and then to evaluate if these targets could be developed into novel therapies for feline trichinosis. That is what Winn has been helping us for many years to do. I don't have time to talk about all that stuff but I'm going to talk about some cool stuff today.

What I'm going to talk about is cellular proteases, and I will tell you what I mean by that, and probiotics. What I am not going to discuss is we have also identified the specific receptor on this parasite that may also be important. I told you that some parasites, some trichomonads, this *T. foetus* is resistant to ronidazole. At what that suggests is like many parasites, like may round worms for example, helminths. They use different ways to cause disease and so to attack them sometimes what we have to do come at them from different angles. Just doing one angle may not work and may induce resistance so that is sort of the goal if our research is to find all of these different targets. All of these things have been supported by the Winn Feline Foundation.

Let me start off by talking about these pathogen proteases. There is a growing interest in the role of cellular enzymes in many different things. For example AIDS, giardia, leishmania, all sorts of infections and we know that those infections establish disease by producing cellular enzymes. One of these cellular enzymes is called proteases. We became particularly interested in them because we discovered by looking at the literature that many intestinal parasites use these cellular proteases and we said "Well maybe *Tritrichomonas foetus* does?" On further reading we found that cystine proteases were identified as virulence factors for bovine *T. foetus*. In previous work on my PhD we established that *T. foetus* also uses enzymes to help establish disease.



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The problem is you can inhibit, you can completely ameliorate, meaning you can completely prevent disease at least in the in vitro model, the lab model that we have by broad spectrum protease inhibition. What do you think is the problem with that if I took a broad spectrum protease inhibitor and gave it to a cat? Does anybody have a problem with that? [laughter]

Cats, humans, dogs whatever also make proteases. You can't just carte blanc inhibit everything, you are going to kill the cat. You will kill the parasite but the cat will also be dead. That is not ideal.

Cats also produce necessary cystine proteases so what we had to try to figure out is which ones are the most important and can we find ones that are redundant in the host but not redundant in the parasite such that when you inhibit that then the parasite is dead but the cat is not. We knew that specific cystine proteases were found to mediate bovine venereal trichomoniasis. We were like well, it seems like it is reasonable then to think that there are specific cystine proteases that are really important for feline trichomoniasis but which ones? We started to search out and I am not going to go too much into this but basically what you are looking at hear is called a zymogram and essentially it is a way of looking at proteases. What we did is we took those feline *T. foetus* parasites and we took all their proteases and we ran them out on a gel and using different methods we established that they produced two different kinds of proteases, those being serine proteases and cystine proteases and we extracted those cystine proteases and said "Wow, there is a lot of cystine protease 30. Let's take a look at cystine protease 30 and see if we can target that specific one and that will be helpful." We looked at that parasite and we had an antibody and basically showed that yes again that feline parasite expresses a lot of CP 30 which you can see in green.

What I'm going to show you here, I'm not going to belabor it too much but what we have here is that our in vitro model so let's just focus in right here. This is our intestinal epithelial cell, this is our intestinal lining. Can you see this in the back? The kind of cobble stone morphology, is that coming through for you guys? Here is that cobble stone morphology when you have these little bugs, this *T. foetus* on top of it, it is completely obliterated. When you then treat that with an anti-CP 30 antibody you inhibit that CP 30 antibody and you restore the intestinal epithelium. Again, what this shows us is that CP 30 is a really, really good target for feline trichomoniasis. However, if you look back again it doesn't look exactly like this. It is not perfect, it is pretty close but it is not perfect. What we showed is that inhibition of feline TP to CP 30 does significantly reduce much of this intestinal damage but not all of it. Other players are probably involved and a multi-targeted therapy approach is probably going to be necessary. Then we said okay, let's find additional targets that could also be used. Then we said probiotics.

The other day I was at a restaurant I ordered a lemon grass tea. The lemon grass tea came and I was opening the package and the lemon grass tea package said "plus probiotics." Probiotics are now in everything. Then we were like well people say that probiotics work for *T. foetus*, let's check it out.

This was funded by the Winn Feline Foundation so just so we are all on the same page, what are probiotics?



