B. gibsoni; What Does it Mean for Your Shelter?

Dr. Sarah Kirk

Video Transcript

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[Beginning of Audio]

Dr. Crawford: Received her DVM from Michigan State University in – do you want me to say the date? 1977. And completed her Certificate in Shelter Medicine from UF in 2013.

She is a volunteer with the Shelter Medicine Department at UF, participating in numerous Operation Catnip spay/neuter clinics, numerous shelter consultations, and even more numerous disaster responses. Most recently, Dr. Kirk served as the ASPCA's Medical Director for Field Investigation and Response, and she was awesome.

And she led the medical care and forensic investigations of numerous dogs rescued from large-scale fighting organizations. You were in charge of the medical care of dogs and cats rescued from Hurricane Sandy, where you all set up a temporary shelter in Brooklyn for that. And puppy mills.

So, in the past year, she has acquired quite the experience as the Medical Director for the ASPCA's Field Investigation and Response Unit. But now she's newly-retired. She hopes to remain involved in shelter medicine by mentoring her daughter, Jill, who on Saturday will be Dr. Jill Kirk. *[Applause]*

Dr. Crawford: And, and this is our first unique combination for the UF Maddie's Shelter Medicine Certificate, in that we have the first mother-daughter graduates. So not only did Dr. Sarah Kirk complete the certificate, but her daughter, Dr. Jill Kirk, did also. So, Sarah, come on up, and talk to us about *Babesia gibsoni*, and what it means for our sheltering organizations. Please. Welcome. Here. *[Applause]*

Dr. Kirk: Thank you, Cynda. Thank you. Well, it's really an honor to stand up here in front of my colleagues. I never would've guess that I would've been given this opportunity. I am not an academician. I'm a clinician, and

have had many, many wonderful opportunities throughout my 37 years, as a veterinarian, and as Dr. Crawford said, I am newly-retired.

I'm sure I will keep busy, but this talk is based upon our recent case of dog fighting with the ASPCA. And I know that when I was first hired by the A, my boss said, "So what do you know about *Babesia*?" And I honestly said, "Well, not much." He said, "Well, you're going to learn." And indeed, I did, and I hope that if nothing else, my presentation will be thought-provoking for you today. Dr. Cannon did an excellent job of providing a history of dog fighting for you, so I will gloss over that, but I will give you a little more history about the ASPCA's FIR Team, as we fondly call Field Investigation and Response.

Again, a brief overview of *Babesia*, and then I'll discuss the specifics of this case, and one of our previous cases, with regard to the medical care of these dogs and then the diagnosis, treatment, and follow-up that we provided for our *Babesia*-positive dogs.

And we'll end with some questions for you to ponder about: what will you do if you encounter *Babesia gibsoni* in your shelter? And we can go through this. The one thing that I wanted to touch on with the different dog fighting categories that I think is really interesting – the street fighter tends to not care so much about whether or not their dog is human-aggressive, whereas the hobbyist and the professionals really want their dogs to be very people-friendly. And that really is the ultimate betrayal of that human-animal bond. When I first became involved with the A, and my first dog fighting case, I really didn't know what to expect. I kind of had this vision of, you know, a snarling pit bull, frothing at the mouth, lunging at the front of the cage when people passed.
And that was certainly not the case. As far as their reaction to people, they were really like mush. They were marshmallows. They wanted us to give them attention. But, as Dr. Cannon mentioned, you can't be lulled into complacency with dogs from dog fighting cases.

I will discuss in, in my presentation, the behavior evaluations that we did on these dogs. But if you have a dog at your shelter that has not had an evaluation, or you don't know the results of that evaluation, but you know that it came from a dog fighting case, you do have to be very, very careful, about when it sees other dogs. Because injuries might occur if you get between a very dog-aggressive dog and a dog over here, and you're in the middle.

And as Dr. Cannon mentioned, the American pit bull terrier is the dog of choice in the US. The 2009 study was pretty convincing that we do see *Babesia gibsoni* more often in these, in the pit bull terriers, and seen most commonly in dogs with a history of fighting.

I had to put this photo in. It is my daughter, Jill. She was able to go out on the scene of this case, and I think everyone's captured by the dog's expression. And I just recognize that ponytail, and know that that's Jill. And again, the 2009 study, 53 out of 157 fighting dogs were found to be positive for *Babesia gibsoni*.

Only one out of the 218 random dogs, and that dog was thought to be a pit mix. So the ASPCA's FIR Team was created in response to Hurricane Katrina. And as you might imagine, a lot of the work that is done by this team is in response to natural disasters. But they also are called upon to deal with man-made disasters.

Puppy mills, hoarding, the blood sports of dog fighting and cock fighting, and other cases of cruelty and neglect. This is a very, very, very busy team. I was with them for a year and a half, and I feel as if I put ten years of time into that team. They travel everywhere, all the time.

There's always a case waiting for their attention, and the A response to – or the FIR Team response to these cases – when they are asked by local, state, or federal authorities to come in and help some of the cases, we were asked by a state's attorney general. Other cases we assisted the FBI.

