So as a little overview of my topic, first I will give you a little bit of background on pathogenic E. coli. I understand that you guys are not all microbiologists, so I'm going to tell you a little bit about pathogenic E. coli. That's E. coli that causes disease in humans in particular. That's what we're going to talk about today and then, some specifics on sub-type of pathogenic E. coli called Enterohemorrhagic E. coli. How enterohemorrhagic E. coli causes disease in particular the shiga toxins that they produce that enable that organism to cause disease. Then I'll follow that with some research that I have had ongoing in my lab at Sac State including looking prevalent studies you see that's pretty much it, prevalent studies in ground beef in the Sacramento area, in free range grazing cattle, in Northern California, prevalence in clinical school specimens also northern to California and finally prevalence in horses and all that's prevalent of these enterohemorrhagic E. coli.
Background on Pathogenic *E. coli* 

So why -- so background on pathogenic *E. coli*. 
So first I want to tell you that there are good E. coli and there are bad E. coli.
Okay, these are the good E. coli and the good E. coli, the flagella is in black you can't see it very well, but they swim around and they have been very, very good. And we have known about the good E. coli for quite a long time. They are very important in colonizing our intestines. They are in all of us. We all walk around with the good E. coli and they are very, very good to have. Okay? They suppress the growth of pathogens because they take up space in food so other organisms that would normally cause disease we call pathogens can't do it. They make vitamins for us, great. Things that we couldn't normally make, they make for us. And they have been a model organism for bacterial genetics, as you guys may know from some of the courses that you've had. A lot of what we know about DNA replication et cetera came from E. coli. And most importantly for decades it has been an indicator species for fecal contamination in waterways and other places.

So my father is a retired civil engineer and he was thrilled to find out I was going to work on Escherichia coli, because this is an organism he learned about when he was in college taking Civil Engineering courses as an indicator species of fecal contamination. Because if you find E. coli in the waterways and some warm-blooded mammal or bird has been there pooping and the problem is not the E. coli but what else came along the way. And I use those words very easily, poop and stuff like that, so just be prepared. It's going to get worse [background laugh]. Okay, so we're not worried about the good E. coli. We're worried about what else came along with the feces, okay, maybe shigella, salmonella, some of these other pathogens that are not good for you and will make you sick.
So here is the bad E. coli, okay? Now as far as the diarrheagenic E. coli, these have only come onto the front page in the last -- probably since just in the last few decades okay. Before that we also knew that E. coli, some of the bad E. coli causes urinary tract infections and in fact it's the number one cause of a bladder infection, hands down, full on number one cause, is uropathogenic E. coli and that's what I was working on in Berlin when I was there. Similar flavor of E. coli also causes meningitis and endocarditis, septicemia which means it's in your blood, goes systemic and can kill you. But these are the ones that we're thinking about today, the ones that are causing diarrhea. And these are the ones that have made front-page news since the big outbreak in the 1980s and the ones that the popular press gets confused when people say there's E. coli everywhere. Well, yeah, there is E. coli everywhere because, remember, we all carry E. coli. It's the good E. coli. So usually, when people find E. coli everywhere, that's the good stuff, so you shouldn't be freaking out about it. Okay.

Okay. So speaking of diarrheagenic E. coli, it's really important to have a couple of community service messages in talks like this, and so you'll hear a few of these. So, when we think about diarrhea, we usually think it's something that we just ate. Okay, so suddenly you come down with big time -- maybe you call it food poisoning or you call it the flu, or whatever you're going to call it, you're vomiting, you're pooping all of the time, big time diarrhea, and you think, well, I just had pizza down at -- I don't know the Bear, does the Bear serve pizza?" It must have been that. Right? Well, in fact most of the time when you end up with diarrhea due to something you ate, it was something that you had a minimum of eight hours ago, if not a week or two ago.
So who can remember what you ate Tuesday, a week and a half ago for lunch? It's kind of hard to remember that. Okay? Some illnesses, some diarrheal diseases take that long. And why is that? The bacteria, [inaudible] is a bacteria has to go all the way down to your stomach, has to survive the acidity of that hostile environment, make it, some of them make it through that. Find their way to the intestines, grab on, come up close, replicate, colonize we call it, and start producing whatever they are going to produce to make you've diarrhea, okay, what we call virulence factors. So that takes some time and E. coli doubles about every 20 minutes so the pathogenic E. coli I'm talking about today, the enterohemorrhagic E. coli, it could take three to four days before you're going to start having diarrhea after you consume this one. Okay? So it's not what you just ate.

Now that being said, there are some food poisonings and they are really food poisoning because it's a preformed toxin that you ate. It was a bacteria that was growing in the food. This is usually a staphylococcus aureus that's growing in some cream base something or other and it's producing a toxin while it's growing. Botulism is the same way. The bacteria is growing in the food and then you eat the food and you get the toxin and then, if it starts you start vomiting almost immediately, which you know that's what it's because it's just a really bizarre -- if anybody has experienced this you know how bizarre it's. You're vomiting, you're vomiting, you're vomiting, and then all of a sudden you're done. You shower off and off you go. Have you ever had that? It's so weird. Okay, but that's a preformed toxin. It truly is food.
poisoning. The only way you would be exhausted and not able to go on off to work is because you've been working [chuckle] already at the porcelain. So anyway I just want to be really clear that it's not usually what you just ate unless you've that dramatic super antigen that's what's doing it to you vomiting experience.

