THE U.S. FOOD AND DRUG ADMINISTRATION

WORKSHOP TO CONSIDER APPROACHES TO REDUCE THE RISK OF TRANSFUSION-TRANSMITTED BABESIOSIS IN THE UNITED STATES

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*   *   *   *   *
MR. GOODMAN: You know, as the day comes on to -- maybe move closer for -- so you have a more intimate feeling with your other speakers here. I'm Jesse Goodman, the director of the Center for Biologics. I just want to thank you all for coming, and the distinguished speakers and others for their involvement.

The idea here is to be proactive and prepared. This is not a signal that we think at this moment in time there is a crisis or an intervention. But this is to try to take stock of where we are with Babesia, what are some of the potential strategies in preparedness needs and how to move the field forward.

And a number, but not all the people who are here were with us in the Blood Products Advisory Committee yesterday, where we talked about malaria and approaches to protecting the safety of the blood supply for malaria, and there are some echoes of that. Most of the people in here
know far more and are day-to-day involved in Babesia.

But as at least a former and not totally reformed researcher in tick-borne diseases, I could make a few introductory comments on the -- you know, compared with malaria, you know, with -- for which it's occasionally mistaken. So in the clinical universe when -- in the Midwest where I was, we would occasionally see patients who had been misdiagnosed as having malaria and inappropriately treated and not doing well.

But compared to that, there are some differences. Obviously, some are somewhat favorable to the patient such that this is mostly pathogenic and life-threatening typically only in people who have one kind of immunologic compromiser, immaturity or another.

That's not to say there aren't normal individuals who've had very serious or fatal Babesia infections, but more typically as you'll hear, I'm sure, it's splenectomized individuals or immunocompromised individuals, and in recent years, for example, particularly people with HIV. The epidemiology is perhaps a little less understood than malaria.

Certainly, there is a lot more issues about
global distribution of the parasites that were outside of the United States as was well understood. You know, perhaps obvious to the transfusion community, sort of disadvantage when compared to facing an in transfusion medicine as opposed to malaria is the sort of fairly blunt as we discussed yesterday.

I mean, the transfusion community is unhappy enough about the deferrals we used for travelers from malaria. But certainly for Babesia, behavior-based deferrals such as on tick exposure or geographic or seasonal deferrals, you know, would be a very blunt instrument, particularly given the rarity of this disease in transfusion.

Some of the -- this leads us to not having an awful lot of obvious approaches, but some of those that have been considered, which again I'm sure will be discussed, include things like pathogen and activation, and of course which could potentially be general or targeted.

And then, of course, diagnostic testing, which as you will hear again, presents a whole variety of interesting challenges, some reminiscent of malaria, some
different. But you know, similarly, perhaps with direct
detection methodologies probably not being as reliable as
one would like. So having just said that, you know, again
I welcome your interest. This is important.

And I just wanted to say in general, one of the
things we are trying to do and I think was a successful
model and we are trying to even move it back a little more
in the cycle of emerging infectious diseases is
preparedness for threats to the safety of blood and
tissues and to be anticipating them rather than just
reacting to them.

One good thing about ticks -- well, there is
many good things about ticks in my mind and Dr. Telford's
at least -- but one of them is that they don't tend like
mosquitoes to have quite the same dramatic and rapid
ecological changes that lead to disease explosion in one
area or another.

The flip side is as opposed to mosquitoes --
whereas we heard yesterday you can do a pretty good job
reducing their population and preventing them from
spreading disease and we have seen progress in that, for
example, in Mexico. And nobody mentioned it yesterday,
but that's why we don't have malaria in this country, mostly is our control mechanism.

As opposed to that, we don't -- even though Dr. Telford has come up with some interesting approaches to reduce tick populations, we really don't have any effective approaches here. And then I didn't know that Sam would be here, but I just want to say that people who study ticks can have a sense of humor so -- although actually we can both say it's pretty unusual.

But one of my treasures -- so we have to consider the other side of the equation here, and I just thought I would show this to get everybody laughing. Sam may even have this, but this is the T-shirt of the International Society for the Preservation of Tick Species.

(Laughter)

MR. GOODMAN: So in your haste to wipe these things out -- and there of course is their slogan. Now, do I really believe that? Well, I don't know. You have to ask me. But all humor aside, I will say I just finished a month of clinical attending at the naval hospital in the summer. And I was actually fairly shocked
by the number of cases of disseminated Lyme disease that we were seeing.

So I think the notion that we have control over tick-borne disease -- and if you look at the CDC curves on Lyme disease, you know, there has been an increase over the last several years. Of course, these are -- this is the worst kind of passive reporting, and we don't really have a good hand on how many cases of primary infection there are.

But providing that the reporting system hasn't changed, we certainly don't see a tapering off of case numbers of the major vector-borne disease spread by the same Ixodes ticks. So I think we need to take -- continue to be prepared and take this threat seriously. So with that, I thank you all for coming.

We look forward to this, and also as a paradigm, as I said, for preparedness. And I will turn it over to Dr. Kumar, and also thank the Office of Blood for organizing this meeting. So thanks very much.

INTRODUCTION TO THE WORKSHOP
MR. KUMAR: Good morning. And thank you, Dr. Goodman, for the welcoming and introductory remarks. As you can see, my name is Sanjai Kumar and work at the Center for Biologics Evaluation and Research at FDA here. So I'm just going to give you a quick introduction to the workshop. I mean, there's not that much to introduce, you can see all in the agenda. So you can get on the main workshop.

Can you see it back there? We need to change lights. How do I do it? Here? No. No, it's not -- okay. Now we can't -- I can't see anybody. So -- don't think I did anything. Okay, I don't know if I did anything. I think -- all right. Let me get it started. So it's a malaria-like illness as Dr. Goodman said, and the similarity is eerie with malaria, but so are the differences.

It seems intraerythrocytic pathogen have -- caused by genus Babesia and you will hear a lot more than I will tell you and -- or ever can tell you. Several species are naturally transmitted in the United States. Clinical cases of babesiosis have been reported in many states in United States and the northeastern states,
Midwest, and some western states.

Clinical diagnoses and laboratory detection are difficult for the reasons you will hear throughout the -- for one reason on blood film it's very difficult to distinguish from falciparum rings and piroforms (phonetic) of Babesia. And the true incidence rate of Babesia cases in U.S. is not known. It's not a nationally reported disease in most of the states in the United States.

I think we worry -- we spend worrying so much time, rightly so, for the diseases which are not even -- which are foreign diseases. Even -- I mean I hate to say this being a malaria researcher, even malaria, but this is a disease which is naturally transmitted here, deeply entrenched. There is stable annual transmission in this country.

So I think this workshop is very -- both very timely and much needed. Endemic transmission continues to occur every year. And there's a large information gap regarding the rate of transmission, number of clinical cases each year, and the asymptomatic carriers which pose the highest threat of causing transfusion-transmitted babesiosis. That is the main reason we are here today.
It's obviously caused by transfusion of blood and blood products from infected donor. And again, they're hard -- true numbers are hard to come by, but about approximately 70 incidents of transfusion-transmitted disease have been reported in the U.S., and actual numbers could be possibly much higher.

In recent years -- and you will see bits of this in later presentations. It seems like there have been sudden surge in the number of fatalities that could be attributed either as primary cause or secondary cause caused by transfusion-transmitted babesiosis that were reported to FDA.

There is no approved laboratory test to detect Babesia infections in blood donors, not in this country and to my knowledge nowhere else. And it has created a great need to discuss novel strategies to mitigate the risk of transfusion-transmitted babesiosis.

And many of us who were present yesterday in the Blood Product Advisory Committee for malaria donor deferral issues, I think if we work together and we learn together and move forward together, then we will probably will not find ourselves in 10 years on the same situation
where we find ourselves for malaria, because there are many similarities.

Even if you identify an infected donor, what to do with it? I mean, how to defer -- how long you keep the person deferred while keeping the issue of donor availability in mind?

So scientific -- so the objectives are scientific discussion on epidemiology, transmission patterns, clinical disease caused by infections with Babesia species prevalent in certain geographical regions in this country, and you will hear plenty of this; assess the risk of transfusion-transmitted babesiosis and methods to identify and defer at-risk donors; and possible approaches to minimize the incidents of transfusion-transmitted babesiosis in this country.

Biology of human Babesia species and their Ixodes tick vectors we'll hear about them, epidemiology, and -- okay, did not move. And then possible approaches to reduce the incidence of transfusion-transmitted babesiosis includes donor testing and pathogen-reduction technology.

You will hear about that as Dr. Goodman
mentioned, algorithms for testing Babesia infections, geographical exposure versus universal screening to allow donor deferral, and how long to keep them deferred and reentry. So basically, we have broke this down into four scientific sessions as you can see in your agenda, and then there will be one panel discussion.

At the end of each scientific session there will be open discussion. So I would request to you to hold off your questions for these speakers, because each speaker is going to serve on a panel discussion also. So you can direct your questions to the panel discussion.

And I request to please, please all session moderators, any speakers, to stick to your time, because first we have a very rich and full agenda here, so we need to get through the agenda. And we have been informed by NIH security that we need to be out of here at the allotted time which is 5:30 for security reasons. So we will probably have 15 minutes and no more to clear out.

Another logistics, the cafeteria is downstairs, so are the restrooms. We will have two or three coffee breaks. There are some restrooms on this floor also, just go down the hall and next to the elevator. Another thing
I would like to say and then I would stop, this -- the parallels have been eerie between Babesia and malaria. So it's -- in the 1880s, when malaria parasites were identified and so was the Babesia around the same time.

And Laveran, the French physician who first identified malaria parasite in an Algerian patient, he also worked in Babesia parasite around '80, '85. But the thing is it took another 60 or so years before the first human case of babesiosis were identified. So it's exactly approximately 51 years before Babesia was identified as human pathogen.