And so, as such, things are very, very confidential. And at first, when I was on the, the team, I thought I wasn't being told things because I was the new person, and they didn't know if they could trust me yet.

And we would be planning for these cases, and we wouldn't be given specific dates, as far as when it might happen and we certainly didn't get information until the very last minute. Which is a little anxiety-provoking, when you have to provide medical care.

And when we are dealing with the criminal cases, we are housing these animals for generally a long period of time. It's, depending on whether it's a blood sport – I would say the blood sport cases, they tend to not sign over their animals quickly, whereas somebody who's a hoarder might; although, I was involved with a hoarding situation that took six months until the animals were finally given custody to the A.

So it's, it's really a unique opportunity; it's sad, certainly sad for the animals, and frustrating, but it's a wonderful opportunity to study infectious disease, because you have this population for a pretty long period of time.

And, so anyway, I found that very interesting. As Dr. Cannon mentioned, initially, with dog fighting cases, it was thought that these dogs couldn't be

trusted, they couldn't be rehomed, and euthanasia was the only responsible outcome. And we know now that we do have to look at these animals as individuals.

And while some of them may not be appropriately rehomed, there are many that can be. So part of our anticruelty group's behavior team does behavior evaluations on these dogs. I should say the entire anticruelty behavior group does behavior evaluations on the dogs.

They try to begin these at around day three, because they don't want too much time to elapse between the time the dogs are seized and they do their evaluation. You wouldn't want a defense attorney to say, "Well, you didn't evaluate this dog for three weeks, how do we know that this behavior, you know, isn't due to your sheltering?"

So, it's useful from a court perspective. It also allows them to identify any dogs that might be human-aggressive. Again, that's not the norm in my experience. But if we do have dogs that seem to be human-aggressive, we want to identify those readily, for safety of our responders.

And we also then can identify dogs that might just be incredibly fearful, and who need behavior modification and/or drug therapy to help them during their shelter stay. And I would say that these evaluations really go on a daily basis.

The anticruelty group behavior team has a very specific evaluation that each dog goes through, but day after day, after day, after day, the daily care workers, the medical staff, whoever encounters those dogs, also has the opportunity to write their observations or concerns into a database, and we always, always have at least one behavior person at the shelter every day.

So it's pretty remarkable. One of the things that, that really struck me about this, FIR Team, was the fact that, that we had a really – we worked really hard to balance behavior, medical, and sheltering needs.

And if there were any changes that were going to be instituted with regard to a particular dog, or a group of dogs, all three representatives from all three areas sat down and discussed it, because they are so interwoven. So, numbers of dogs seized can range from a few to hundreds.

Typically, when the ASPCA is involved, we're in the hundreds range. And when we're dealing with a disease such as *Babesia gibsoni*, this can be a real logistical and financial challenge, as far as diagnosing and treating.

You know, some folks might think of the ASPCA as having really deep pockets, but when you're involved with many, many different cases that go on for a long period of time, the cost of housing these animals climbs really rapidly. Again, Dr. Cannon mentioned this.

The typical dog yard with these poor dogs chained out here 24/7 unless they are in the process of being trained for a fight, and that's a pretty grim existence.

And again, *Babesia gibsoni* is an intracellular, red blood cell parasite. We know that at least nine species affect dogs. Two species affect cats. Depending upon the species of *Babesia*, you might have a different method of transmission. And Dr. Cannon touched on this as well. Ticks are really important for *Babesia canis*, but it doesn't seem to play, at least we haven't identified the tick vector for *Babesia gibsoni*.

And my presentation will show you my findings with regard to vertical transmission. Most of the dogs that I encountered were asymptomatic. And on, I'm going to discuss, two different cases here. Each case had only one dog that became symptomatic.

But these are all of the signs that you might see. So if you have a pit bull type dog in your shelter, and it develops anemia, I would hope that you might think that, you know, "Gee, maybe we should do a *Babesia* PCR on this dog." But you might see any of these clinical signs.

Anemia, thrombocytopenia, hyperglobulinemia, hypoalbuminemia, icterus, hematuria, splenomegaly. And a dog that has otherwise appeared healthy might manifest clinical signs if a splenectomy has occurred, or if that dog is given corticosteroids.

So when we ask, "How might this organism impact your shelter?," I think you have to ask yourself, "How many pit bulls do I have in my shelter, or pit bull mixes?" In this old study, already 14 years old the ASPCA conducted in 2000, 35 percent of shelters in the U.S. took in at least one pit bull per day.

And 25 percent of shelters said that pit or pit mixes made up more than 20 percent of their canine population. A more recent article in the *Chicago Tribune* said that 40 percent of the dogs coming into Chicago's Animal Care and Control were pit bulls.

And we heard the other day that pits have become such a popular dog as far as ownership is concerned, this is not surprising that we're seeing, you know, 40 percent of the dogs coming into a shelter might be pits or pit mixes. And so as you ponder, might I have this organism at my shelter, I

think you have to ask yourself, you know, questions such as, "Do you handle cruelty cases?"