Okay, so when we talk about diarrheagenic E. coli there are quite a few, did you know? It's not just one. There are many different. This is the one that you get when you go to Mexico. It's the number one cause of traveler's diarrhea -- enterotoxigenic E. coli. It produces a toxin much like cholera toxin. It gives you foamy watery diarrhea. I told you it was going to get worse.

Okay, this is the very first pathogenic E. coli ever identified. Who has studied microbiology in here? Okay, cool. So you guys know about MacConkey agar, at least the 75% of you that raised your hands. So, here's the thing -- MacConkey agar is a media that people used to isolate bacteria and it was identified by a fabulous doctor, MacConkey figured out that you could identify diarrheagenic bacteria because the one's that cause diarrhea don't ferment lactose, so you could use this really great media and that with a pH indicator and find out all -- you could screen essentially, screen away all of the E. coli, because E. coli ferments lactose. It doesn't cause diarrhea. Back in the day, that's what they thought. And -- but you would pull up salmanila shigella all of these other fabulous media. Well, here comes enteropathogenic E. coli, summer diarrhea, in a lot of kids. They couldn't figure out what was going on. This was in the U.K. They were running these MacConkey's and
everything is coming up black positive, black positive, black positive and then somebody, chance favors the prepared mind, Louis Pasteur, said, "Huh, I wonder if that's the one that's fermenting lactose that's causing the diarrhea," and it was. It was enteropathogenic E. Coli enter the scene. E. coli can cause disease. They carry a special bag of tricks, unlike the good E. Coli, okay? So that's enteropathogenic E. coli, still around and still causing summer diarrhea in children.

This is the one that we're going to spend a lot of time on. This is the one that's new for people who are microbiologists and pathogenic microbiologists. You want to pay attention to this one, Enteroaggregative E. Coli; brand new on the scene. Now we know it's the number two cause of travelers diarrhea, identified by its ability to when it colonizes the intestine it looks like a logjam or stacked bricks and so they call it aggregated occurrence. Stay tuned we’re going to talk a little about this towards the end. Enteroinvasive E. coli causes a shigella like illness in its pathogenic mechanisms where it invades like shigella does the intestinal lining and then diffusely enhances. Quite a long list, huh?
Here is the EI. It causes acute prolonged watery diarrhea greater than 14 days. Okay? That's prolonged, eh? We're finding it in HIV patients and infants. It's both in developed and developing countries. Lots of really large studies in developed countries, as well as, underdeveloped countries. In Guadalajara, Mexico 43% of the hot sauces came up positive. Just saying. Okay. Associated with malnutrition in children, this is primarily in third world countries. They are thinking because of this prolonged watery diarrhea, that they're malnourished, so they are stunting their growth. Interesting. Okay. And then I already told you that piece.
Okay, onto the big star, for me anyway. Enterohemorrhagic E. coli has lots of different names. EHAC of course, everything has an acronym. These organisms produce shiga toxins as I alluded to. And so you could call them shiga toxin producing E. coli which is a larger sub-grouping. EHEC is a part of the shiga toxin E. coli. Then we have the people who first identified the shiga toxins and called them verotoxins. So we have two classes of people that identified the toxin within the same year. Some persisted on calling them verotoxins and those of us who studied in the other camp called them shiga toxins. And nobody can come to the middle so we have two names for this organism. And then 0157H7 is the one you hear about in the media all of the time. That's the sub group of EHEC and it's a very specific strain on enterohemorrhagic E. coli that causes the majority of the outbreaks here in the United States. At least that's what we think because that's what we're looking for.
And it really made its debut in the national media in 1993 -- that was very fabulous for me, because it was precisely the year I was defending my dissertation on enterohemorrhagic E. coli and nobody knew that E. coli caused disease, not even my dad. He said, "Wait a minute I thought you were working on pathogenic microbiology?" "I am -- I'm working on E. coli." "That's not pathogenic." See how things have changed since 1993? Okay, so this was on the front page of the Oregonian where I was headed for my post-doctorate to work on salmonella and there was this fabulous, from the very bizarre odd perspective of mine, outbreak of enterohemorrhagic E. coli. I couldn't have done better timing for me. It put me in my work -- not me, but it put our research on the map of how important it was. Okay. Children were dying.
And then, even equally fabulous timing, in 1996 I was teaching a brand new Emerging Infectious Disease class and we had an outbreak of enterohemorrhagic E. coli. And so we got to spend the semester talking about how California and the United States was managing this outbreak and about my favorite pathogen, enterohemorrhagic E. coli.
Of course, Popeye played a big role in that outbreak, that spinach outbreak. [Background laugh]
Okay, but it's been more than spinach since 1993. We talked about the hamburgers, but there have been many, many outbreaks of enterohemorrhagic E. coli in the United States. Raw milk -- oh boy is that a problem. Pennsylvania has had multiple pathogens coming out of their raw milk. A lot of litigation there right now. It's a cocktail of fabulous things, if you like being sick. Wisteria, E. coli, salmonella I think gets some time in there too -- so all kinds of good things if you want.

Then interestingly there was an outbreak of apple cider and this was in Vermont. And nobody believed it because we felt that you know this is a fairly acidic condition and how could E. coli even survive that? But sure enough there was an outbreak. Then there was a following one, that made more national news with Odwalla. Now because of these outbreaks it has completely changed in our area, Apple Hill. In the gold country people go up and gather apples and get the cider. It's completely changed the way that apple cider is produced and so that's good to know.