So it's rather a new human disease. But we have the advantage that most of the people who have worked and contributed most to Babesia research, at least on this side of Atlantic, are present in this room here today. So it's a very fortunate experience here, and hopefully we will take most advantage of it.

So -- just some acknowledgements are due. Members of the Scientific Program Committee who have helped us to put this agenda together, and many of you who are present here in this room and you know who you are, so thank you. I must thank Dr. Nakhasi, Dr. Paul Mied, Dr.
Asher, and Dr. Epstein who helped tremendously to bring the entire issue together and move the -- this workshop forward.

I must thank Noel Gerald who is from my lab, and Olga Maximova who used to be with us in another lab in our division, but has now moved on to NIH, to help us design -- helped me design this poster you see, which many people are inquiring about. So thank you.

And Jennifer Scharpf, who is our associate director for policy, and Rhonda Dawson, who works with Jennifer, and Darlene Best, we couldn't have done this workshop without their help. Darlene helped us with the travel arrangements for all the speakers here. So thank you all. And I think it's time to switch it to Dr. Barbara Herwaldt who is going to -- now I can't get out of it. I think I need help.

SPEAKER: (Off mike).

(Laughter)

MS. HERWALDT: Yeah, then if you can get me, Herwaldt --

MR. KUMAR: We can get it okay -- a divine voice advised us. Okay. All right, thank you.
(Laughter)

SPEAKER: -- just go down that way.

MR. KUMAR: Okay.

OVERVIEW OF BABESIA SPECIES AS HUMAN PATHOGENS

MS. HERWALDT: Well, thank you very much. I'm very glad to be here today. And this is going to be an overview talk. And I'm here to talk about the other intraerythrocytic parasitic disease. And I'd like to actually start with some basics, to make sure that we get something straight from the very beginning.

First of all, these are intraerythrocytic parasites, but they are not plasmodium species. Yes, Babesia species are tick-borne in nature, but they are not bacteria. And yes, Babesia species can be blood-borne, but they are not viruses, and the viral paradigm does not apply in many respects.

I would like to start actually with my morals or conclusions so that before you fall asleep some of my main points come across. And of course for most of you in the audience, these are very straightforward and self-evident.
Don't assume ring forms equal malaria, consider babesiosis. Don't assume tick transmission, consider blood transfusion. Don't assume acquired in the Northeast or upper Midwest, consider other regions. And don't assume the species as Babesia microti, consider other species.

Now, Sanjai alluded to the history. I'm going to start with the temporal dimension. We are going to have temporal and spatial dimensions. And this is the big picture, temporal dimension.

Way back in 1888, Victor Babeș, after whom the Babesia genus was later named, investigated febrile hemoglobinuria in so-called Romanian cattle caused by an intraerythrocytic microbe. And then in 1893, Smith and Kilbourne, switching continents now over in the U.S., were investigating cattle again, Texas cattle fever caused by a tick-borne parasite. And this was the first demonstration of an arthropod vector.

And switching back to Europe, almost 50 years later or more than 50 years later, 1956 was the first well-documented zoonotic case, meaning human case in what was then called Yugoslavia. Switching back over again to
the U.S., 1966 was the first documented U.S. case, and it was in California. And the species was not determined, and we can't determine it in retrospect, either.

It wasn't until 1969 that the first documented case on Nantucket was identified, and that was caused by Babesia microti. And then 10 years later, the first documented -- I want to keep emphasizing "documented" -- blood-borne case, again in Massachusetts, caused by Babesia microti.

So this is the big picture perspective from which we are now dealing. Now I'm going to shy away from showing maps with the actual distribution of species and cases for various reasons. But I want to start with just a blank map, so to speak. And you see all the states here and they look united and quite innocuous.

And as I mentioned, the first documented U.S. case was in California in 1966. And as I said, we don't know the species. Then there was another case documented in 1979 in California also in an asplenic person, and we don't know the species. But then at least temporarily, California seemed to have fallen off the babesiosis map.

And the center of action shifted to another part
of the country. France continental over to the Cape Cod area, you will see a little bit of Martha's Vineyard and right down here, thanks to NASA, we can see Nantucket. It looks quite small and innocuous, doesn't it?

But if you are taking your infectious disease, board exams and there was a question about, oh, someone was on Nantucket, you don't have to read the rest of the question, you would know the answer is babesiosis. There is that association in a lot of peoples' minds between Nantucket and Babesia infection.

Babesia microti is the species that most of the speakers will be devoting their attention to. And a lot of my talk will be about the other species -- make sure everyone is on the same page and knows the various organisms. But I will initially start with a little background about Babesia microti.

I like this slide in part because this is actually from a public health notice, and it's like note, take note, and that is why we are here today. This is a public health issue. And this came from a public health notice before my time, and I deleted the written part of the notice.
But it had to do with some cases in Nantucket, but actually nicely shows some of the offshore coastal islands that we sort of still in our heart of hearts think of as the Babesia heartland, but Babesia infection is no longer, you know, concentrated or just in these coastal islands, or even just in these states.

But here we see Massachusetts, Rhode Island, Connecticut, New York, and some of the key offshore islands. And Peter Krause will be telling you about Block Island and other islands, and I'm sure Sam Telford will be talking about Nantucket and you know, a lot of other places as well.

But again, this is some historical perspective, and I like the idea that Providence is right here in the middle of the map. I like to think of Providence being here as part of the public health issue and directing what we do here even though it's not center of babesiosis heartland, so to speak, in terms of the geographic place.

Now, when we think about babesiosis in a general sense, we think of it as tick-borne zoonosis. Etiologic agents, multiple Babesia species -- as I will be talking about later, the tick vectors, various Ixodes species,
reservoir hosts, various wild and domestic animals. Now, there is specificity amidst the diversity.

And for some of the species, as we will be talking later and as Sam and others will be mentioning later, there are particular tick vectors and reservoir hosts. For others we don't have clue what the tick vectors and reservoir hosts might be. But this is just broad strokes.

Now, in terms of geographic range, in terms of what's been documented, which may be very different than what has occurred, the big picture perspective is that the vast majority of reported or documented cases of babesiosis has been from the United States, particularly from what I loosely call the "northeast" and parts of the upper Midwest, some from Europe, and a few from elsewhere.

And during the panel discussion, we can talk more about some of these other areas. And to simplify some of the complex issues that Sam will be talking about, when we talk about Babesia microti in U.S. foci, some of the dimensions include the parasite, of course, and tick vector. And Sam may get into the nuances of dammini and scapularis and various animal reservoir hosts.
And of course, there are other ecologic vectors, and there are other dimensions that are very important too. But humans are not part of this cycle normally, they are incidental hosts. Now, when we think of Babesia microti, we have to keep in mind it's not necessarily just a homogenous species. There may be different strains which may have importance in terms of virulence.

And then also particularly in other parts of the world, there may be Babesia microti-like organisms that may or may not ultimately stay called the Babesia microti, but for now our Babesia-like or part of the Babesia microti species complex. But when we think of Babesia microti and these organisms as a whole -- and again, I am focusing on human cases and human Babesia infection.

The U.S., far and away in terms of what's reported, you know, is the major site of action for Babesia microti. And I'm throwing in a couple other places, this is by no means complete. Just to make some points that Babesia microti-like organisms have been found in other areas of the world, far-flung areas of the world, and not just tick-borne, but also blood-borne.

And one of the recurring themes of my talk is
that blood-borne transmission often follows soon after
tick-borne transmission is documented, or it may be the
context in which people initially find out that there is
Babesia infection in their locale. For example, in Japan,
the first case -- first documented case of human
babesiosis was documented in the context of blood-borne
transmission.

And quite recently in Europe, what is being
called "B.microti" and may or may not be the same as what
we call "B.microti," has been documented in a person in
Germany. And the most likely mode of transmission is
thought to be blood-borne. If indeed that's true, it is
the first to my knowledge, case -- documented case of
blood-borne transmission in Europe.

So, and I'm going to be giving you sort of a
whirlwind tour of various Babesia species, and it's -- and
again, this is from a human perspective, and it is not
exhaustive, it is not every single one, and I can't do
justice to the various organisms. But I just want to give
you a sense, and I'm doing this for a reason, and it's
relevant to why we are here today.

babesiosis can be caused by so-called novel
parasites that are not detectable by serologic or molecular testing for Babesia microti. And again, that's very relevant to the issues of screening and blood safety issues. And by the way, my talk is not about whether, when, how, where to screen the blood supply, nor am I talking about risks or rates or regs, okay?

But some of these issues are ultimately relevant. Some of what I'm talking about are relevant to some of those bigger picture points. So Taxonomy 101 for -- in other words, phylogeny for poets. The taxonomy of the Babesia species -- a number of them you are already familiar with -- were emphasize points here at the bottom.

People always say there are about a hundred species that infect animals or mammals that have been described. Now, so many, how many, we don't know, maybe synonymous. Traditionally, taxonomy has been based on morphology and host-specificity. As I like to joke -- and again, it is a very serious matter, but to give some perspective, you know, if someone found Babesia species in a skunk, they would call it "Babesia skunkei" (phonetic).

Now, parasitologists would know the actual term, it is not called "skunkei." But it's just to make the
point that traditionally if you found something in an animal -- of course I'm exaggerating and oversimplifying -- then you would name it -- or it wasn't always named, but now our tool bag for characterizing organisms has more tools in it.

And I don't at all mean to imply that molecular techniques are the be all and end all for characterizing organisms. But it's an important issue when we talk about newly characterized organisms. For all we know, they might have been previously described, you know, 10, 20, 50 years ago. But we don't necessarily know what the characteristics were of some of those other organisms.

So I may be talking about what I point some new-age species. And by "new" again, I do not mean that they are new organisms, and I'm using the word "species" very loosely. I'm not and so implying that everything I'm going to be mentioning here constitutes a separate species. But I want to help make some sort of sense of what may seem like an alphabet soup of various names of organisms.