Or do you accept transfers from other agencies that handle cruelty cases? Or are you seeing pits, or pit bull type dogs that come in heavily scarred? Or, these would all be things that should prompt you to be thinking about the possibility of *Babesia gibsoni*. There are basically three available tests, or types of tests.

You can do a simple blood smear. You can do serology, or you can do PCR. The smear obviously is very easy to do. You can look at it yourself. However, I've never seen it myself doing a smear in-house, and we only see *Babesia gibsoni* during acute infections.

You're less likely to see it with a chronic infection due to low levels of parasitemia. You're more likely to see it with capillary blood, so you know, get a toenail, or an ear. And turnaround, if you send it to an outside lab, is only about a day, and the cost is relatively cheap. But, heck, you can do it yourself.

I mean, if you really are limited with regard to your resources, and you just are curious and want to see, you know, if you get a positive, well great, you've just made a diagnosis. If you get a negative, you have to keep looking. Serology detects anti *Babesia* antibodies. So it tells us that the dog has been exposed.

And it may be useful in cases where your parasitemia is too low for molecular detection. However, it may cross-react with *Babesia canis*, so it's not as specific as PCR. And this is very important with regard to treatment. Because the treatment for the two species is different.

It's pretty expensive, at $120.00, and has a long turnaround time of five to seven days. We elected to rely upon PCR in our shelters. It has high specificity, so you can start the appropriate treatment. A positive PCR tells you that you are, your dog is infected, because it is detecting the *Babesia* DNA. Cost is not horrible.

It's 40, about $49.00, and turnaround time is one to three days. We do have to consider that testing is just a snapshot in time, and I kind of liken this to when we do a retrovirus test in cats. If you test cats coming into your shelter, and you only test them once, all you can do is say that at this point in time, this cat was negative.

Same thing with the PCR testing. We know anecdotally anyway, that the time from the dog bite to a positive test seems to be four to eight weeks. So when we bring in these dogs from these fighting cases, I don't, we

don't necessarily know when all of these dogs may have been fought, if any of them were fought.

So, it's a moment in time. And do you do all three tests? Well, that would be ideal. Very few of us have that kind of – those resources to do that. Some folks are advocating serology and PCR as the best of both worlds. So now is the opportunity that I have to laugh at myself, because I'm going to delve into our southeast dog fighting case. And this is a moment of levity, because I was told to expect 100 to 150 dogs, from multiple sites. And again, these cases are highly, highly confidential.

And it seems like whenever we were planning for cases, you know, the first thing I wanted to know was, how many animals to expect, what species are they, and it took me a while to realize that the individuals who are providing this intelligence for us, are not the people that are back at the shelter.

So their whole focus is on a whole different – on prosecution, or the scene, or whatever. Their focus is not, "How many vaccines does Dr. Kirk need to have on hand?" So, you know, these figures, I knew from our previous case not to trust the figures. Initially we were told 100 dogs. Then they said, well, 100 to 150.

So I thought, okay, fine. I'll have enough vaccine for 200 dogs. I'll be golden. Well, the warrants were served on a Friday morning, and Friday afternoon, around 3:00, we finally started getting phone calls back at the shelter. I was, I was always back at the shelter, not out on-scene.

But I, we finally start getting phone calls, and they say, "Oh, well, looks like we've got about, you know, 20 dogs at this scene," and "Oh, well, we were expecting 50 at this scene," and "there's 115." And the numbers just kept climbing. And so around 3:00 on a Friday afternoon, we were finally told to expect about 250 dogs.

I had enough vaccine for 200. Now, I was freaking out, because to me, they have to be vaccinated immediately. So I made some phone calls, and thankfully, Dr. Isaza had some vaccine that she could loan me, and she saved me that day, because I was hyperventilating, thinking that I wouldn't have enough vaccine for all these dogs.

We were able to – a couple of weeks ahead of time, I was able to call the state vet, of the state where we were housing these dogs to discuss with him what we were doing, because I was really hoping that we would be able to transport these dogs just as soon as they arrived; do all of their vaccination, what have you.

And he said, "No." He said, "I'm sorry." He said, "Each dog of the appropriate age must have a rabies vaccine to cross into the state." I'm like, and they needed a health certificate. *[Audience groans]*

Dr. Kirk: So clearly this very nice gentleman had never been out on a scene like this. And I said, okay, I really tried to nicely get him to understand, you know, that that was really going to be difficult, and it could potentially be putting these dogs at risk, because it was during a time of year in the southeast when it was very hot and humid.

And, it was, "No, they needed to be rabies vaccinated, and they needed to have a health certificate." So, that's what we did. Upon admission to the shelter, and this is the dogs arrived anywhere from 10:00PM until probably 3:00AM the next morning and so as soon as they came off those transport vehicles we had two, as you can see, two cages set up, and we gave them their DAPP and their intranasal bordetella.

They also got, the majority got Advantix and Heartgard until I ran out, because I had 253 dogs. And then, they got Advantage Multi. And all of the dogs were started on Panacur for five days. This admission exam was also an opportunity for us, you know, first opportunity to lay eyes on these dogs, so that we could determine – would we put them in general population, or if they had any indication of respiratory disease. We did a PCR swab on them. These dogs – this dog, this was at the end of the case. This dog looks great. These dogs did not look this good when they first came in.