Also water systems, there was a big outbreak in upstate New York and that was a problem. It was at a big fair. There have been multiple outbreaks with water systems -- but there was a big fair and people had loaded up their food trucks with water from a well and there hadn't been any rain in a while and a whole bunch of rain came and the wells from the ground water I guess had gotten contaminated with enterohemorrhagic E. coli and they carried those in food trucks to the fair. The big worry was where are those trucks going next. Luckily the outbreak was identified before it was passed on to yet another fair.
Okay, and then here is the best part for sprouts. People say, "Okay, I'm a vegetarian. No worries here." Well, we had the big spinach outbreak right? But we have had multiple outbreaks of enterohemorrhagic E. coli in sprouts and I have to say a cooked sprout is not a very nice or yummy sprout. So vegetarians are kind of out of luck here. It is in vegetables and unless you cook these items to 165 degrees, you're going to get sick. Okay, so one of the largest outbreaks was in Japan, school lunches, sprouts.
So additionally in addition to food, we have considerable person to person spread throughout these outbreaks, lakes and swimming pools, daycare centers and nursing homes. These are all what we call fecal oral-related. Don't you love that? Fecal oral transmission just brings all kinds of pictures which weren't in your head. But what this tells us is that this organism has a very low infectious dose. In other words it only takes a few cells to make you sick. And that's very interesting because we start to look at the pathogenic mechanisms or how this organism causes disease, we realize that these E. coli have something called an acid tolerance response where it turns on all whole sets of genes and enables them to get through acidic environment. And so that's how it gets through the stomach okay? So just a few cells -- it only takes a few cells and it can make it through that stomach and colonize and cause disease. Now why does it suggest a low infectious dose? Well, as far as the lake and swimming pool, this we know from experience that if one kid at the one end of the pool has a code brown and a kid at the other end of the pool ends up coming up with enterohemorrhagic E. coli there's a huge solution factor there, so it must be a low infectious dose. Okay, and so we've seen that. It came to light big time national news when a major football player's son got it at a water park.
So here's just some pictures. This is one of my favorites. It's a Chili Fest with the petting zoo. So what are you going to do? You're going to pet the pet and then you're going to go eat. Okay? You'll now notice that we have had multiple outbreaks at petting zoos and you'll now notice if you ever go to a place that has a petting zoo they always have the Purell outside or a hand washing station. Use it. I'm not kidding. Use it. That's why. We've had multiple outbreaks from that.
We also know that geese carry enterohemorrhagic E. coli and hole-waterways. Kids when I was working in Portland Oregon there was a place called Blue Lake which is a fabulous watering hole where people would go and swim and all of the time kids would -- about once every couple of years, a child would come home from Blue Lake and they would get enterohemorrhagic E. coli a few years later. Here's the feral pig that was the scapegoat for our spinach outbreak. Okay, and I think it was probably these guys that were involved in the spinach outbreak in a farm upstream, but that's just my view.
And here's some water parks -- just thought I would show you. This one is in Japan. None of these have been implicated in outbreaks. I just thought they were interesting. Daycare centers, I thought nursing homes -- so why daycare centers and nursing homes? It has to do with ineffective hand washing. Okay, if you're changing a diaper and a kid has got diarrhea and you don't wash your hands before you do any food handling, you can imagine a low infectious dose, you can get some transmission pretty easily. Okay, so what's going on?
Well, we now know that cattle are colonized with enterohemorrhagic E. coli in their intestines and if those intestines are accidentally slit during slaughter, then the meat gets contaminated and that’s what’s demonstrated here. This came from the Washington Post in 1993, right when I was getting ready to give my dissertation defense. So hold on to those slides and make sure you reference every single one of them before you put them and display them in any way, because you might use them again [background murmur], okay, just a little aside students. Okay, so anyway so we have bacteria sitting on the outside of the steak and actually the steak does not have too much of a problem, because if you sear the steak [inaudible] with high heat, you'll kill everything. Right? Cool. No worries. Big -- well, no worries about E. coli. I don’t know, we have got maybe a parasitologist and maybe we could talk about some other things you might want to worry about.

But if it's ground beef now it's a whole other story because you could have one infected cow or infected meat from one cow and that's thrown into a vat of multiple other cows, ground beef okay and it's all ground up, all ground up. So one contaminated cow meat is now all ground together and all of the meat is now contaminated. And then you're going to go make that ground beef patty. If you sear it on both sides, okay you kill what was on the outside but what's on the inside? Okay. So this is the issue. This is why they say use your meat thermometer and get it to 160 or 165 degrees okay? So that's why suddenly a steak is now ground beef hamburger.
Okay, so let's say it has enterohemorrhagic E. coli in that hamburger and you eat the hamburger. It's going to get through the stomach, colonize your colon -- different pathogenic organisms colonize in various places. This one colonizes the large intestine or the colon and so here we go here. So three or four days later, you start getting the runs and for some of you it may be whatever you've done. Others of you it might be bloody diarrhea. Okay. And that's what we call hemorrhagic colitis, bloody diarrhea -- very classic to this organism. In about 11% of individuals who have the bloody diarrhea, they will go on to something called Hemolytic-uremic syndrome, triadic features for HUS, hemolytic anemia, thrombocytopenic purpura and kidney failure. Not good. Okay, and this is what kills people. This is why this can be a fatal illness.