And I'm going to start with the WA1-type parasite and explain about the christening to Babesia
duncani and how that applies to the WAs and the CAs, how it does and doesn't apply, and then talk about some of the B.divergens-like organisms. And again, I can't do justice to them. I'm just going to briefly go over them. And for some of you in the audience, this is just basic; you know this very, very well.

For others, it's new to you. You are used to thinking of plasmodium species and the various species names, but this alphabet soup which keeps changing, by the way, can be very confusing. So first of all starting with the WAs -- and it is "WA" for Washington, this is sort of a precedent we established -- and by the way, when I say "we," it's meant in the collective royal we sense.

All these investigations have been collaborative, multiagency, multidisciplinary, and some of you in the audience have participated in some of these investigations. So the WA1 parasite, that was from the index case patient in 1991 -- very convenient -- WA1, 1991, in Washington State. So here we are back on the West Coast.

And then the second case that was documented -- back to this theme of blood-borne transmission often
following shortly thereafter or being one of the early cases documented, the WA2 case patient. And when I say "WA2," these are actual isolates from hamsters or gerds (phonetic).

And actually, this organism can be cultured in stationary red cell cultures in contrast to Babesia microti. We still cannot culture Babesia microti. So these are actual isolates from the infected patients. There was a blood recipient in 1994 and it was called WA2. And the blood donor, the isolate -- same year, of course -- the isolate was called WA3.

And this slide also gives me an opportunity to make the fundamental point that although this organism, these WA1-type organisms -- and they are essentially the same -- although they are very virulent in hamsters and gerds in contrast to Babesia microti, the fact that there was blood transmission and an asymptomatic or essentially asymptomatic blood donor shows you that just like with the Babesia microti, there is a spectrum.

Now, there haven't been enough cases caused by this organism that have been documented to be able to say with any confidence, you know, what proportion of people
are ill or asymptomatic, but at least shows you that not everyone is necessarily ill. And by the way, none of these patients were splenectomized.

Now, to give you a perspective about the WAs and the CAs -- I have already told you about the WAs, the index case-patient and then WA2 from the recipient of red cells and here is the donor. And now is the CAs, very convenient, this wasn't intended -- CA 1,2,3,4 -- 1991, '92, '93, and '94. "CA" for California, that's simply all it meant.

And these are all presumptive tick-borne cases. There was one person, you know, who did actually recall a tick bite. These are all in asplenic patients. And one of the persons died. And by the way, I hate talking about cases. These are real people as everyone knows, and so when we talk about cases and these isolates, again these are real people.

And we -- again the royal we, have never been able to get an isolate in animals or in any culture system of any of the CAs. And then there were a couple more CAs, CA5 2000 -- year 2000, another blood recipient, red cells, and CA6 was the blood donor. And the blood donor was
probably exposed either in California or in Oregon, it's not clear.

And these CAs actually behaved like the WAs, okay? Is it getting confusing? And then Conrad, et al., including Sam and myself and others, a number of years later -- 2006, so a couple of years ago, actually ended up christening the organism, the "WAs" -- you know, I will get back to that in a moment -- Babesia duncani as applies to the WA-like organisms.

And so it's a description of B.duncani and the species, and it's differentiation from other piroplasms. And by the way, it cannot be differentiated on the basis of morphologic criteria. Either light microscopy or electron microscopy or careful measurements of the various stages of the organism.

This is based on other types of criteria which I don't have time to go into. And despite concerted efforts on the parts of lots of people, the tick vector in animal reservoir hosts have not been identified. So Babesia duncani, you can think of as being synonymous in a loose sense with the WA1-type parasite.

So to simplify matters, the christening ended up
in the following. WA1, WA2, WA3 we can think of now as being Babesia duncani. These CAs, like WAs, are also Babesia duncani. And these CAs have not been subsumed under the new name "Babesia duncani." They might be part of the same species complex, they do tend to behave at least on the basis of a few cases, do tend to behave a bit differently.

And they are not restricted to California per se, by the way. But just so you are clear that when we talk about WA1 type, that's what we are now referring to as "Babesia duncani," but we still are going to be using the terminology "CA1" or "CA type." And also another important point is because there was or is an isolate of these WA and CAs that are WAs, there is IFA testing available for the WA1-type organism.

There is not for the CA1-type organism, though there is some cross reactivity. So you know, at CDC and other places when there is serologic testing done, it's done with the WA1-type IFA and those antigens. Now, for the other new-age species I'm going to be talking about, all of them are B.divergens-like organisms.

And again, I'm not going through an exhaustive
list of all of the organisms, but I just want to touch on several. The MO1 index case patient was identified in 1992, and "MO" is for Missouri. And unfortunately, the person died. And in 1996, we described the patient's clinical course as well as efforts to characterize the organism in many, many respects which I'm not going to go into here.

And one of the reasons for the title identification of another piroplasm that infects humans is because this was during the same period that the WAs and CAs were being identified. And when I say the "index patients," there have been a number of cases in various states that have been characterized by some of you and others as well as people at CDC, and are not limited to Missouri.

But when people refer to the MO1-type organism, they are referring to something that on the molecular level and in other respects looks like and behaves like this organism. And it's B.divergens-like in various respects that I don't have time to go into, but it is not Babesia divergens.

And when I talk about the fact that you can't
base specie identification on -- solely on morphologic criteria, I don't mean to imply that there are no morphologic clues, although the same species can look different in different host. But I thought I would show some of the morphologic features of this MO1-type organism.

And by the way, they are consistent with the B divergens-like organism for both literal and symbolic reasons to think about developmental stages, sort of the development of what we are doing here as well as today. An early ring form and then a more advanced one, a trophozoite and then some budding merozoites and some merozoites joined by residual body.

And here a paired (phonetic) piroform at an obtuse angle, a divergent angle is where the name "divergens" comes from. This divergent angle and then actually there are more extreme examples where it's truly, truly very divergent and tetrad, and I like to think about crisis forms and degenerate forms.

I don't want what we are doing here today to decompensate into -- Sanjai talked about this is not a crisis. But we do want to develop and become more mature
without degenerating. And we want to come to some conclusions about where we go from here and how we develop and what we can do that's best for public health and best for patient care.

And so for Babesia divergens-like what do I mean? I'm contrasting it with Babesia sensu stricto. Babesia divergens sensu stricto in European foci and in North African foci in animals is a bovine parasite. And Ixodes ricinus is thought to be the predominant vector. And of course, there are various ecologic niches.

But if it's a bovine parasite -- and I can't do justice to describing it, but what we are talking about is not Babesia divergens per se. Now, maybe it is part of a species complex, and we are not here to quibble about what constitutes a different species.

Now, in general in Europe -- we have already talked about the first reported case, and you know, we can quibble about what the species was for that -- in general, more than 30 tick-borne -- presumptive tick-borne cases have been recognized. And most were in asplenic patients and reportedly were caused by Babesia divergens.

But the type and quality of the evidence vary
tremendously. Often it was based on serologic criteria or morphologic criteria, and in some cases undoubtedly were, you know, truly caused by Babesia divergens. But some of them are really not that clear that in retrospect that they really have been or were.

And in 1998 and 2000, the index cases caused by EU1 -- again following our precedent, this is for European Union 1 -- were identified in asplenic patients in Italy and Austria. And these were the first documented cases of babesiosis in those countries. And with the help of our colleagues in Europe, we characterize organism in various ways, and described it as a non-Babesia divergen organism, but divergens-like in multiple respects.

This was described in 2001. And again, here we see some divergent angles and -- but still are famous tetrad. Now, back to Washington. I'm going in chronological order here, and by "Washington" I mean Washington State. It has also been a Babesia divergens-like organism identified there.

And we didn't give it a alphabet soup-type name, because we'd already had, you know, WA1 and WA2, WA3s, so what could we call it? And again, we are not at the point
of being able to say whether these various B.divergens-like organisms are different species. And so we are being very conservative in what we are calling them.

And again, just in terms of the morphology, you can see some of the features, some, you know, that you wouldn't be able to differentiate for Babesia microti or from Babesia duncani, and then others that are quite characteristic, they're not asserting diagnostic for these B.divergens-like organisms, I mean one of these appliqué forms, for example.

And again, these obtuse angles, and here again are some crisis or degenerate forms. I'm just going to show one phylogenetic tree which does not show all the organisms I have been talking about. This is an unrooted tree, and it's just to give you some sense of, you know, B.microti here, B.duncani here, again unrooted tree the theilerias over here, and then these B.divergens-like organisms.

And MO1 is not in this phylogenetic tree. This was a phylogenetic tree that we published in one of the papers. Here you see EU1 and a cousin, Babesia odocoilei. And by the way, we don't have caprioli and we don't know
where that fits in the scheme here. Here is Babesia divergens sensu stricto, and here is the Babesia species from the Washington State case patient.

And by the way, all of these patients to date, at least the ones who have been clinically ill -- and by the way, these Babesia odocoilei this is from animals. And this is sensu stricto; this is the Purnell isolate from cattle in Ireland. But these are -- these are from people, and this would be from an animal.

The people that have been infected with EU1 and this Babesia species in Washington State and the M01-type organisms have been splenectomized, asplenic patients. And I'm just briefly mentioning, I don't know much about this case patient, but just to give you a sense that there are other -- there's other work like this going on.

Korea, they recently published 2007 a description of an organism, and I can't speak to the characterization, because I'm not familiar with it. Now, what I just did -- and I'm just going to briefly transition into some transfusion issues, and then I'm going to be done, is just give you some signposts or stakes in the sand, and talk about some of the index case
I haven't gone into -- as I mentioned before, I haven't gone into the details of the characterization, I haven't gone into what's happened since, you know, what's happened, for example, with EU1, where it's since been found, other case patients and efforts for the identification of tick vector and reservoir host.

Now, in terms of case counts, everyone wants to know case counts, case counts, whether it is for tick-borne cases, blood-borne cases, U.S. versus elsewhere, these other species versus Babesia microti. And it occurred to me let's use the log scale. Let's use the log scale.