And, you know, lots of skin disease, lots of hair loss. "Um, was it ringworm?" Who the heck knows until you do a culture? So, we were able to get these diagnostic tests started right away, and we were able to determine where in our warehouse these dogs should go.

This photo shows you our shelter design, which I just love. It worked really well for us. We had a perimeter fence around all of these cages. And then they were set up anywhere from, well, once we had 253, this shelter was great. We had it all figured out, and then they gave us 100 extra dogs; so, we were scrambling.

But anyway, we would have anywhere from probably seven to eighteen cages in one pod. And that was dependent upon how – where these dogs came, what scene they came from – for example, if we had dogs coming from Texas, we didn't want to put them in the same pod as dogs from Mississippi.

So we're trying to, as, as best possible maintain populations together so that we could see what kind of infectious disease might manifest. These

pods also, each pod had an indoor exercise pen. So every dog in that pod would be able to get out of its kennel and go into the exercise pen every day.

And this pod approach also enabled us to have – obviously all of our cleaning implements stayed in that pod – certain personnel were assigned to certain pods. You can see in this photo, for the first two weeks, when we held, had our quarantine period, full PPE. And I got a lot of grief about this. "Um, is this really necessary?" And we were so glad that we did this when distemper reared its ugly head on day 13. But in any case, we – this photo is of general population. We also had, apart from this, in a different section of the warehouse, we had a maternity, pediatrics section.

We had respiratory iso, we had a ringworm iso, and when we needed acute care for our distemper positive patients, they were housed in one of our trailers, completely out of the building, with personnel, the personnel that took care of those distemper positive dogs, that's all they did.

They did not come back in and deal with anybody else. So it's a pretty nice setup. You can see the crates are on top of each dog's magnum cage. You can see the visual barriers that were put up between the cages.

Once our quarantine period was over, and once we were through distemper, and once we were through corona, and once we were through ringworm, we were able to – we had outdoor exercise pens. And those were also pod-specific. So, dogs from pod A would go to the outdoor exercise pen for dogs from pod A.
So the shelter design was always evolving. As diseases came and left, and, as dogs got older, and moved out of pediatrics and so forth. So like most shelters, you know, it's never constant. The intake exam, as Dr. Cannon mentioned, was the time when we did a really complete, full forensic exam.

And that occurred anywhere from the day of arrival, to five days post-arrival. It just took a long time to get through all of these dogs. It was a full forensic exam. And blood was collected for PCB total solids, the SNAP 4DX, and the *Babesia* PCR, among other thing. So our total population ended up being 324 dogs.

We had that initial group of 253. We had 16 additional dogs transfer in. 55 pups were born because we didn't have enough dogs apparently. And so we had 324 total. And we managed to get *Babesia* tests on every surviving dog.

So 253 from our initial seizure, six different sites, the 16 that transferred in from two more sites that came in about four weeks later. And believe me,

I was not real happy about this transfer. I had my population, I was dealing with distemper, and now they're saying, "Oh, we've got dogs coming from two more sites."

And I'm like, "Oh my gosh. I've got to start quarantine all over again." But it all worked out fine. 42 dog – pups were tested. 40 live, 2 deceased. The only reason why the other deceased pups weren't tested was probably because we were dealing with so many other things, and it just wasn't in the front of my mind to test these guys.

I wasn't thinking in terms of doing a presentation, so. But that's pretty good, so our total number tested were 311. We hid, had 88 that tested positive. So 28 percent of the population. And we ended up with four different treatment groups. Group one was necessitated by the dog that became symptomatic.

Group two consisted of 39 dogs who were asymptomatic, and it took a while. I mean, we had to identify these dogs, we had to get their current weight, because of course coming into the shelter, their weights, you know, they're gaining weight each day as they're in the shelter.

And we had to order the drugs, and wait for them to come in. Group three was a group of seven dogs that were asymptomatic treated later. Four of these were dogs that were younger at the time they were first identified.

And I knew from my previous case that the younger dogs didn't seem to clear as readily as the adults. And then three were transfers. And then our final group was two pups that were born on-site that were asymptomatic. This slide I thought was really interesting.

It just shows the prevalence per site, and it ranged from zero to sixty percent. And from the literature that's out there now, one might wonder if these very high percentage sites were really the dogs that were most heavily scarred, and were the dogs that were more – had a history of fighting.

Unfortunately, when I was writing this presentation, I did not have access to the scar charts, 'cause that would be a really cool thing to take a look at. So the zero prevalence that was just a group of four dogs. So kind of a small group, but nonetheless. Zero percent in that group. So, what was our treatment plan?

We had identified 88 dogs. We decided that we were going to treat all positive dogs whose behavior would make it most likely for them to be adopted. So this ended up being 49 dogs total. And that included the two

pups that were born on-site. We did not treat positive dogs who were not likely to be adopted.

And there were 39 of these dogs. Obviously if any of these 39 had become symptomatic, they would have been treated. But it seemed like, if we felt that these dogs were not going to be rehomed, why put them through the treatment, why put our organization through the cost; it's, it just was the best solution we thought.