Other kinds of diarrheal diseases people die of anybody know? Dehydration. In fact, cholera, that's why people die of cholera. It's because they don't get the fluids back in. They die of dehydration. Anybody -- I'm suddenly on my community service soapbox. Anybody know when the first instance of dehydration? A headache -- you think it's because you're vomiting. No. It's because you're dehydrated. That's why you have a headache. That's why you need to get those fluids in and it's not just water. Its water and salt and a maybe a little sugar for coat transport in. Get the water back into your cells, not just into your body. Okay? What's the second sign of dehydration? One past a headache? Its turgor - - the skin -- what's it called -- skin turgor -- skin turgor. Okay. So if I was dehydrated when I went like this my skin would stay up. Someone who is severely dehydrated, it literally stays up. But there are variations on that right?
And here's another aside. My brother-in-law who is a physician, an ER doc, he works really hard. He's a marathon runner. We were visiting. He pulled a call and then he got up in the morning and went running and I got to see him and, "Hey how are you doing?" And he was on his way to bed, but he took off his glasses and he rubbed his eye like that and his skin stayed there. And I was like, "Jonathan, have you had anything to drink today." And he said "Aw, yeah, I had a little orange juice," and then he rubs his other eye and his skin stayed there and I said, "Okay, this is not normal." I said, "I really think you might need to get some fluid into you." Well, we ended up driving home and got a call when we got home to learn that he was in the ER for dehydration. Okay? So there are variations on -- on this. So pay attention. Now you know the signs and symptoms -- back to enterohemorrhagic E. coli.
Okay, so EHEC 0157H7 also and shiga toxin producing E. coli. 0157 is the most common serotype in the United States associated with outbreaks. However, there are other serotypes that have caused okay 026, 045, blah, blah, blah. What does that mean 0157H7? What is that?
Well, for those of you who had microbiology remember there is something on the cell membrane called lipo polysaccharide. The polysaccharide, the multiple sugars [inaudible] on the outside is the O antigen. In other words they make antibodies outside of bacteria and then they can strain type them. So we know who's coming through again in the clinical lab okay? And this particular outbreak strain, it took 157 antibodies of testing, testing, no, no, no okay here's one. They call it 157. Okay? So it's the 157th. So it's O for O antigen of LPS, O157, and then E. coli had this cute little flagella that helps them move from place to place and it's the same thing where they type that antigen with antibodies. And that's the H antigen and it was the 7th one for this colonel strain that caused outbreaks, first in 19 -- first in the late '80s and then in 1993 in the Pacific Northwest. Okay?
Now what else about enterohemorrhagic E. coli? They have a locus on their chromosome called the EAE Gene and that codes for something called intimate and intimate enables the bacteria to attach and it also causes this interesting casing lesion and I'm going to show you a picture of that again, a picture of that. And that's on the intestinal epithelial cells where it colonizes in the gut. It also produces shiga toxins. Okay, two of them either Shiga Toxin One or Shiga Toxin Two.
Here's the picture. This was taken by somebody in Finley's lab up in Vancouver BC, colorized our intestines. It doesn't normally look like that nor does E. coli. And this is so cool from a really bizarre perspective, mine. These bacteria basically get to the colon, they put out a long thin ray for attachment and then they come in tight and they inject into the host cell, something that's stimulates actin polymerization on the part of the host cell, causes the host cell to form this pedestal underneath it. I'm king. Give me a throne. There's E. coli. Okay? So this is not normal intestinal epithelial topography. Okay. It induced actin polymerization on part of the cell to form this pedestal and in the process of this scanning [inaudible] picture, they actually took some pictures where the E. coli got knocked off in the process of getting the -- getting the prep for it and there's this indentation where it was before it got knocked off when they went to spray mount it. Okay? And there's the E. coli. Then I told you about an effacing lesion so these micro villas there is like a forest of micro villa. They're gone. Okay? Enteropathogenic E. coli remember that one? The prolonged summer diarrhea in kids versus E. coli diarrheal disease identified also has the EAE gene locust. So we know this alone will cause diarrhea. Okay?
But EHEC produced the shiga toxin and it's a toxin that has an A sub unit and five little B sub units, and the B sub units are involved in binding the toxins to the cellular receptor and the A sub unit has enzymatic activities.
So it binds to as it turns out, kidney cells and we know that the receptor for that toxin is the glycolipid receptor.
So it binds to the glycolipid receptor, gets into the cell and once it's in the cell, the A subunit then cleaves an Adenine residue on 28S ribosomal RNA and inhibits protein synthesis. This cell says, "Wait a minute, I can't make protein. Something's wrong. It's time to die." And so they undergo apoptosis –
Okay, program cell death and so the cell dies. Okay? So that's the process of a shiga toxin.
Now glycol lipid receptor GB3 is found on kidney cells. Remember what the problem is with after hemorrhagic colitis you get this potentially 11% hemolytic uremic syndrome, dried up features -- kidney failure. Okay, so this is why -- the kidney failure. And so let's back way up to the early '90s, my PhD work early '80s, we were, the pathogen has just been recently recognized in the early 80s and I was working with Allison O'Brien who identified the shiga toxin and we were trying to come up with some sort of small animal model to figure out how this organism was causing disease. How is it that it's causing disease? We couldn't get a handle on it okay? And so we went to a -- orally fed mouse model and, we found that if you give the mice this bacteria orally they die and the only symptom of that mouse was damage to their renal tubules. Now in humans it's the renal glomeruli but that was it. It was just kidney damage. That's it and they were dead.
So this is a picture of the renal tubules and a normal renal tubule okay, looks like this but you can see the cells have gone away and you sort of have these huge vacuoles, here in the kidneys of a diseased dead mouse. So we thought well since I was working in Allison's lab and they working on the shiga toxins well I wonder if it's the shiga toxin that's making them sick, right? Logical step -- so how are we going to test that? So what we did is we took antibodies to the shiga toxins and we passively immunized the mice with antibodies to Shiga Toxin One or antibodies to Shiga Toxin Two, or both, and then we fed the mice and none of the mice died and none of them had kidney damage. Oh it was so exciting.
So let's talk about shiga toxins. Two families as I said, Shiga Toxin One and Two, bacteria can produce one or both but what we know from retrospective studies is that those that produce either Shiga Toxin Two or Two in combination with One, are more likely to cause hemolytic-uremic syndrome. And interestingly in that mouse model, strains that cause just -- that just carry the Shiga Toxin One didn't touch the mice so that was kind of interesting.
Okay, so my question to you; 0157H7 makes the news headlines, dah, dah, dah, dah. You know what 0157 means and now you know our results from our mouse model study and so my question is, which is more important to virulence, the license plate or the gun? Does that make sense?