And for Babesia microti, presumptive tick-borne Babesia microti in the U.S., let's say log scale grew up here, more than 1,000 cases reported, and "reported" in quotation marks. We can talk later in the panel discussion and in other talks. What it -- what does it mean to be reported or documented, because it is actually important to know what terms we are using here.

In terms of blood-borne cases, again, I'm not talking about risks or rates here. In terms of numbers,
of "documented" cases on the large scale, let's just say we're around here some place. And on the log scale in terms of these other organisms that I mentioned, some of these B.divergens-like organisms and also B.duncani on the log scale we're down in here some place.

But that doesn't mean they are not important, but it just gives you some sense of how many cases have been documented. And in terms of cases -- total numbers of cases documented in other areas of the world on the log scale is presumptive tick-borne, you know, we are down in here somewhere for Europe. And down in here, you know, occasional cases in other parts of world compared with up in here for the U.S.

And so briefly into the transfusion issue, this is again an overview talk and others are going to be going into this in much more detail, so I'm just going to be ending with some points that are second nature to everyone in the audience. Again I'm putting "documented" in quotes. This even totally avoids the issue of how many have occurred.

But even how many have been documented, what does it mean to be documented? That also is an important
issue. You know, how much is hearsay and how much is confirmed and how much is possible and how much is probable and was an investigation really done. There is always a story in terms of how and why cases come to peoples' attention.

And when we do get into the issue of risk, there is not only the risk of transmission occurring by blood transfusion, there is also the issue of what is the likelihood a case would be diagnosed. And what is the likelihood that the possibility of transfusion will be considered, what's the likelihood that an investigation will be conducted?

And I'm just trying to quickly give an example. When we talked about count and how to count and what to count, and just so you may remember this situation, four donations were associated with a cluster of cases in Missouri -- excuse me, in Minnesota, and it was the third patient who is the index case patient.

But here we have donor and here we have recipients. Therefore the donor in retrospect, probably a tick exposure back in June of '99, his first donation -- and by the way, he was differed at one point for donating
because his crit had dropped below the cutoff of 38. It's only in retrospect that this was known.

It's not as if I'm saying everyone whose crit drops below 38 should never be allowed to donate again. It's just that it's often a helpful clue, not often, I mean sometimes a helpful clue when you are doing transfusion investigations to ask, you know, look at the donor records and find out was this person ever differed.

And it sometimes gives you a sense of when they might have become infected. And there was a platelet recipient who did not become infected, and a red cell recipient who did. Now, keep in mind that although in a sense these are internal controls for each other, you have to keep in mind the host factors, inoculum factors besides the difference in components.

In a second donation there was the red cell donor who became infected, so two red cell donors consecutively. The third donation, this was the index case patient, the platelet recipient did not become infected. And by the time the donor was implicated, the donor already donated again, and this platelet recipient became infected.
And so here are four consecutive donations, and several months later, the donor was still demonstrably parasitemic. So again demonstrating the long period during which someone can be infective or infectious. And so protracted, unrecognized parasitaemia and asymptomatic donors into regional shipment of blood products, travel to and from areas of endemcity, translate into the potential for blood-borne transmission anytime, anywhere.

I'm not saying the risk is uniform. I'm saying there is the potential. This is in contrast to tick-borne transmission, which, in simplistic senses, is generally regional in foci within those regions, and generally seasonal.

Blood-borne transmission obviously also may be more likely to occur in particular regions, and to some extent particular regions but -- and seasons, but it's not inherently restricted by region or season. And so as I close here, in this era of planes, trains, and automobiles, we essentially have babesiosis without borders.

Now again, that's not to say the risk is uniform, but just to illustrate the point, again getting
back to some of the West Coast states, and the fact that Babesia microti can show up there because of blood-borne transmission.

Why? Well, for example, there was someone in Rhode Island who became infected in Rhode Island, went to Washington State to do some military training and donated blood there. And there was blood-borne transmission, so B. microti showed up in Washington State.

What about California? An example. Someone from the northeast donated blood in the northeast and their blood was exported, so the person didn't go to California, but the blood was transported to California and B. microti showed up in California.

And people summer, and people winter -- someone in Texas summered in Cape Cod went back to Texas and donated blood and B. microti showed up in Texas. Someone in Wisconsin went, became infected there, wintered in Florida, and blood-borne transmission occurred there.

And blood -- people and blood products travel much more quickly and widely than infected ticks, as Sam will talk about later. And I want to close with an important point, because this is relevant to why there can
be platelet transmission by -- especially random platelets -- and by the way, in the Minnesota situation, those were full platelets, those were not apheresis platelets.

But this is an intra-erythrocytic parasitic, but there can be extracellular organisms. Obviously, when red cells rupture, the parasite released -- is released, and you know, has to get into another red cell. But at least for Babesia microti and for Babesia duncani to date -- and I emphasize "to date," -- no exoerythrocytic stage has been documented.

Is it to say that it won't be documented in the future? And by the way technically, if one is documented, then you need to go into the Tyleria genus. Why is this important? Is it important in terms of the issue of organ transplantation, tissue transplantation?

Now, of course with organ transplantation, there has been multiple cases, but it has been because of blood transfusion in the organ recipients. And theoretically there could be cases, but they haven't been documented yet to date where it's actually because of the organ, because of residual blood despite flushing. But to date, that hasn't been documented for whatever it's worth.
So in closing, back in 1888, we had Victor Babeș working with cattle, and 120 years later we have the first FDA workshop about babesiosis. And I think that we should give it some sort of -- you know, following our alphabet soup we should call it FDA, and then in contrast to (inaudible) this is a workshop. So it would be "WS" for workshop, number 1.

And whither Babesia? That's what we are here to talk about, and if only we could just make it disappear. And with that I will close. And our next speaker will be Peter Krause -- I'm sorry, Sam. We switched the order, that's right. Sam, and then Peter.

(Applause)

MS. HERWALDT: Let's see here -- you -- that's you and then I can't remember if I'm supposed to double-click on you -- probably not. Great. Thanks.

MR. TELFORD: Well, I am happy that Barbara introduced this. And in particular I'm happy to have seen the log scale, and I'll have to borrow that in the future for slides, because what I'd like to do -- I came here and specifically with the point of addressing questions one, and touching upon question five in the schedule.
And so like Barbara, what I'd like to do is give you the take-home message upfront, and then go through the talk. The first is that endemic areas is really too broad a term. Vector-borne infections, and there are no exceptions, are focally distributed.

If you chop up this room into a chessboard and then overlay it on something like Nantucket Island, square two or three may have a 100 percent of all the ticks infected, and you wouldn't get a single tick infected in the rest of the squares.

However, when you treat this statistically you pull all the data, you collect all your ticks and you put them all in the same vial, and then do the infection rates you get an average over those squares, and that's fairly misleading. For the poor sucker who steps in those two squares there is a very, very high likelihood of being exposed. Whereas the person who randomly walks through the rest of the 98 squares, or whatever number it is, there would be very, very low risk. And so that applies directly to all scales of our characterization of the distribution.

Furthermore, the infection maybe enzootic but not zoonotic, that is, enzootic refers to natural transmission,
the life cycle in nature between the tick factor in animals. Zoonotic is when it's bridged to humans. And so an agent may be present in the environment but pose no hazard whatever to humans simply because the vector doesn't have the characteristic of biting humans.

And this is classically seen in the south -- in the Southern U.S. where Trypanosoma cruzi is enzootic in many, many animals. There is a vector that maintains it among those animals, but you rarely -- you have never seen or very, very rarely seen apart from these cases of Trypanosoma cruzi as a result of transmission by Triatoma sanguisuga simply because the vector doesn't defecate on the host. And therefore humans who are exposed the bug crawls off and defecates in the wall somewhere. The magnitude of risk we can map on a very broad scale, but we don't have the data to address this issue of focality, funding largely driven by question-oriented -- hypothesis-oriented research funded by NIH we could never get good enough data to be able to rely upon a good map for so-called endemic areas.

And finally, the risk distribution is dynamic. What we saw 20 years ago is different than what we see
today. And it -- not only is it dynamic over that course of time, but because of number two, the seasonality of transmission there is a component of variability within -- between years. But one thing that may bear upon our deliberations later is the idea that perhaps you can screen depending upon -- relying upon the seasonality of transmission.

However, if you look at very detailed studies -- we've done very detail studies of the population dynamics of the vector Babesia microti -- although I usually say risk is between Memorial Day and Labor Day, there are years when it actually extends much further than that. So at least from tick data risk can be anywhere from epi week 15, third week of April or so, to epi week 40 which is in October.

And in fact given the extended duration of infection by Babesias in their host it would seem that seasonality may actually be irrelevant to an evaluation of transfusion risk, because people are chronically infected. Someone could be infected in June and go back home and donate and have a unit come up positive in February. That doesn't mean they acquired infection in February, it just
means that they've had them persistently infected.

And finally, the vector-pathogen interface, I alluded the fact that the human biting behavior which is critical to zoonosis varies from place to place. And not only that the vector relationships for virtually all the Babesia species are very poorly defined. And that's why I'm going to talk about Babesia microti.

And in fact because of that log scale, that's really the one that should direct our discussion, and in fact the global nature of Babesia microti has long been suspected, the vector of Lyme disease, the Ixodes persulcatus complex ticks is distributed in a circle throughout the Northern Hemisphere ranging from California all the way across to Hokkaido and parts of Japan. And this tick has usually as its host for the immature stages larvae and the nymphs, a rodent, and that rodent serves to infect the tick. The tick relies upon larger animals such as deer for reproduction that is that's where the blood meal comes from to generate new ticks. And that pattern is seen virtually everywhere across the world.

Normally, this tick specious complex maintains a system of infections, that is, it's not just Babesia
microti or other Babesias it maintains the agent of Lyme disease, Granulocytic ehrlichiosis, and tick-borne encephalitis virus. Pretty much wherever you want to look you will find those four members what I call the four horsemen of this specious complex. And so in theory the distribution of something like Babesia microti is global throughout what we call the Holarctic, the Northern Hemisphere.