So our treatment plan, we identified these 49 dogs that we wanted to treat, and we utilized the standard protocol, which has been published, for atovaquone and azithromycin for ten days. We monitored PCR at 30, 60, and if necessary, 90 days post-treatment.

Current thinking is that two consecutive negative PCRs, 30 days apart is considered clear of the infection. When you look at published literature, you will see that they didn't begin their post-treatment PCR testing until day 60. And that's wonderful if you're dealing with a dog in a private home.

The reason for the delay is that we know PCR detects both live and dead organisms, and the worry was, if you do it at 30 days and you have a positive, are you detecting a, a dog that's still infected, or is that just dead organisms that haven't been eliminated yet?

But in a shelter setting, the sooner we can determine where we are the better. So we said, "What the heck? We'll start at 30 days." And again, here's the protocol. Atovaquone. I've seen anything from 13.3 to 13.5. For whatever reason, we decided on 13.4 mgs per kg, PO TID in a fatty meal, and commercially available azithromycin at 10 mgs per kg PO SID, given together for ten days.

Our shelter operational period was probably very similar to most of yours, and it was basically from 8:00AM to 5:00 or 6:00PM. We felt that it would be really important for us to try to get this to be an every eight hour treatment as much as possible.

Keeping in mind that this meant we would have to send a team of two people back to the shelter after they've been there all day, working hard, they go to the hotel, relax a little bit, have dinner, then they have to get back in the car at 10:00 and head back to the shelter, treat the dogs, and then go back and wake up the next morning.

So it was in, pretty intense and we tried to rotate which two people went in so we wouldn't have the same people with an exhaustive schedule. There

is a commercially available atovaquone. It's called Mepron, and it is a human product. And as such, it is priced as a human product.

It only comes in a liquid; the tablet form was discontinued in people, as in people, they felt bioavailability was better with the liquid. But we had used compounded atovaquone in our earlier case and had some success.

And knowing that we had 39 dogs to come in and treat at night, there was just no way we felt we could do, you know, administer a liquid, via syringe anyway, to 39 dogs. So we had capsules compounded in 50, 100, and 250 milligram strength, and each dog was dosed as close as possible to the 13.4 mgs per kg.

What we did, because people were coming back at night, we would go ahead and prepare their little food trays during the day, and put them in the fridge so that at night, when the team came in, all they would have to do is take these out of the fridge, put it on their cart.

They were organized by pod, and it was a fairly simple matter to just go pod by pod and slip these under the appropriate cage door. Our average dog weighed about 17 kgs. These, as Dr. Cannon mentioned, these dogs are really pretty small, that these professional dog fighters use.

Again, I had so many eye-opening experiences. They didn't have their ears cropped, they weren't these huge, bulky, what I would consider a typical pit bull. They were pretty small dogs. So our average cost for these drugs for the compounded atovaquone and commercially available azithromycin was about $130.00.

And you compare that to the reported cost of a commercial Mepron and azithromycin for a 20 kilogram dog, which was $356.00. So there's significant savings both in time, effort and money, if you go with a compounded atovaquone.

So we were happily planning that, okay, at some point we're going to start our *Babesia* treatment as soon as we get this distemper thing somewhat under control. And as soon as they stop transferring in more dogs, and I can actually think about things. But before I could get started, we had one dog develop clinical signs.

This dog had her intake exam five days after admission. And she was a lactating female with three pups. Look at that body condition score. One out of nine. Teeth were severely worn down or missing. Her pack cell at that time was 29. So not great, but not horrible.

The rest of her exam was within normal limits, and she had her blood drawn for her *Babesia* PCR. On 8/30, she was identified positive for *Babesia gibsoni*. And she was positive for Isospora on 8/31.

And again, we had 87 other dogs that were positive for *Babesia gibsoni*, so you know, we just had her monitored like, on our daily rounds, like we did everybody else. She had routine treatment for her coccidia, and got her booster DAPP, and again, at this point, everybody's, if they didn't have distemper, they were doing' pretty well.

And by the way, I should say this whole distemper talk, Dr. Crawford has to talk about that. This was amazing. We only ended up I think with 12 dogs coming down with distemper. And it was really brief, thanks to Dr. Crawford and her team. But, in any case, it was very nerve-wracking, when I knew that I had over 100 dogs at risk because they had no antibody titer.

Clinical signs in this little dog were noticed on 9/12, during daily rounds. And actually daily rounds was more like twice-daily rounds.

Her mucous membranes were pale. She was lethargic. She was dehydrated. Temp was up a little bit, but again, we're in the south, in the summertime, and so 102.8, eh. Her in-house PAC cell on 9/12 was 16, and her total solids were 8.8. Whereas 15 days earlier, PAC cell was 29, total solids were 7.6.

So again, she, I mean she wasn't like wiped out horrible, she was just like, something's going' on here. And I will say that we had also a little pup that I thought for sure was having a *Babesia* issue, and it just had horrible, horrible hookworm. So, she didn't, she looked bad, but not like on death's doorstep yet.