So off I went to my post doc, worked on salmonella did some really fun stuff, really enjoyed it and then I came down to Sac State and -- this is the way it works right? It's money, right? Okay, that's why we have some food next Friday. But if you want to do research, you've got to figure out where is the money? So here I'm in the Department of Biological Sciences and I'm doing this really, you know, when you do it's is cool because you're doing it because it is cool. So I was doing this really cool research on salmonella and how it causes disease and -- and there was some money that somebody had endowed to the university and if you did a research on a survey, a biological survey, there was a larger pot of money for that than for doing any molecular biology research, because the money was given a long, long time ago and there wasn't a whole lot of DNA work going on at the time.

So I thought if I applied for the salmonella work, I can get about $800 but if I applied on the survey pot and not a whole lot of people are applying for that pot of money, I could get $2,500. I wonder if I could do a survey. I could do a survey for the prevalence of enterohemorrhagic E. coli rather than doing a survey of -- you know, pheasants or ring neck mammals and things like that [background chuckle] which are
all very good but it wasn't my field. So I got the grant and off we went to make some surveys. And it was based on my studies for my PhD where I felt that the toxin had some relevance to and everybody instead was looking for the presence of 0157H7 and I thought you know it's got to be here. We're just not looking for it.
So current ID techniques used in the U.S. Diagnostic Laboratory -- so these are laboratories that are trying to figure out what you’re sick with, would do biochemical tests looking for the fermentation of Sorbitol. So they are looking for the presence of enterohemorrhagic E. coli right? But they did it completely looking for the presence of not enterohemorrhagic E. coli, but of 0157H7. Their methods were totally skewed to looking for this collotype because we know that 0157H7 does not ferment Sorbitol. So they created a new media which is very smart and instead of using MacConkey agar with lactose they used MacConkey agar with Sorbitol. And so you have this nice screen again. Okay? So you place the stool sample on that, and any plate -- any colony that's white is going to be potentially enterohemorrhagic E. coli. You identify it and in fact it's E. coli and you go on. But again this is only looking for the 0157. There are other EHEC that cause disease and they would not be identified using this method the current labs were using.
In fact we know and some grass roots organizations have gotten the ear of finally the USDA who just announced this past fall the “big Six” are now going to be tested by different labs across the United States instead of just the 0157. They're testing for many of these others that are found in beef trimmings. Okay? And there's a reason why I'm telling you 0104 isn't listed, because that's an outbreak that if I have time I will tell you about.
Okay, so this brings me to my research that I was telling you about. I found this little pot of money bigger than my other little pot of money and off we went to look for the prevalence in ground beef in Sacramento. And then to tell you about free range grazing cattle, horses and clinical stool specimens.
Okay, so here is Julie Oliver and she was the first graduate student to work on this project and then Phillip Barruel is the second one that followed her in this project.
The objective was to determine the prevalence of all EHEC or shiga toxin-producing E. coli in retail ground beef in the area. And we're going to use a molecular method looking directly for the Shiga Toxin gene, not the 157. I want to see who's there -- who's there. And then Phillip came along for whatever causes we found and he attempted to isolate the EHEC that we identified was there and then to characterize its serotype to you know what is it. Okay.
Before we had done this, there were a couple other studies done. These were both from the Pacific Northwest. If one was doing colony blot hybridization and they found 23% of the samples came up positive looking for toxin. And then Phil Tarr who spent an integral part of a lot of research on these pathogens tested just looking for O157 and found 0% out of 1,500. Well, that's kind of a nice study because you didn't come up with anything. So that's looks really good but this suggested we might come up with just a few more if we look for the toxin. Right? So we were excited. Okay, we're going the right way with this -- plus.
So Julie got this -- she figured out her route to the university and she was going to take one route on Mondays, Wednesdays and Fridays and then another route on Tuesdays and Thursdays, stopping at the various supermarkets and quickie-marts on the way picking up ground beef. This is a picture of some of the ground beef that she bought.
And then she would take that ground beef and put it in LB and this was a very quick and dirty assay -- put it in the LB, grow it up all night to enrich for all the good stuff that's in that ground beef, bacteria-wise.
Then she just took a micro liter out of that, threw it into a PCR tube with all of the reagents and amplified it up for the toxin genes. Okay? Quick and dirty, no DNA isolation -- we just put -- and how can you do that? Well, the first step of PCR is 98 degrees so these bacteria are popping, opening, and releasing their genes to whatever primers are going to be there to amplify up whatever you're wanting to amplify. So it worked great. So there's our little PCR machine.
And here is what we designed primer so that you would get two different sized bands. If it was Shiga Toxin One, it would come to 210, if Shiga Toxin Two was present, it would come at 484. So this is our positive control and these were our first two positive samples. When we first started, she did a handshake with a number of the butchers at the grocery store chains, "Hey I'm doing this project. Would you do it?" You know, "Oh sure." And they actually had it ready for her, little golf ball size amount. They shrink-wrapped it for her. It was great. We got it free -- again, not much but - great and then, one day one of the guys said, "So how's the study coming?" She's, "Oh really good." "Are you coming up with any positives?" "As a matter of fact, yes." And the next time she came, "I'm sorry, we can't help you." [Laughter] So that's when we started buying. Like that's okay, we can take care of that -- we'll just get out the grant and start buying our own ground beef.
Anyway, okay so those that came up positive, we went back and tested the ground beef milieu for EAEA genes to see if they had that and many of them did.
So here's our results -- she tested 200 ground beef samples of all different fat contents from eight different grocery store chains, all different levels of chains from high-end markets to quickie-markets. 11% of our samples were positive for Shiga Toxins. 11% of the ground beef that we were buying at the grocery store -- I got to the point where I was, you know if I bought some ground beef, "Hey Julie, here's some extra [laughter]. How'd it turn out?" [Laughter] So regardless of the grocery store chain, regardless of the fat content, 11% of it was coming up positive and our PCR sensitivity was less than 1,000 CFUs per gram. So there it is. Now it was a crude essay. We think that the sensitivity's pretty low, pretty low. So we think that this is an underestimate in fact.
Okay, 22 confirmed positive samples, 60% had Shiga Toxins One and Two. 36 had Two only. These are the ones that we worry about as far as potentially causing hemolytic uremic syndrome. 90% had that attaching and facing gene that would -- these are all genes we were identifying -- had the gene for the lesion that could cause in the intestines.
So then Phillip came along and found the needle in the haystack. It was a significant effort to pull these out of the ground beef [background noise] milieu. In fact, 22 positive samples -- we were only able to get 16 out no matter how many times we tried. He developed an assay system that worked out really great and got 19 isolates at the end of the day. Now this is really interesting -- I want you to use some cerebral energy on a Friday afternoon real quick. 16 samples, 19 bacteria -- Shiga Toxin producing bacteria out of those 16 samples, what does that mean? Yeah, some of our ground beef samples had more than one Shiga Toxin producing E. coli, and we knew that because it had a different Shiga Toxin profile. So maybe one had both toxins and the other had just one of the toxins, okay? So that's a little reason.
Okay, so then Phillip took those and went to test what the serotype was, in other words what their license plate was and only one of them was 0157H7. So that's why we realized, "Oh during the initial selection to pull the needle out of the haystack looking for 0157, that's why that didn't work very well." Well, we spent about six months on that method using immunomagnetic feeds for 0157 -- that didn't work. That's why -- there was only one. Okay, 25% of the samples, they weren't able to serotype. There wasn't an antibody that would react against it and these were the remaining serotypes. This is one of the Big Six, that's why I highlighted that in red. That's been known to cause -- known to cause outbreaks.