Now, to go back to this point about enzootic and zoonotic starting in the mid 1970s the late Andy Speilman at Harvard started working on the ecology of Babesia microti on Nantucket Island, at that time called Nantucket fever, and described what he called -- he considered to be a distinct specious of tick, Ixodes, and he named that Ixodes dammini after the prominent pathologist at Brigham Hospital, Gus Dammin.

And subsequent molecular studies do indeed show that there is a distinction between northern and southern populations of ticks. However, subsequently the tick was sinonimized with -- what was a well-known specious Ixodes scapularis, the black-legged tick, and is now considered to be one and the same.
That is prior to 1990, Ixodes dammini was considered to be the northern deer tick and the vector of Lyme diseases and babesiosis. Subsequent to 1990 with the synonymy of dammini with scapularis, the black-legged tick is now considered to be the vector -- the main vector of Lyme disease and babesiosis.

And maps have changed correspondingly, that is, this set of maps on the left comes from -- I don't know what it is -- the 1990 -- 1982 to 1998 is the summary, these are MMWR slides. And here is a map of ticks considered to be vectors of Lyme disease in that period 1992 or so.

Subsequently, this is a more recent map of Lyme disease incidence in 2005, but here is the CDC map of the vector. And so now by equating populations of ticks that clearly show no vectorial capacity, no human biting ability. In fact I'm the only one who continues to use the name Ixodes dammini simply because there is a public health distinction -- public health merit in doing so.

That is, populations of black-legged ticks down here do not bite humans as nymphal ticks. The ones up north do and as we'll talk about towards the end of this
there is a -- that is the major feature of the epidemiology of Lyme disease as well as babesiosis due to Babesia microti.

And so this terribly damaging controversy about the name has epidemiological impact, and also should be brought to bear upon discussions of regional risk. That is, if you look at this map and say okay, these things occur as systems, you have Lyme disease, babesiosis, Ehrlichiosis, and tick-borne encephalitis; perhaps we should consider this area where Ixodes scapularis is endemic as a risk area.

That's not true to my knowledge. There has never been a report of Babesia microti from rodents or from ticks from anywhere south of Maryland. So again, we have a situation where even if the parasite were present in rodents and ticks down south, because of the human biting nature of the populations of ticks down south you would rarely see human cases, because the tick just doesn't feed on humans.

Now, even if we had the data there is no way to really adequately represent this on a map. That is transmission is focal, even county level maps are not
useful. This happens to be Lyme disease from Massachusetts in 2005. So if you look up here Massachusetts is blackened, and your assumption would be that all of Massachusetts is risky.

If you look at the case, the incidence, it varies greatly where places -- there is a very low background of infection and then there are areas where it's intensely zoonotic. If you overlay this on a map with the darker green being places with greater incidence and then overlay where just out of convenience sampling we found Babesia microti of rodents wherever I travel I pick up mice and I do blood smears just from heck of it.

There are Babesia microti in animals in central in western Massachusetts, where you see very little to none -- no human exposure. And so here is a situation where the parasite is endemic, it's enzootic, but we don't see human cases simply because in these sites -- and Lyme disease is a great tracking mechanism for this -- the tick densities have not reached a point where it's a hazard for humans.

And so once the tick populations start increasing these sites will probably become zoonotic for the more rare infections such as Babesia microti and granulocytic
ehrlichiosis. My office is somewhere in there. Ten years ago if someone had said, oh, there is Lyme disease and ticks there, I would have said yeah, all right, there -- you know, we know that the ticks are localized to the islands and coastal areas.

But now I can go out, you know, right behind my office and drive for ticks, and oddly enough there was Babesia microti there which put into question something that I'd assumed was gospel which was from these original sites of transmission of coastal New England.

And I had made some sort of theory of glacial origins that tick populations have been fragmented by the ice ages, and then that the tick survived in refuge south of those ice ages and have since spread northward and spread out of that and recolonized areas such as the terminal marine islands more readily and therefore babesiosis should not be seen in site where the tick is newly introduced or where the tick is newly recognized, that does not seem to be the case.

For the longest time we suggested well, even though we know Lyme disease -- so this is 1987, this is 2002 there is a clear expansion in the range of the tick as
well as Lyme disease incidence. The reason for that is that this tick is transported in sub-adult stages, larvae and nymph, by migratory birds.

Not only that, certain migratory birds are capable of infecting ticks with the agent of Lyme disease. However, Babesia microti does not infect birds. Therefore, and because Babesia microti is not inherited by the tick, how would it travel from this area inland on the backs of migratory birds because it would have to acquire infection as a larvae on a mouse, turn into a nymph, hitch a ride on the bird as a nymph, and then drop off and perhaps it might be infected as an adult tick, what would the adult tick feed on that would be capable of sustaining a Babesia microti infection because they feed on something like deer?

Very early studies done by Joe Piesman, now at the CDC, when he was a graduate student, showed that deer are incapable, they are incompetent host for Babesia microti. Dogs apparently are incompetent host for Babesia microti. And so we just couldn't understand how Babesia could move from site to site.

And now we're having a crop up in places and the alternative hypothesis is that it's long been maintained in
very, very small cycles of rodents and host specific ticks such as the mouse tick, *Ixodes muris*, which has a distribution roughly from here all the way over into the Northern Dakotas or so, and then from there it's replaced by *Ixodes angustus*, which also ranges from here all the way into Siberia maintained by *Ixodes angustus* or *Ixodes muris* in very small pockets never seen by humans, because these ticks don't attack people.

And then when human-biting tick such as *Ixodes dammini* is introduced, those feed on the very same animals and you have a new cycle established where you have that so-called bridge factor capability. Not only that, the other point about distribution is that they are dynamic over time, a nice study done by Tom Mather's group looking at Rhode Island, their case reports from Rhode Island clearly the amount of black is changing over time, although it's not necessarily a progressive relationship. Sometimes you have hot years, sometimes you have cool years.

This slide would seem to have no bearing, but just to come back to a point that Barbara made regarding Babesia and its representation in the parasitological fauna. Babesia are the most common blood parasites
encountered for any mammal anyway. If you got on just catch mice on this campus, or catch skunks or raccoons
there are very, very good chances you'll find Babesias on them after Trypanosomes and Bartonellas.

You can survey blood parasites all over the world. Trypanosomes, Trypanasomes, and Babesias are the most commonly encountered things on a blood smear. And these animals there are at least a 100 specious. And I would actually say with molecular techniques we're going to actually see that double. There is more unrecognized diversity than appreciated by the classical methods.

Well, the reason I show this slide, this is a veterinary journal, JAVMA (phonetic), a Brightwords (phonetic) group did a survey of blood samples from dogs and found that in all these gray areas you had dog samples coming up positive for a Babesia not necessarily one that is important to humans. But on the other hand, as we're finding with the Rickettsia, 181 animals in the -- one animal symbion may actually become a parasite in the right host.

And so companion animals, of course, the people are becoming more and more likely to have cats or dogs in
their house. And the juxtaposition of animals which are frequently parasitized by Babesias could also serve as a risk factor. But the fact of matter as this slide shows, as Barbara pointed out, there really is no, at a very macro scale, no area of where we wouldn't consider the possibility of babesiosis being transmitted.

On the other hand, there are enzootic Babesias which don't seem to be of any public health concern whatever for reasons that are unclear. That is, early surveys that we did with microscopy showed that ticks from places such as Wisconsin, or Maine, or Massachusetts had ticks with -- a lot of them had sporozoites in their salivary glands. And I'll refer to the life cycle in a little bit. And then with newer technologies -- this is a very crude one at the time, but at the time it served its job -- when you further analyze the map samples from microscopy it turns out that the bulk of those sporozoite positive ticks were actually infections by Babesia odocoilei, which is a common parasite of white-tailed deer.

Wherever you see white-tailed deer you will see Babesia odocoilei. In fact, it was first recognized in Texas by Gail Wagner's group, Texas A&M. And Texas A&M
continues to be a real resource in the study of this organism. In fact, the odocoilei-like parasites found in caribou ranging throughout the Arctic or going through -- passing through the Arctic. But anywhere where you see deer you see Babesia odocoilei.

It's very, very common on Nantucket. In fact, for some strange reason we didn't get any hits for microti with our survey, they were all Babesia odocoilei. Well, when we started working on the Babesia divergens like agent known as MO1, we were very interested to see whether people were exposed to MO1, and so we went, and because of it's relationship to Babesia divergens which in turn is very closely related to Babesia odocoilei we had an isolated Babesia odocoilei at vitro, we had the Purnell strain from Pat Holman at Texas A&M and looked at the number zero from -- mostly from Nantucket Island and found that there was indeed some reactivity to the Purnell strain of Babesia divergens.

We went back and repeated some of this using the in vitro cultivated strain of it's -- it is MO1 but it's from a different -- it's from a rabbit source and got a conservative 2 percent estimate with conservative cut off 1
to 64. And zero percent were reactive to Babesia odocoilei even though it's the most common tick-borne infection -- tick-borne piroplasm in our area. Our conservatism is reflected in the fact that the same sera gave us about 5 percent for Babesia microti.

So there is a piroplasm in a human biting tick very, very common, but it doesn't -- people don't seem to react to its antigens. There is risk associated with other common elements of the fauna. Cottontail rabbits are widely distributed in U.S. They are found around people's houses. And we previously showed that Lyme disease like spirochetes as well as HEE (phonetic) were maintained in cottontail rabbits between them and their ticks.

But we quickly focused on the presence of M01 or something extremely similar to it within rabbits on Nantucket Island. We called it Babesia divergens at the time because it was the most parsimonious thing to do based upon molecular and morphological data.