So blood was sent to IDEXX. She was given fluids to correct her dehydration, and then the next morning we got our CBC results. Her white count was within normal range, but she did have some toxic neutrophils. And then we had these lovely red blood cell parameters.

So her red blood cell count of 1.77, hemoglobin of 4, hematocrit now is 12, nucleated red blood cells is 9, and elevated reticulocyte count. And all of these wonderful terms that, and we can remember from our clinical pathology classes in vet school. She had it all, except notice that they didn't see any parasites microscopically.

That really surprised me, because this is a reference lab. But that just goes to the point that, you know, unless it's an acute infection, you may not see

them on the smear. So, before we sent her to UF for her transfusion, we gave her first dose of atovaquone and azithromycin.

When she got to UF on 9/13, her PAC cell was now down to 14. They kept her overnight, and following her transfusion, her PAC cell was 24. Up from 14. So that was good news. They told us to monitor her for a transfusion reaction or continued hemolysis due to *Babesia* infection.

So we had to look for lethargy, pale mucus membranes, and hematuria. And they suggested we obtain a PAC cell total solid the day following discharge and monitor her as needed. When they discharged her, they did note that she had had a little vomiting and diarrhea, cause could be the treatment for *Babesia*.

We see this sometimes when we start treatment. Could've been stress. It was not that big a deal. She was treated symptomatically. And then on 9/15, the day after she came back, she was more alert and active, no further vomiting, and by 9/16, she was eating well, no vomiting, and normal stool.

So, she was treated from 9/13 to 9/22, with our standard protocol. Possible side effects of vomiting and diarrhea for one day only. I laugh when I see her follow-up PCRs, because we had a 30 day, a 48 day, and a 79 day. And you're like, "Huh?" And if you haven't deployed with the ASPCA, it's a wonderful, wonderful opportunity to do so.

As a medical director, I mean, we're always looking for veterinarians and vet techs and vet assistants, and people who like to do data stuff. But, because people have regular jobs, they can't usually drop everything for two weeks; somebody might come for three days, somebody might come for five, somebody might come for two weeks.

As such, constant turnover in your medical team. It was pretty rare to not have one person leaving and one person coming in every day. So, if things got a little bit jumbled in translation that was understandable. So 30 day post treatment, which actually looks like it was about 30 days was negative for *Babesia*.

For whatever reason this dog had it submitted again on day 48, and that was also negative. And then on day 79, it was negative again. So this dog clearly had at least two negative, consecutive PCRs 30 days apart. And as such, there was no further treatment or, and no further PCR was done on this girl.

Just to look at her, her PAC cell over time, and again, she had her PAC cell, her blood drawn on her intake exam, which was 8/28, why she had it

done again on 9/4 and 9/5, I have no clue. But there you go. You can see where she bottomed out, before she had her transfusion.

Then we were real good. They did the, the post-transfusion PAC cell at UF. We were pretty good; we got it two days after she came back, but then nothing. She was doing so well, we didn't repeat it. But when I was back at the shelter in March, I thought, I wonder what her PAC cell is like. And it's 49. So it's pretty good.

This just shows you again, our four groups, how many dogs were treated in each group, and the treatment dates. You know, ideally, because you have to have people come back to the shelter at night to do this treatment, it would've been great if groups one through three could've all been treated during the same time, but it just didn't work that way.

So we kind of treated them in different waves. So total treated, 49. 42 were available for follow-up, and that consisted of 40 adults and two pups. We lost seven to follow-up. Three were euthanized, and four were transferred to outside agencies.

And depending upon the agency, and the type of transfer, even though we always requested a follow-up PCR, and would email them, and call them, and say, you know, can you do this? Sometimes it just didn't happen.

And my best guess is that these might've been dogs that were actually signed over to us, so they were free to be actually transferred to another agency and then adopted. And we all know, once that animal leaves the shelter, which is what we want follow-up sometimes doesn't happen.

Again, side effects of treatment, partial anorexia, vomiting, usually lasts about one to three days if it happens at all. I usually just say dogs that have been treated, they're just kind of like, meh. It's nothing horrible, but they just look like they're not feeling great for a day or two.

We did treat six dogs with Cerenia, because of vomiting. Two of the six had renal disease, so I'm not sure if they were, you know, vomiting because of the renal disease or because of the *Babesia* treatment. Again, to the best of current knowledge, two consecutive negative PCRs, 30 days apart indicates the infection has been cleared.

And depending on the literature that you read, some folks feel that they never really eliminate the organism entirely, but that the levels in the blood are so low that it's beneath detection. And they also propose that if that is the case, that the likelihood that this dog will be much of a risk to other dogs is pretty low.

So, of our 42 dogs available for follow-up, we got some really great results. We had 37 out of 42 that were cleared by day 60, or 88 percent. This is higher than published reports that indicate 80 to 85 percent will clear their infection. So that was pretty exciting. And then, two more dogs cleared by day 90, for 93 percent.

That was really exciting. And then of course we did have three dogs that didn't clear. And, for seven percent. But those are really, really great numbers. If we break it down into adults that were treated versus pups, numbers get even better. 93 percent of our adults were cleared by day 60.