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**What are the other Serogroups?**

- Only ONE out of 19 isolates was O157:H7
- 25% were not reactive to the over 167 sera used for serogroup testing
- Remaining isolates were identified as:
  
  O8, O34, O39, O55, O83, O85, O110, O112, O121, O139, O166, O171, and O175
Okay, so he also confirms -- so this was -- we presented this at the National ISM Meeting and everybody said, "Well, that's very nice but who knows that they're actually causing disease? So you found it there but I'm sure they don't cause disease. And -- and you're only finding the gene so just because the gene's there doesn't make it -- mean it makes toxin." So he went ahead and confirmed that in fact if it has the gene, does it make any toxins. So he did the cell culture assay and sure enough, they were all toxic. He also found a really disconcerting that 42% of the isolates had antibiotic resistance to at least one antibiotic.
So here's one of our strains of multiple antibiotic resistances.
That was kind of worrisome.
Okay, so conclusions this portion -- we knew that it was in the ground beef, many sera groups other than O157. They had the adherence capability and they have the toxin. Therefore we really felt it can cause disease and that these strains are not the isolated under the current techniques that most clinical labs are using. So this is -- yeah this is worrisome.
So then Michelle showed up and Michelle is looking for a lab to do her Master's Degree research and we got to chatting about it and she said, "Oh, I live in the country." I said, "Oh, you live in the country?" She said, "Oh, yeah, I live in Placer County, you know, with all the cows." And I said, "Ooh, with the cows. Do you have any cows?" "Yes, I have cows." "Welcome to my lab." [Chuckle]
So Michelle joined the group and she spent some time looking at the prevalence in cows. This was some research that was done before she came, wide-variety — anything from 7% to 70% depending up on what type of cow we were looking at. A lot of them were slaughterhouse — these were previously — previous publications, okay? So slaughterhouse, feedlot — she said, "Please don't send me to the feedlot on I-5." I said, "That's fine, you don't have to do feedlots." So she did free-range grazing cows.
Okay, the question is we know that -- we knew Julie and Phillip's work 11% of the ground beef at a minimum. So was it feedlot? Was it pasture? This is what she focused on.
And okay, she looked at adult cattle. It turns out that the cattle that you see kind of hanging out along the side of the road. These are the ladies who are giving birth to the baby calves that are going to be sent to the feedlot for your good, good steaks. These ladies work hard for about four to five years and then they are sent to the slaughterhouse, okay? These are the ladies that she tested.
They’re the ones that will enter the market. They will not be finished with special diets before they go to the slaughterhouse and 50% of them will go to ground beef. So this is a good population of cows to test. And the majority of outbreaks as you guys know were due to consumption of ground beef. So this makes sense.
So what she did is she took fresh fecal samples, truly fresh, okay? Cows apparently have this interesting habit of nestling down for the night and then when they get up, kind of like us, huh? When they get up, they do their thing and so she would look from her window. Okay, it's time to go. She'd get on her horse and they'd go out and go collecting the horse from this not amusing in anyway, this routine, but that they would go and collect the fresh fecal samples from the cattle and she would bring them to the lab, put them in LB broth to enrich. And this time, now we knew that stool specimens carry a lot of inhibitors for enzymes used in preliminary's chain reaction. So we knew -- we knew we needed to purify the DNA before we did PCR and dell electrophoresis.
Okay, she tested 152 samples from 11 different herds, got DNA from about 138 of them. Okay, so it's tricky to isolate the DNA out of them. 72% were positive for Shiga Toxin. [Whew] We were blown away by this result.
So the profile of those positives, 44% had One and Two, 25% had Two only. So quite a few were potentially worrisome with the homiletic uremic syndrome.
Now what was interesting, the question was well what about the size of the herd? Does that matter? And it did not matter; there was no statistical significance whether it was the herd of 1,000 cattle versus the herd of 18.
There was a similar prevalence. And then what about the size of the field in which these -- these cattle are hanging out? And she said, "No, there's no difference there either." It was her thought that because these cattle liked to kind of hang out together, so even if you have 25 acres, they're all going to hang out together and share whatever they've got. Okay? So it really didn't matter.
So 72% of the cattle were positive. Herd size didn't matter. Neither did the size of the acreage. She also looked over several seasons, that's important for field biologists you know. She looked over several seasons to see if there was a difference in prevalence and there was not. And one of our huge take-home messages and it should be for you as well, let's think about this for a minute. 11% positive in the ground beef, back up to the cow that made that ground beef -- 77% positive in the cow. So there's obviously something we are doing right in the processing of that cow for ground beef. That's a good thing, right? So this -- there's good thing with this study -- okay. So pre and post processing measures -- it suggests that these are critical and also said, "Hey you know what? We're doing something right here if that little gets in." So then Donica comes along and she had read a study that was very interesting to her. She said -- my -- another Master's Degree student and she read a study out of the UK where there was this guy, older guy and he had a horse and he came down with hemolytic uremic syndrome and they found it actually in his horse and they were able to do the molecular typing and it was the same strain. So she said, "This is interesting because a lot of people who have horses, they are very friendly with the horse -- a bite of apple for me, a bite of apple for the horse." She was worried that if horses were in fact carriers of this, that it would be a place of spread to humans.
So she wanted to look at the prevalence of this in horses. Big industry in the United States and in California, they're used for all sorts of purposes, okay -- companion to work.