And the prevalence in rabbits is fairly high, that is, anywhere from 10 to 30 percent. And subsequently blood from such rabbits were used by Pat Holman to grow the parasite in vitro which served as a standard inoculum for
cattle infectivity testing which demonstrated that it really truly was not Babesia divergens.

But we focused then on Ixodes dentatus, a relative of Ixodes dammini, as the vector for this rabbit parasite. And about 5 percent of Ixodes dentatus molting from infected rabbits were infected. And everybody would say, well, you know, Ixodes dentatus is narrowly host-specific tick, it only feeds on rabbits and migratory birds.

On the other hand, we did a study of so-called Lyme disease in a coastal Maryland community and found that if you ask the residents to save the ticks for us, out of 1,500 ticks submitted to us a half a percent were actually Ixodes dentatus, and we were only getting about 2 percent Ixodes dammini. So a quarter of the ticks that we were getting from people that were Ixodes were Ixodes dentatus.

And so there is probably a very good chance of exposure to MO1 like parasites due to the very wide distribution of cottontail rabbits and Ixodes dentatus up to about the Dakotas and then replaced by Ixodes spinipalpis over to the California coast. So here is something that may emerge as a more aggressively human
biting vector, comes along, although interestingly Ixodes dammini and scapularis don't really like rabbits all that much.

So thankfully -- and that's reflected in the fact that we didn't get enough to really do any vector competence work with this parasite. Finally, to sort of home in on seasonality as a potential risk, something we should factor into risk, the life cycle of the deer tick is at least 2 years. You have adults in October and November looking for deer. They feed successfully. They lay eggs in July -- in March or so, the eggs hatch in July, the larvae feed July, August, September, get infected over winter again and then molt to nymphs in March, and then seek hosts from about May until the middle of July.

Well, the reason why we focused on between Memorial Day and Labor Day is because the nymphal stage is most likely to feed -- in contrast to the adult tick, is most likely to feed to a point where parasites have differentiated enough to be infective. That is, there is a so-called grace period with all the agents transmitted by Ixodes ticks.

For Borrelia burgdorferi there is 24 to 48 hours that
are required for the spirochetes to migrate through the gut wall to the salivary glands and up regulate a critical outer surface protein. For Babesia microti it takes 48 to 62 hours for sporogony, for the production of infective sporozoites to occur, and so adult ticks even if they are infected are less likely to feed in place long enough for sporogony to occur.

In brief, the life cycle of Babesia microti here I allude to a potential pre-erythrocytic or exoerythrocytic stage, probably somewhere in the reticuloendothelial system, maybe even dermal such as in Langerhans cells, and so maybe that’s not really a source for transfusion issues. The reason I say Langerhans cells is we do see some tantalizing things on the scan.

This is the classical picture of the epidemiology both Lyme disease and babesiosis. If you plot out the cases these are all from Nantucket Cottage Hospital. This was a paper published in 1987. Again, the Memorial Day to Labor Day thing seems to be very, very prominent, and that fits very well as the -- with the nymphal ticks, and their appearance in time as being the main vectors. And note when the adult ticks are around and this time you see very
few cases of either.

This is just on Nantucket Island. However, that was just for a couple of -- you know, the tick data was just for a couple of years '81 and '82. We've been following tick populations for 20 years on Nantucket and this is actually Martha's Vineyard for 10 years. And if you look at the red, this changes from year to year. That is, some of them have very low numbers, some of them have high numbers in mid summer, and some of them actually continue out towards epi week 40. And so it's -- even though the majority of risk is indeed between May -- in May and June, that does not mean there is no risk even after Labor Day.

Finally, what can we use, at least as ecologists, as predictors for -- try to understand these issues of not only seasonality and changes from year to year, but also from place to place? Can we look at mouse populations?

This is a plot of cases from Nantucket Cottage Hospital from 1975 to 1994, 1995 or so. The same microscopist was in the lab during that time, the lat Pat Snow (phonetic), and she kept very good records of what she found. This is all passive case detection. And you'll
notice here, and then I juxtapose that with mouse trapping data to see whether we can use mouse populations as a predictor for we're going to see lots of human babesiosis cases.

There is no such relationship. However, you'll notice right about here we're starting to see a real spike in the number of cases and that was related to a diagnostic advance and -- does anybody really want to pass out a guess as to what that is? Everybody says it's PCR, it isn't.

These guys were so poor at the time that they just bought a new coulter counter and the tec was really smart, Pat was really smart, she said, "Well, you know, when I look at blood smears there aren't a lot of platelets, there aren't a lot of white cells. I'll just tell the machine to flag, note low platelet, low white.

At the same time, any sample that comes in low platelet, low white I'm going to spend 30 minutes on the microscope slide. And so the number of cases she found doubled or tripled. So a simple measure like that - and, you know, we might want to talk about this later as a general screening method of a simple CBC was enough to help us document to what extent things were being undetected out
there.

Unfortunately, it screws up my data. I can no longer use -- you know, now I’ve got all this data with the new days, and in fact Victor Berardi at Imugen has greatly advanced our detection of this infection since taking over of the lab testing out there. And so unfortunately our longitudinal series of ecological data won't be very useful with these three different periods in diagnostics in the lab.

We also did serology for a number of years trying to look at (inaudible) subjects, and made the very gross simplification that IgM reactivity suggests recent infection and try to use that as a predictor with entomological inoculation rate, the number of ticks multiplied by the number of -- the proportion of infected ticks per minute or per hour to try to see whether there is a predictive -- whether that was a predictor or not.

And unfortunately, there was no relationship. But again, this underscores the great seasonal -- the great annual variation not only in babesiosis but the other infections of this system as well. And that tick data really doesn't tell us an awful lot in terms of a
predictive value. And so trying to get a handle on risk factors based upon ecological work is going to be very, very difficult when it comes to, for example, doing some mapping for so-called risk areas.

So finally, to make some acknowledgements, we've been fortunate, at least the late Andy Speilman for -- who contributed much to this field to babesiology, the study of Babesia microti, was lucky enough to have NIH funding for a very general purpose to look at tick population dynamics, and we were able to do a lot of studies that serves as the background to this talk.

And I've been very fortunate; NIH has been very nice to me. But none of this has been Babesias specific, and unfortunately trying to get better data does actually require a funding. And so we've just been lucky to steal from our NIH grants to do other work, but that is one of the problems with working with a so-called a rare infection is that support, to get the data that we need is very difficult. So with that I will cede the floor to Peter.

(Applause)

MS. HERWALDT: And I'm not going to give a formal introduction, but Peter Krause, clinician and colleague and
friend for many years will now tell us about some of the clinical manifestations in epidemiologic manifestations. Thank you.

MR. KRAUSE: I’m going to take the speaker's prerogative to ask that everyone stand -- if you’d like to. You’d been sitting for a while. If you like to stand, please feel free to do that while I get set up here. Stand but do not leave. No, so -- if you like to leave, that’s okay.

And actually you can stay standing if you want during the first few slides or all of them.

MS. HERWALDT: You think, I was a cheer leader or something like that --

MR. KRAUSE: Is there a pointer?

MS. HERWALDT: Yes.

MR. KRAUSE: Oh, this one, right.

MS. HERWALDT: Yes.

MR. KRAUSE: Yeah. Okay, thanks.

MS. HERWALDT: And then to advance poster.

MR. KRAUSE: Okay, very good. Thank you very much, Barbara. I did want to thank Barbara, Sanjai, and Jesse for inviting me here today. It’s certainly an honor
to be able to -- and a pleasure to talk about babesiosis.

Actually I’m in transition from University of Connecticut School of Medicine to the Yale School of Public Health, but just to explain this first slide. I was asked to talk about three topics - epidemiology, clinical manifestations and host immunity, and of course talking about epidemiology is bringing close to Newcastle and that Barbara and Sam really are, I think, better equipped to talk about that in the newer specs than I am.

In any event, Babesia are protozoan parasites in the phylum Apicomplexa. They target tissue or red blood cells, transmission over by the Ixodes ricinus family of ticks. Blood transfusion cases, of course, occur and there have been just a few cases of perinatal transmission; that’s really a rare mode of transmission. And so, here’s a thin smear with infected red cells. This is B.microti and you can see this particular case is relatively heavily infested with about 10 percent parasitemia. Most cases are far less parasitemic with generally 1 to 2 percent is what we generally see.

The species of Babesia causing human infection and have been reviewed and so I won’t dwell on this, but I
will say this is a disease of worldwide distribution.

And this is a primitive map of the Babesia species in the United States. Barbara has already covered this. I will say that the Babesia divergens like organisms have been identified in human cases in Kentucky and Washington State, and I will say that the slide is somewhat inaccurate in that New Hampshire and Vermont there have been no human cases in those states. But there are these general regions of endemnicity with these three species of Babesia.

So the National Academy of Sciences has stated in 1992 that babesiosis, erlichiosis, and Lyme disease are emerging threats to human health in the United States.

I want to just talk about this theme of emergence because Babesia is truly, I think, an emerging infection.

This is data from New York State showing -- by Meldrum et al., showing the number of cases in New York. In the dark parts, you see reported cases and the light part is hospitalized cases and is as often occurs in an early reporting system. Most of the cases that one sees are the most serious cases and hence relative similarity between reported and hospitalized cases which you can see
that although the number of cases are small, the general trend is upward over time.

And this is some additional New York data that comes from the state -- the New York State, Department of Health. And here we see the number of cases increasing over time, over a period of, I think, a little over 10 years.

And the other point that I would make in this slide is the age distribution. The severity of cases is much greater in older individuals than younger individuals. We know with Lyme disease, a lot of -- the majority -- well, probably the highest percentage of cases occur in young people because they are out and about in high risk areas, wooded areas compared with adults.

I think the same would be -- is probably too for Babesia as well. But these are reported cases. These are symptomatic cases and there is a fair amount of asymptomic disease that I’m going to touch on shortly.