95 percent were cleared by day 38. And two, or five percent, remained not cleared. We did have seven dogs give birth during this deployment, for a total of 55 pups. 40 pups survived, 11 were stillborn, and four died during the neonatal period.

The *Babesia* status of the mom dogs at that intake exam identified four of these moms as positive, and three as negative. We decided to treat three out of the four positive mom dogs. One was treated during the last trimester, and two were treated when the pups were about one week old.

I looked in *Plumb's*, I'm like, "Well, is this going to do anything?" And we just felt, let's go ahead and treat. We did not treat one of the positive mom dogs due to behavior concerns. She was not likely to be placed and she had only delivered one stillborn pup the day before we began that treatment for group two.

So this was before we knew what, if any benefit pups would gain from their moms being treated, so we just thought, okay, she's in the not treatment group. All of our treated, positive moms were negative at 30 and 60 days post-treatment.

And thanks to Dr. Levy, who was elbowing me, she said, test all those surviving pups at four and eight weeks. Let's see what we've got. So we had 40 pups that survived that we tested. 18 were born to negative moms, and 22 were born to positive moms. 16 out of the 18 born to negative moms were negative on PCR at four and eight weeks.

But two were positive. And all 22 of the pups born to positive, treated moms were negative at four and eight weeks. This was really exciting. The two that were positive was not exciting. They happened to be litter mates, born to a mom who was identified as negative at her intake exam.

So of course we retested her, and she was positive when we retested. No surprise. I'm sure every one of you in this room thought that when you saw the previous slide. So now, great. We have two positive pups. But

all 22 of the pups that were born to positive, treated moms were negative at four and eight weeks.

So one has to ask the question, does treatment during pregnancy or lactation confer protection to offspring? Or you know, is there vertical transmission from the mom? Well we know there's vertical transmission from the moms.

We know it's incomplete, but looking back at our previous case, where we had four positive mom dogs, none of whom were treated, we had positive and negative pups from each positive mom, the only exception being mom C. She only had one humungous pup who just happened to be negative.

But you can see that with the other litters, we had anywhere from 25 to 80 percent of the litter was positive for *Babesia*. So again, these moms were positive, they were not treated, and we did have pups that were positive.

And if you compare both of the cases, looking at number of positive and negatives, again, you know, the only time we had positive offspring from these two cases was if the mom was positive and not treated. Is this going to happen every time? I have no idea. But this is what we saw.

So when should we treat these two newly-identified positive pups? Our previous case suggested that young pups didn't respond as readily to treatment, and minds that are much smarter than mine indicate that it's due to their immature immune system, and does it not respond to the drugs that we're using?

And, you know, I'm ever-optimistic. I, I wanted to delay treatment as long as possible for the best possible outcome for these pups, but we never know on these cases when we might be given custody of these dogs. And when we're given custody, we want to get them transferred out ASAP.

I did not want to be treating pups and have them in the middle of treatment, and then have them transferred out. So we delayed treatment until these pups were ten weeks of age, and the results were not great. Only one of, one pup was positive, one was negative at day 30.

They flip-flopped on day 60, and then by day 90, one of the pups had had two consecutive negative PCRs and was considered clear. The other one did not clear. So again, this graphic just shows you. And with an n of 2 *[inaudible]*, you know, I don't know. We ended up with two groups for possible retreatment.

We, while I was still with the A, we did retreat the two adult dogs. The pup, I had suggested be retreated. I'm not sure if it was. But we didn't do

well on retreatment. And this kind of is the same thing that others have discovered. If you retreat with the same drug combination, you're really not doing anything beneficial.

I was very excited at day 30 when these PCRs were negative, but by day 60, actually one dog, day 48, they were positive. It's presumed that the dogs that, you know, one mechanism that might be at play here is that *Babesia*, might have a gene mutation which renders it resistant to atovaquone.

So if you're going to retreat dogs, you should use a different drug combination. And again, a controlled study indicated some dogs never clear the infection. 80 to 85 percent. And those that don't clear are a potential source of infection for other dogs. They may develop renal disease down the road.

And of course they may suffer a hemolytic crisis at some point in their lives. And again, do any dogs really clear? I think that question is still out there. And there are real researchers *[Laughs]* working on this.

I have all kinds of random thoughts that go through my mind when I'm at, at my shelter and thought, well, would we see more pup mortality in our *Babesia* positive moms than we did in the *Babesia* negative moms? Or did somehow the treatment – it either really helped the pup, or it killed the pup?

So I just wanted to look at that. And again, no statistics here, it's just the raw numbers. It's kind of all over the place. The dog that had the highest mortality for her litter actually was that mom that wasn't treated. And she also was herpes virus-positive. She had four out of seven pups were stillborn.

One died, I think the day after birth. So, you know, certainly one would question whether the herpes was playing into that. We had two interesting, two moms that had no mortality. One was treated during lactation, one was a negative mom. Ideally, wonderfully, they each had 11 pups.