So each little star is a different place where she tested in northern California. 156 horses, 19 different ranches and what she was interested in was looking at the -- not just the prevalence but also how much was on the coat of the horse -- on the -- for the companion animal aspect of it. And she wasn't able to come to any conclusions on that but she was a very good scientist. And the reason why was because people spray all kinds of interesting things on their horses to make their coats nice. And some of them are homegrown cocktails of stuff that work out really well for the 4H Fair and they don't tell you what's in there. So we were having a hard time picking up bacteria from -- from a lot of those samples. So that's kind of cool -- so it kills the bacteria. But -- so that was her original focus, right? She did -- I will almost say thousands of samples because she did -- she did a swipe there and another swipe on this side of the horse. So for every horse, she had multiple samples. And then she also took a stool specimen while she was there and she paid attention to what was going on around that horse. That day when she went to the ranch to go gather those things and she logged all of this, okay? We get all -- two years down the line, we get the data -- this high data is coming up with absolutely nothing. It's all over the map.
But we start looking and we note -- as it turns out, half of the -- well it wasn't quite half, but she went on to more ranches to make it work. So that half of them had interaction with some sort of ruminant animal and the other half didn't. Okay? So let's see what happened. She also had to isolate the DNA completely different type of stool than a cow. Cows are ruminant animals, horses are not. They have a lot of grass in there -- in their stools, okay? She got down 120 CFUs per half-gram feces for her sensitivity assay.
21% of the ranches she tested were positive, four of those horses, okay, were positive at a 2.6% rate. So that's much lower than the cows, right? But here was where that interesting piece came in and you already know it because I kind of gave you the punch line already.
Of those that were positive, they were the ones that interacted with ruminants. None of the ones that had no interaction with ruminants were positive and ruminants included cattle, deer, sheep, goats and llamas, even. This is California.
Okay, so here are four positives and here is what happened, the type of ranch and what the scenario was with the horse -- so this one actually had diarrhea. The owner said that it was because the horse was eating a lot of these berries in a high bush that was near the -- where the stable was and, "Oh that happens every year, he gets a little diarrhea." We wondered really was it the berries or was it the E. coli? This one she didn't find any ruminants but she found cattle feces on the property. This one had deer scat around the property and that horse died when we went to go back to see if it was still positive, it was dead. We don't know why, interesting. And then this had cattle which we had tested were positive for Shiga Toxin. So this was very interesting to us.
We went to -- and we were able to isolate two of the -- two of the E. coli out of that -- the needles out of that haystack. The other two we were not able to and there was Shiga Toxin Two producers only and no O sera typing was possible, no antibodies would react with those.
Okay, so Part Three, prevalent -- prevalence in horses especially those interact with ruminant animals. We had other questions -- the duration, how long it's in the horses, and does it cause any damage to the horse? So we still had those questions outlined.
Then I have to tell you about Margaret’s work. Okay, so she came along -- she's a clinical lab scientist I met from NCASM -- be part of your professional society, whatever it is. And she said, "You know, my daughter's starting college at Berkeley and I need to do something too so I think I may go for a Master's Degree. You know, just to keep busy." So she was working as a clinical lab scientist and I said -- I said, "Well, Margaret," I said "Well, that's -- that's kind of cool. Do you ever have any stool specimens?" "Oh my gosh, we're swimming in stools," she said. "Welcome to my lab." Because this was the piece that was missing in our story, right? People are saying, "Well, yeah you found it but it doesn't cause disease." We can't do Koch's Postulates. We can't feed it to people. "Would you like to participate in my study? I'll give you some E. coli and we'll see if you get diarrhea and maybe hemolytic uremia..." No, people aren't going for that. So -- but we could go to the clinical lab and look -- use our assay and see is it there, okay? So she did that.
She tested 200 clinical stool specimens for the presence of Shiga Toxin One and Two and -- and she wanted an assay system that she could do right away. So she got -- isolated the DNA directly rather than enriching overnight, okay? And then she ran for additional PCR.
So she took the clinical specimens, she's working as a clinical lab scientist, and 2.5% were positive using that traditional 0157 screening that they do in that lab. And then she would bring the specimens down to our lab and run our molecular assay. Those same specimens, 27% were positive for Shiga Toxin producing E. coli. So this was huge to us. This said that about 24% of those samples are people, patients were going undiagnosed. Now this is a problem because enterohemorrhagic E. coli produces Shiga Toxins, right? And if you give somebody who has bloody diarrhea enterohemorrhagic E. coli antibodies, you're going to send them to hemolytic uremia syndrome. So you don't want to treat these people with antibodies -- antibiotics, sorry. Okay? It won't -- it will increase the risk of their disease. So they need to be diagnosed.
Okay, this was the profile a lot of them had the Shiga Toxin Two which we're worried about. Some were Shiga Toxin One only which can also be involved in diarrheal diseases et cetera but it's not as disconcerting with the HUS.
Okay, so conclusion -- it's prevalent. It's causing disease. Much more prevalent than current methods that are used and sick patients are being left undiagnosed.
So current testing I said, looking for the 0157.
The CDC came out and said, "You need to start looking for the Shiga Toxins. They're called non-0157 Shiga Toxin producing E. coli." And I've been doing the rounds now to different clinical lab talks. This is '09; one lab that I know of is doing this [chuckle]. Okay? So it's -- it takes a little bit and some labs are still not even testing for enterohemorrhagic E. coli. Okay, what else?
All right, what else? Okay, the old methods, there's a Sorbitol-MacConkey agar.
Other methods and now they need to do –
The CDC says you need to go ahead and do an enzyme absorbent assay or lateral amino assay or PCR on your stool samples. Okay? One lab so far I know is doing that. So when I get sick, I want to be there.
Okay, so what's my take-home message for you guys? Sprouts or spinach, cantaloupe -- we had a big Wisteria outbreak in the fall. I don't know if you were following that. It's entirely up to you what you want to eat but it's really important you use good food handling. You heat your meats 165 degrees.