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Probiotics are live micro-organisms that are administered and the hope is that they confer some sort of benefit to the person or cat that they are administered to. The idea is that we hope they affect the host immune system so that it has a favorable environment. For example if you have Crohn's disease the hope would be that it would make it an anti-inflammatory environment. If you have cancer, the hope that it would be an anti-neoplastic environment. All of these we hope that they confer some sort of benefit to you depending on what is going on. The other thing that we know about probiotics is that they compete with pathogens. They are often times bacteria and those bacteria basically kind of kick out other bacteria or pathogens. Most probiotics contain lactic acid producing bacteria. When you go to the grocery store and buy probiotics or you are prescribed a probiotic through a veterinarian they are usually going to contain lactic acid producing bacteria like *enterococcus*, *lactobacillus* and *bifidobacterium*. The reason that they have those is because those are normal inhabitants of the colon and we know, especially in inflammatory diseases like IBD those tend to be lower. We were particularly interested in looking at lactic acid producing bacteria for feline *T. foetus*. We knew that resident intestinal bacteria probably play a pretty significant role in this disease because clinical signs of feline trichomoniasis improve with antibiotic therapy. That would suggest something is going on with bacteria. Diarrhea recurs during times of stress or diet change and that is probably because of a result in the level of resident bacteria. We were like "Let's look at some probiotics, which probiotics should we look at?" Well we decided to look at enterococcus. The reason we decided to look at *enterococcus* is because well there is already a probiotic available that we can look at which is really nice. It is called FortiFlora or *Enterococcus faecium*. That is an already commercially available probiotic for cats. Then we said, "Well what else can we look at?" We decided to also look at a bug called *Enterococcus hirae*. They are both *enterococcus* but they are different species of *enterococcus*. The reason why we looked at *Enterococcus hirae* is because that was cultured from healthy kittens. There have been studies that looked at kittens that were healthy but euthanized because of over population in comparison to kittens that died as a result of severe diarrhea. When they looked at those two different populations they discovered that the healthy kittens had a lot more *Enterococcus hirae* compared to the kitten that died of diarrhea. *Enterococcus hirae* was really interesting to look at.

What we wanted to do was evaluate the effect of these *enterococcus* probiotics on *T. foetus* growth, on that adhesion that we were talking about and eventually we wanted to look at cytotoxicity. We wanted to compare the effects of this commercial probiotic, this FortiFlora to this novel probiotic called *Enterococcus hirae*.

Here what you see is a *T. foetus* growth curve and this is the number of *T. foetus* on the Y axis and time and culture on the X axis. What I can tell you that I don't have pictured here is when you add *enterococcus* of any flavor to the *T. foetus* cultures so both are added at the same time, *T. foetus* never grows. It completely inhibits its growth. They we were like, "Well we need to see what is going on." We decided to let *T. foetus* kind of have some time in culture before we add the *enterococcus*. When we did that we saw that again it has a negative effect on growth. Here is *T. foetus* alone and here is *T. foetus* after you add the *enterococcus* so you can see it has a negative effect on growth. We were like "Why is that happening?" Is the bacteria outcompeting for nutrients, is it producing something awful in the environment, is there some sort of direct interaction? What we did was we took the bacteria and we heat killed it. We made it nonviable. Then we added it to the



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cultures. What we showed is when you add the *enterococcus* that are non-viable which is this line here. This is *T. foetus* alone, *T. foetus* with non-viable *enterococcus* and then *T. foetus* with live *enterococcus*. What you can see here on this growth curve is that when *enterococcus* are dead, it doesn't have any effect on *T. foetus* growth. What does that tell you? For the veterinarians in the room, what does that tell you about probiotic administration first of all?

You got to make sure it is viable, and that is a big problem with using probiotics that are not from reputable companies. When clients tell me they want to buy Joe Schmo probiotic from CVS, I'm like, you can do that but I would rather you use companies that actually produce studies that show me there is good quality control. You may just be giving a really expensive placebo. What we can see is that this basically says you are giving a really expensive placebo if those *enterococcus* are not live because this is an active process. Something is going on where they are actively inhibiting the *T. foetus* from growing.

Let's take a look at that, what is happening?

This is what is called scanning electron microscopy. This is one of those *T. foetus* that I showed you before. Remember the flagella? Right here, it has three flagella on the front and a big old flagella on the back. This is its undulating membrane that kind of makes it move like this.

This is *T. foetus* plus *enterococci*, what can you see?

It is surrounding that flagella, that's right. Basically for lack of a better word opsonizing, it is attaching to the flagella and interfering with that parasite. Then over time, check that out. That is *enterococcus*, where in the heck is the *T. foetus*? It is spludged all over the place which is pretty frickin cool.

We were super excited about these results, we were like "Dude, this is the next coming, it's amazing! Let's check this out in our model." So we did and what happened? Nothing!