So I mean, I just thought it was interesting. What might be causing the mortality in these pups? And it could be any number of things. So, what will you do in your shelter now that I've raised the specter of possible *Babesia gibsoni*? Will you test all of your incoming dogs?

I think it's the rare shelter that has the resources and time and money to do that. Would you test only certain dogs? Will you test all the pits and pit

mixes that come in? I'm guessing probably not. Again, that, for a lot of shelters, that's a significant number of dogs.

Will you test dogs that are in your shelter from alleged dog fighting cases? That would be a really good idea. Will you test dogs that have scars suggestive of fighting? That would be a really good idea.

And again, the one study indicated that dogs with scars are 5.5 times as likely to be positive for *Babesia gibsoni* as dogs that do not have scars. Will you only test dogs if you have one that becomes clinical for babesiosis? That may be, that may be all you can do. Or you may not test anyone.

I mean, every shelter is different with regard to its resources. So, I think you have to look at, at your shelter and decide what's best for you. But if you do test, and you get a positive, are you going to treat it? Or just adopt it out with a waiver? Your waiver, if that's what you're going to do, you want to be sure your waiver is pretty thorough.

And you need to discuss the possibility of, you know, what clinical signs these adopters might see, if there's an acute hemolytic crisis. They should be made aware of the fact that renal disease could occur. And they should also be told that this newly-identified positive dog is considered a risk to other dogs, if a significant bite occurs.

And the question is, that's the big question. What constitutes a significant bite? It's thought that casual contact is not a concern. We do know that with dog fights, that's a significant, that's a significant bite in those guys.

And again, oral bleeding occurs, there's an exchange of blood with all of that, and that's thought to be the primary means of transmission at this point. So there are little, little bites, and there are big bites, so. But if somebody's adopting a positive dog that hasn't been treated, they need to be made aware of potential issues.

We did have one dog that we treated successfully in that she cleared her infection. But she did develop renal disease about a month or two later. And who know? I mean, was this due to her having had *Babesia*? Possibly.

When I asked the question, could necropsy tell us if this renal disease was due to *Babesia*, I was told no, probably not. So this dog was not necropsied. If you do treat positives, are you going to hold them for two to three months until you get those negative PCRs?

That would put a strain on most shelters, I would think. Again, in our setting, when we have these dogs for three to six to nine months, we can do that. But in your shelter setting, is that something that you can do? Or will you treat that dog and then send it out to foster until the dog's considered clear?

Or will you adopt it, telling the new owners that the dog has been treated, but we don't know the results yet, so you have to consider the dog positive until we get these results? And if you, if you do send them out with this information, will you, or will your agency cover the cost of the follow-up PCRs?

And then the question is: will they really come back? And it, and, you know, it all depends. Again, the cost is not insignificant. Drug cost is about $130.00. Cost of follow-up PCRs is about $49.00 per test, and you're going to have at least two.

So, the cost of drugs and follow-up tests alone ranges from $228.00 to $277.00, and that doesn't take into account the cost of food and shelter. Other things to consider, if you have a positive dog, either it's positive and not treated, or positive and treated but you still don't know the final result of the tests, if they're adopted or fostered, ideally there would not be resident dogs.

Then at least you don't have to worry about another dog contracting the disease. Again, casual contact is probably not a concern, but a significant bite would be. So what some agencies do is have them wear a basket muzzle until they're comfortable with what kind of dynamic is going on between the resident dog and this dog that's being fostered, or the newly-adopted dog.
If you're going to keep a positive dog, positive, treated dog at your shelter until you have your PCR results, you really have to consider the behavioral needs of that dog. You know, we can't just put them in a box for three months and expect that they're going to come out of this behaviorally intact.

They do need to have opportunities to play and to interact with like-species. So, you know, what we would do in our shelter, for example, if we, if we had two, *Babesia*-positive dogs, and if they had gone through the treatment we would either put them in a play group with a basket muzzle, or we might let two well-behaved *Babesia*-positive dogs play together without muzzles.

That's taking a little risk, obviously, if a bite does occur, and one's in the process of clearing, and one isn't, but you know, everything's a balance,

and you really, you have to let these dogs interact and play, otherwise, it's just not fair to them.

And while you wait for your post-treatment PCR, be sure, even though we haven't yet identified a tick vector in the U.S., still, you do want to use products with for tick control. I would not let these dogs go to dog parks, and as Dr. Cannon mentioned in her talk, these dogs from these cases, even if, if you get the two negative PCRs, I would not use these dogs as blood donors.

I think there's just still too much we haven't figured out yet, and they would not be a good choice for blood donors. So I ended a little bit early, but I do want to give my thanks to Maddie's Fund®. I have, I and my daughter have been beneficiaries on many levels, thanks to this fund. So it's with much appreciation that I thank them.

Also, great thanks to the ASPCA, and in particular the Field Investigation and Response Team. While my total time with the team was only two years, it was an amazing experience, and one that I'm profoundly grateful for. And also great thanks to the Maddie's® Shelter Medicine Program at UF, particularly Drs. Levy and Crawford. They were such a huge help to us, as I know they've helped many of you, as well. So, that's all I've got. Thank you very much. *[Applause]*

[End of Audio]