You want to be very careful with cross-contamination. So what are we talking about? When you grill, we're coming into grilling season starting after the rains end this weekend, right? And so when you take food out to the grill on a platter, you want to make sure you take a fresh platter. If you put your contaminated meat, which it is, on the grill, you want to make sure when you take it off the grill cooked that you put it on a fresh platter, okay? The same with the marinade, when you make your marinade to marinate your chicken, you want to make sure before you add all of the marinade to the chicken, you have a little bit on the side and that's what you're going to use for basting when you're at the grill.

And luckily Sunset Magazine and some of these other magazines have picked up on this and they're putting that in their recipes that go out to people because imagine you're using that same marinade that's a nice soup of campylobacter and [inaudible] and salmonella and enterohemorrhagic E. coli. And it's nice and cooked and you do one last little splash of that fabulous cocktail and I have talked to people who have gotten sick because of that in retrospective thinking -- even came down with gian beret and was in the hospital for like two months. Yikes, okay? That's after campylobacter infection. So don't do that.
And then also with the -- have separate cutting boards for your meat and your produce at least within a meal -- one meal preparation. You're not going to use the same knife to cut up your meat and then go and cut up -- don't smoke while you cook. I know somebody who got a really bad case of campylobacter because they were smoking and they were cleaning the chicken [inaudible] okay? And it's on your hands and now in your mouth. Okay? You shouldn't be smoking anyway, so you know that. Okay. Wash your hands and if you're not getting sick regularly, you're doing something right so no worries, right? That's the best take-home message.
Thank you and congratulations.
[ Applause ]