Here is an adhesion of *T. foetus* to the intestinal cells. Here is *T. foetus* only and here is *T. foetus* plus *enterococcus*. You can see that *enterococcus* does do a little bit to interfere with adhesions but it is not that convincing. This is when they are both added to the cultures at the same time. What happens when you add *enterococcus* first? Let the *enterococcus* do its thing for a minute on top of the intestinal cells and then add *T. foetus*? That is what happens, you almost completely inhibit the parasites ability to adhere to the epithelium, to the intestinal lining. What does that tell you as a veterinarian? What do you think is the best place to use probiotics? Pre-emptively! If my cat is going to a cat show and I am worried about *T. foetus*, that cat is getting probiotics. If I am the veterinarian of a shelter and there is an outbreak of diarrhea the ones without diarrhea are going to get a probiotic because there is a potential that I might be able to stop the infection before it takes hold. Again this is a stretch because this is lab stuff that I'm talking about. I don't know, is that true when you actually take it into a cat? I don't know but it is convincing at least in our laboratory model.

This is basically kind of showing us that. We are looking back at our, this is what we call immunofluorescence and I show this because they are prettv pictures. The intestine. again. remember



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*foetus* jamming all over the place on the intestine. When there is enterococcus that is allowed to adhere to the intestine maybe it occupies some of those receptor sites, I'm not sure exactly what is happening but it is largely preventing the ability of the *T. foetus* to adhere to the intestinal epithelium. Then what we did is we said, "Okay, we looked at *Enterococcus faecium* and we are really interested in *Enterococcus hirae*, is there any difference between the two?" The answer is yes. Repeatedly what we see is that *Enterococcus hirae* no matter what parasite you use and we have many different trichomonads in the lab that we attempted in cats including ronidazole resistant strains. It doesn't matter if it is ronidazole resistant or not, the same effects are observed. Where it does decrease adhesion is if you add the *enterococcus* before infection and that *Enterococcus hirae* is always better. What does that tell you as a veterinarian? I'm not going to say anything about FortiFlora, what it tells me as a veterinarian and I consider myself a gastroenterologist. What it tells me is just because the owner tried a probiotic and it didn't work doesn't mean that probiotics are not going to work. It may be that the bugs that were in that probiotic didn't work. You tried bifidobacteria, maybe you needed something with *enterococci*. You tried *enterococci*, maybe you needed something with *lactobacillus*? You tried *Bifidobacterium breve* and maybe you needed some other *bifidobacterium*, right? I think that is really important as well.

We showed this in a ronidazole resistant stain as well which I'm not going to talk about as I'm hoping I'll have a few more minutes, 10 minutes or something like that? [No, you have 20.]

What are the conclusions?

I've really just kind of quickly summarized a few of the things that Winn has supported our lab doing. There is a lot more I could have shared if we had, like 20 hours.

Feline trichomoniasis is a highly prevalent disease. No current therapy is 100% safe and effective. Through Winn funded research our lab has identified several novel targets that show really great promise for the treatment of feline trichomoniasis. I think sort of the next step is to take it all together in the lab. What if you add an anti-CB30 antibody with *Enterococcus hirae*? What does that do to adhesion? What does that do to cytotoxicity? We haven't done those experiments yet but that is coming. A multi-targeted approach will likely be necessary.

Now I'm going to shift gears really quickly to talk about some of our clinical research. My bench top research that I just showed you is really about infectious diarrhea in the cat and then my clinical research is about finding gastroprotectants in cats and dogs. We just finished a study with Winn support to look at the frequency of oral famotidine administration to see if that affects tolerance in cats. How many of you use famotidine, how many veterinarians in the room? What happened to all the veterinarians? There seemed like there was a lot more before. Famotidine is Pepcid. How many of you who are not veterinarians or who are veterinarians give Pepcid to your dog or cat when they vomit or do something like that? Pepcid is frequently given to cats by owners, veterinarians, veterinary technicians and the reason is because cats get a lot of GI diseases, they vomit, they have dysrexia, they have pancreatitis, they can get metabolic diseases. In fact in one study 27% of cats that have chronic kidney



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disease were getting Pepcid, but the efficacy of long term use of this drug is really unknown. It is really common that veterinarians give this drug but we don't know if it works and we have a lot of concern that it actually may not work very well over time. The reason we think this is because of some stuff in humans and a paper that we published in 2017 that was on dogs. Essentially what we did is we measured their intragastric pH using a very small, noninvasive pH capsule that the use in people to detect GERD. You can do the same thing in dogs and cats. We measured the pH and when we looked at their pH over time, here is placebo, here is Pepcid, what we found is Pepcid is really good at suppressing acid on day one and two. It is actually a really effective acid suppressant. However, by day 12 and 13, it starts not working anymore. These guys are developing what is called tolerance which means the more you give it, the more tolerant they become to the drug. We look to see when does this actually happen and we think it probably happens as soon as day three in dogs on therapy.