In any event, in terms of reported disease, it’s 40 and older where one sees the majority of cases. And the same upward trend is seen in other states. This is the State of Connecticut between 1991 and 2005 reported cases.
And again we see this increase in the number of cases. So what has caused this emergence? Certainly some of it is not biologic, it’s simply increased recognition and diagnosis of these infections.

I think that just in my experience with this disease over time, I’ve seen that laboratories are now offering testing where they did not in the past. And I think physicians are becoming more aware of the diagnosis and the general public as well.

There has certainly been an increasing amount of building and human habitat where ticks are located in wooded areas and there certainly has been an increase in the deer population over time. And that’s been due to the eradication of predators and at least in Northeast with an increase in the woodlands.

At the turn of the century, there was a much of - New England was denuded of wood for building purposes for farming and when farming moved to the Midwest and other sources of energy became available, the woods returned to New England and with the return of the woods came the return of the deer, and with the return of the deer came the increased number of ticks because deer amplified the
Also there’s an unequal distribution of Lyme disease and Babesia. That is there are many areas in the country where Lyme disease is present, but Babesia is not. And the question arises why is that if both are transmitted by the same tick, and the answer -- at least one answer is that, and Sam had touched on this, that Babesia -- that is Borrelia -- that is birds can serve as a host for Borrelia, but not Babesia. And therefore, there’s a saying that Andy Spielman used to say to me and that is that Borrelia spreads on the wings of birds and Babesia on the backs of mice. So there is this uneven distribution. There’s many areas where Lyme disease exists where Babesia does not.

So I wanted to talk a little bit further about the epidemiology of the disease citing some studies that we’ve done over the course of about 10 years, actually it has now been about almost double that. This work has been done in conjunction with Andy Spielman, Sam Telford, Rick Pollock, and others. The study sites are Connecticut, New England, Massachusetts, that is the island Nantucket and Rhode Island, primarily Block Island.

We developed a study cohort or started to study
the diseases -- these diseases, Lyme, Babesia and Anaplasma
on Block Island in 1991. And we have -- we’ve been doing
studies there ever since.

What we do there is an annual serosurvey and
case-finding of Lyme disease, babesiosis and anaplasmosis.
And we have a large cohort there that comprises a little –
about 70 percent of the Block Island population.

The island is an ideal setting for epidemiologic
studies and clinical and pathogenic -- pathogenesis studies
because -- especially epidemiologic work because the study
designed and the relative small -- it’s a focal area.
People spend most of their time on the island. When they
become ill, they go to one medical center on the island.
All of this allows us to -- and I think both the serosurvey
and the case finding have allowed us to arrive at
relatively precise understanding of what the number of
cases consist of.

So this map shows Block Island. It’s about 13
miles off the Rhode Island coast. It is part of Rhode
Island. This is a typical house on the island. You can
see there’s a lot of woods. There are a lot of deer.
There are a lot of ticks. There are a lot of mice.
There’s a lot of Lyme and the Lyme disease and babesiosis, a little bit less Anaplasmosis. But all three diseases are found there.

There is a single medical center on the island, on Block Island Medical Center and we’ve worked over the years with the physicians at this center in a collaborative way that I think has been very helpful in terms of our studies.

So as I mentioned, we had two major approaches on the island. One is a prospective longitudinal serosurvey. We do active surveillance for B. microti, B. burgdorferi and A. phagocytophilum.

In the autumn and spring of each year, we go to the island and we offer free blood testing to the residents. And we do antibody against these three agents and PCR and selected blood samples. We do a prospect of longitudinal case finding and a sort of passive surveillance where patients who are suspected of having any of these diseases are asked to enroll in our study by the physicians on the island, and we get a standardized history and physical exam and do blood testing which consists of thin smear, antibody, and PCR.
So this shows the results of 10 years worth of work on the island. This shows the Borrelial infection here with the circles and the Babesial infections in -- with the boxes.

And basically you see that there is quite a disparity at the beginning of our studies and over time the Borrelial infection varies year to year, but ultimately statistically there was really no difference over this 10-year period in terms of the incidence of Lyme disease.

On the other hand, Babesia -- Babesial infections increased over that time period and we could discuss why that is at a later point. But the thought is perhaps Babesia was introduced at a later time point than Lyme disease.

This data actually shows the same data, but the actual numbers and it shows the incidence of Babesia -- Babesial infections, Borrelial infections in various age groups. If you look at the total group here, which you will notice is we had 140 cases over this 10-year period of babesiosis and 179 cases of Borrelial infection. And that ratio, this is about -- there are about three quarters as many Babesial cases as Lyme cases, not as obviously a
tremendous difference between what we find on Block Island and what we see either nationally or even in state reported.

And so what this -- and one of the reasons for this difference is that we were able to pick up through our methodology not only symptomatic cases, but also asymptomatic cases. And so when one is able to do this and do careful epidemiology, at least in certain sites, at least on Block Island, we found that the disparity between the number of cases of Babesia and Lyme is much more narrow than one would suspect from current reporting.

We asked the question as to whether this might be just Block Island or a more generalized phenomena and what we did is look at hospital admissions for babesiosis in Southeastern Connecticut, compare those with hospital admissions from Rhode Island for babesiosis.

And the hospital that we used for this study is Lawrence Memorial Hospital, the largest hospital in Southeastern Connecticut and their catchment area.

And so this is -- the Lawrence Memorial Hospital is in Southeastern Connecticut and when we look at the admissions, one of the things we see of course is that
younger individuals, there were no admissions for babesiosis among that group. There were some in this age group, but most of the admissions were in 50 to 89-year age group.

And if you look at the incidence of admissions for babesiosis in these two sites, they are relatively similar and that suggested to us that this relatively high rate of babesiosis that we’re seeing on Block Island was also found in coastal areas as well or at least in Southeastern New England.

So just to summarize the points I’ve made about epidemiology, babesiosis has international distribution. It’s the most commonly reported, it’s most commonly reported in the United States, compared to Europe or other sites. It’s an emerging infection with an expanding distribution. The distribution and incidence of Lyme disease is greater than that of babesiosis.

At certain sites, the incidence of Lyme and Babesia are similar and national reporting of human babesiosis, I think should be considered. I know this has been a topic over time and I know Barbara has -- there are many good reasons why that hasn’t occurred, but I would
put in a plea again for this at this time and I’m sure it’s a very complicated issue, but that’s something I think that should at least be considered.

So let’s move on to clinical manifestations. There’s an array of presentation of this disease as with all diseases. Asymptomatic infection is actually not -- the majority of cases are not asymptomatic. And the work that we did that I showed you -- didn’t show you the slide, but about half of the children were asymptomatic with babesiosis of those 140 cases that we had and about a quarter of adults. So in fact the majority of cases are symptomatic when one looks at this very carefully, although there is of course a large component of asymptomatic infection.

The classic diseases are viral-like illness with fever, chills, sweats, headache and fatigue, and then varying other symptoms as well, but these are the most common symptoms that one sees.

Fulminant illness occurs in people who are over the age of 50, those who are splenectomized, those who have HIV aids or those with malignancy or other immunosuppressive conditions.
And about in several studies, it appears that about 5 percent -- there's about a 3 to 5 percent mortality following infection and almost all of those who have fatal disease are immunocompromised.

And finally there’s a persistent relapsing illness that's been described that we had described with colleagues and I’m going to discuss this a little more detail shortly, but these are individuals who are highly immunocompromised who have malignancy and splenectomy and are on immunosuppressive drugs or have HIV/AIDS.

Complications of babesiosis were studied in a study by Hatcher et al. from New York. They looked at 34 consecutive hospitalized patients with babesiosis and they found that -- AR -- that acute respiratory failure, ARDS, was the most common, about 21 percent complications; disseminated into vascular coagulopathy, about 18 percent; congestive heart failure, 12 percent; coma and lethargy in 9 percent; renal failure in 6 percent; and in their series 9 percent of these hospitalized patients died.

Another problem with babesiosis that's been alluded to is the whole concept of persistent asymptomatic parasitemia. We did a study back that reported results in
1998 where we looked at two groups of patients. There were about 20 patients in each of these groups. One group consisted of patients who were not treated, the other treated.

At that time, at the time we did this study, the only treatment for babesiosis was clindamycin and quinine. Quinine has a very high reactivity rate, many patients cannot tolerate it, and at that time the recommendation was if a patient had mild babesiosis that they should not be treated, because the treatment seemed to be worse than the cure, and these patients generally resolve to do without any therapy.

So we looked at -- what we had noticed in our studies on Block Island, we found that when we did follow-up PCR on some of these patients, we were finding that they continued to be positive. So we looked at this formally in a prospective study and what we found was in fact that patients remained PCR positive for months, following the infection. And in fact, there was a difference between treatment and non-treatment as you would expect.

We had one patient who went 27 months with
asymptomatic parasitemia. He allowed us to draw blood on him for 18 months. Every 3 months we would draw blood, do a PCR, and we found that he continued to be positive. At 18 months he said enough, I don’t want this anymore. We suggested that he get treated. He decided not to. Nine months later he was hospitalized in early spring with quite severe babesiosis, was treated and cured, but it also -- we also found that he had developed a renal tumor and that probably accounted for this recrudescence.

I will tell you, I think this is a very rare event, this recrudescence. Most patients who have the disease, if they have mild disease, whether treated or not will probably resolve. And certainly patients with more severe disease require treatment. I would recommend all patients now be treated, but we can talk about that in the discussion if you would like. But in any event, patients generally do well with this disease. There is generally good recovery. But the problem with persistent parasitemia, after they are treated or without treatment is that they are feeling well and they may then go and donate blood, and from there, transmit the disease to others.
So in addition to the -- so in addition to this persistent asymptomatic parasitemia some recent experience has shown us that patients can have persistent symptomatic parasitemia. And I received a number of calls from all over the United States, actually from the world, about cases, difficult cases. And after receiving a number of these calls and hearing about patients who had difficulty clearing their disease, a group of us decided to do this together, put these cases together, and do a formal retrospective study. And this was a retrospective case control study of human Babesia microti infection.