We were interested to see if this happens in cats and if it happens in cats is there anything we can do to help it not happen in cats because Pepcid is used so frequently. We came across this study in people that looked at daily and every other day administration of basically Pepcid. What they showed was, this was pre-, this is day one, this is day 21 and essentially what we have is basically the higher the number, the higher the gastric pH, the more acid suppression. What you can see is the Pepcid was really effective on day one but again by day 21 it wasn't effective anymore, people developed tolerance. However, when these guys got it every other day then they didn't develop tolerance. We were like "That's cool, is the same true for cats?"

That is what we did with this one Winn Feline Study, we said, "Okay, does daily famotidine cause tolerance in cats and if so, if you give it every other day can you prevent that from happening?" We hypothesized that it will cause tolerance in cats if you give it every day and if you give it every other day it won't cause tolerance. We did this in a crossover two way study. A crossover study just means that all animals receive all treatments. They serve as their own controls. It was a really nice study because you kind of minimize that inherent variability that happens across animals. We use 16 healthy cats and randomized them to either receive daily treatment with famotidine for 14 days first or every other day for 14 days. Then they crossed over and received the opposite treatment. How we do this is really simple, they have sort of a mild sedative that we use for all of our cats that come into the hospital at UT. We sedate them because it makes life much better for them and for us especially when they are getting radiographs and ultrasound. They get a mild sedative and they can collect blood and we place the capsule essentially as I'm going to show you with radiographs.

This is what this looks like, it has handles, a delivery device and the pH capsule is a small bit that you can see there. Essentially what we can do, they are sedated and we can actually pass it orally just like you were passing a stomach tube for example and here is that capsule in the stomach of a cat. Then we can remove the delivery device after we have adhered it and there it goes. The nice thing about that is it will stay in the stomach for several days and then it just passes over time. They poop it out, no problem. It doesn't cause a problem for the cats; you don't have to retrieve it. It is really nice. It continuously records pH really frequently.



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These are the treatments that we were looking at, daily or every other day. We fed them twice daily. We monitored their physical exam. We monitored their clinical signs like vomiting, change of fecal consistency, attitude, appetite and what we showed again here is the pH at or above 3. It was really, really effective on day one at suppressing acid. Then by day 13 it was terrible. No different than what we would see for basically a sugar pill. If you give it every other day they don't develop tolerance. This is a really nice finding for all of us veterinarians who want to use Pepcid in cats and dogs that we might just be able to give it every other day and prevent that tolerance effect from happening. Again, this is just kind of the same thing with a different pH indicator and again we see the same is true. You give it daily, you get tolerant, you don't give it daily, you give it every other day and they don't develop tolerance. When we looked at daily famotidine over time, just like we saw in dogs, we think that starts to happen somewhere around day two or day three.

Conclusions from this research; famotidine is a weak acid suppressant in cats. It is probably not an effective treatment for ulcerative disease in cats. It might help with things like bilious vomiting, vomiting syndrome, maybe chronic kidney disease and that sort of thing. It does have a diminished effect over time but every other day prevents tolerance. We give this orally, do we think the same is true with subcutaneous or intravenous routes? We do so if you are going to do something like that we would recommend using something else other than famotidine so using proton pump inhibitors for example or of it is something that you can use every other day then to use it every other day.

The other thing that I just wanted to mention that I think that the board knows but maybe you guys don't know as much is that Winn helps to train the next generation of clinician scientists. My PhD was supported by someone actually having a little bit of money to pay me to be able to do a PhD. It is really challenging to get money to pay for veterinary student research, to pay for graduate students, to pay for resident projects and so behind the scenes Winn is actually helping us to fund those projects and to get them exposed to research so that they too might want to become clinician scientists and help feline medicine. Here is just a list of all the students that have been in my lab that have been affected in some way by the Winn Feline Foundation research which is pretty awesome.

I think I am going to stop there just so I leave plenty of time.

**Mr. Steve Dale:**

Thank you! [applause]

**Transcript provided by Veterinary Information Network (VIN)**

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