We reviewed clinical and laboratory information from 14 case subjects, patients who experienced this persistent atypical Babesia -- babesiosis. And over that same time period we gathered together 49 -- all the cases that we had over that same time period of patients who had typical babesiosis, whether they had resolution of symptoms over the course of a week or two. All of these subjects resided in New England, except for one case in New York, and one case in Wisconsin, and they were enrolled between 1991 and 2005.

If we look at the clinical characteristics of
the subjects we see that of the 14 cases -- I'm sorry, that the mean age of the cases with severe babesiosis or persistent babesiosis and the controls with typical babesiosis, the mean age was similar. But there was a striking difference in terms of their immunocompetence, that is those that had persistent babesiosis had B-cell lymphoma, a high percentage had B-cell lymphoma or other malignancy. Ten of these individuals had, in addition to their lymphoma -- or in addition to their malignancy, asplenium. And a high percentage also received rituximab, which is a monoclonal antibody directed against B cells which is used in B-cell lymphoma therapy.

There were other immunosuppressive agents in two cases. When you looked at the controls one had malignancy and two were asplenic. But clearly those who had this persistent disease were much more likely to be immunocompromised and severely so.

So then we looked at the severity of infection among cases and controls and we found that the median peak parasitemia was 8 percent in the cases; 2.5 percent in the controls. That the median number of antibiotic courses was 4, ranging anywhere from 2 to 10 courses of antibiotic
in these persistent parasitemic patients versus 1 in the controls. The median number of hospital admissions, 1.5 versus 1; median weeks of therapy, 13 versus 1 with a range here, in the cases, of 4 to 102 weeks. Complications were much more common in those that had the persistence, as shown here, and we had 21 percent -- 3 of these 14 patients died.

This shows that anti-babesial therapy that these subjects received, the first three in red are those who died. You will notice from this slide that these patients receive multiple courses of antibiotic therapy. The general situation was -- what we generally observed was there would be a course of standard therapy. They would get better, they would get so much better, the antibiotics would be stopped because traditionally one gets 10 to 14 days of treatment. And the Babesia would return and the patient would become symptomatic again, the parasitemia would rise, they would get another course of antibiotic. After that second course, and that was typically a little longer, this therapy would be stopped and then it would come back again and it required multiple courses of therapy.
What we finally found out -- what we finally determined from analysis of the data was there are a variety of sort of home-brewed regimen was tried and there was no single regimen that seemed to be superior. It was a matter of duration of therapy and treating until the patient was non-parasitemic and continuing that therapy about at least 6 weeks, those patients are cured.

Those that -- where therapy was stopped when they had a very low parasitemia or if they didn’t continue it long enough after there was no parasitemia, those patients recrudesced.

So we concluded that people suffering from broad-based immunosuppression are at risk of persistent and relapsing Babesia microti infection. Generally they require anti-babesial therapy for at least 6 weeks including 2 weeks after the Babesia no longer were detected on blood smear.

The broad-based immunosuppression conditions included HIV/AIDS, malignancy with or without asplenia, with or without immunosuppressive treatment and that adaptive immunity B and T cells, both B and T cell factors appeared to be important for cure. And we were impressed
with the fact that many of these patients had -- and these were unselected patients really -- that a high percentage had B-cell lymphoma, and in fact a high percentage had received rituximab which is again targeted against B cells.

Now, this suggested that B -- that humoral immunity was important in this disease, and one would expect so. However, mouse data would suggest that sort of T -- cell-mediated immunity is more important and this segues into the last part of the talk which is host -- immune response of the host.

So studies in both humans and animals have suggested the importance of the spleen and certainly patients who are asplenic are at increased risk of severe disease and any one who is diagnosed with this -- that is, babesiosis in an asplenic individual, that individual needs to be followed very carefully and they are at high risk of severe disease or even death. However, there have been asplenic patients who have had a typical course of Babesia and resolved very well with just standard therapy.

How does the spleen work? How does it get rid of the Babesia? No one knows precisely but it does appear
that splenic macrophages are important and in fact the spleen does filter the blood and it does sift out those abnormal red cells and in the process causes hemolysis and perhaps death of the organisms.

Adaptive immunity has been shown to be important again in mouse studies and in humans. T-lymphocyte activity seems to be very important especially CD4 cell activity, and as I mentioned, humoral immunity.

So this slide simply shows these -- just a general slide showing how antigen may be captured, organisms may be ingested by macrophages and that these get to lymph nodes, draining lymph nodes where the antigens presented to T cells in which case then the -- that immunity through CD4 activation becomes -- or one has an active then -- process identification of the Babesia and perhaps killing of the Babesia. And of course, the antigen or microbes can go through the spleen, be picked up by macrophages and perhaps dendritic cells within the -- or at least macrophages within the spleen.

And one can imagine that there might -- this sequence of events may occur that splenic macrophages could pick up the Babesia, that then this -- the
macrophage may they present antigen to a T cell, helper T cell, releasing of -- and then with a resultant release of cytokines especially, perhaps interferon-gamma and then you may have an intercellular killing of Babesia. This is hypothetical, I don’t think there have been any specific studies to show this, but this is a likely scenario.

And that in terms of B-cell response you would have B cells that would recognize Babesia through external IGG molecules that there is then presentation of antigen to a helper T cell, leading to cytokine release and activation of B cells to release antibody that would go ahead and kill the Babesia.

This particular mechanism here with CD8 cells seems very unlikely because Babesia are found of course in red cells that are not nucleated cells. They do not express MHC class I molecules, and therefore, this mechanism is unlikely.

I wanted to just briefly talk, near here to the end of the talk about some work done by Edward Vannier (phonetic) at Tufts, Sam Telford has also been involved with these studies. Some very interesting studies looking at immunity and especially immunity in regard to ageing.
So I want to -- this is just a general protocol that was done for these studies. Nymphs infected with B. microti were allowed to infect BALB/c skid mice. High parasitemia ensues. Those organisms then were diluted and then at a fixed concentration injected into other laboratory mice and I will show you that data shortly.

And the there were tail snips every 2 to 4 days to determine the parasitemia, and in fact, parasitemia was used as a marker of disease severity as the mice do not become ill, or at least the strains used in these experiments did not.

So this shows strain and age-related differences and resistance to B. microti and it turns out that DBA/2 mice have an age-related severity of diseases. They are similar to humans and that the younger mice do not become particularly ill, but the older mice become highly parasitemic, and this shows 2 months to 18 months of age. Eighteen months is -- mice who are 18 months of age are aged mice. So you see this increased parasitemia as these mice get older. This was not seen in black 6 mice nor in BALB/c mice, this aged-related severity.

So an interesting experiment was then done to
infect skid mice and then to do a transfer of splenic cells from DBA mice or BALB/c mice back into these infected skid mice. In fact that was done prior to the infection to determine -- to see if these splenic cells would affect the parasitemia and in fact they did.

And if one looks here, these are -- this is time post infection on this axis, this shows the present parasitemia on this axis. Here are the DBA/2 mice and the BALB/c mice. And remember, the DBAs are the one that have this age-related severity. So in this particular slide, you see -- this is the parasitemia and the control skid mice that had no splenic cells transmitted. But when you transfer splenic cells from young mice, you see the parasitemia drops dramatically. These are splenic cells from older mice and you can see that they drop but not to the same extent. In the BALB/c mice on the other hand there is no difference in age-related effectiveness of the splenic cells and so again this demonstrated that this phenomena that one is seeing is those genetically related and also related to the transfer of splenic cells.

So the conclusion of this study was that the resistance to B. microti infection is conferred by the
adaptive immune system and that it is genetically
determined and associated with age and the authors
postulated that there is an age -- that there are age-
related differences in the expression of alleles critical
for adaptive immunity to B.microti.

Currently Dr. Vannier is doing F2 cross
experiments to isolate the gene or genes that are
responsible and we’re also doing collaborative studies in
humans to look at the same issue in people. I wanted to
end with just this comment -- some further comments about
the host immune response to Babesia and the fact that
cytokines, although they may be protective may also be
damaging.

The pathogens of babesial infection is thought
to result from two major events -- cytoadherence of
Babesia-infected erythrocytes and excessive production of
proinflammatory cytokines. If we look at erythrocyte and
cytoadherence, adherence of Babesia to vascular
endothelium has been shown to occur for several Babesia
species, but not all.

Cytoadherence is most thoroughly studied in
B.bovis, a cattle Babesia. Cattle that are infected with
B. bovis may die from encephalopathy as erythrocytes obstruct cerebral blood vessels, similar to what one sees with cerebral malaria. Cytoadherence probably is thought to occur in mild cases, that is the Babesia-infected red cells adhere to the side of vessel walls, and this may be a survival phenomena for the Babesia so that they can go through their lifecycle without ever cycling through the spleen. That’s a hypothesis; it hasn’t been shown. But that is what people think in terms of why there would be this cytoadherence.

And it is thought though that excessive cytoadherence when you have, let’s say, a very heavy infestation, may in fact cause vessel obstruction, leading to a CNS infection and death in cattle that are infected with B. bovis.

Interestingly work at Pat Conrad’s group has shown that B. duncani, the same phenomena is occurring in the lungs of hamsters infected with B. duncani; that is the cytoadherence can lead the pathology.

Severe disease without cytoadherence, erythrocytes cytoadherence for certain Babesia including B. microti and it suggests that there are other pathogenic
mechanisms that are operative. And inflammatory cytokines such as tumor necrosis and interferon-gamma have been shown to protect the host against babesial infection, but if produced in excess, have been shown to cause severe symptoms or at least associated with severe symptoms and complications.

This is a very simple cartoon showing these -- just demonstrating these phenomena. So in asymptomatic case one would have very low levels of cytokines and it turns -- low levels of cytokines, this shows a mitochondria relatively healthy and a blood vessel where blood is coursing through quite well, you have no symptoms.

With an increase in some of these cytokines that is shown to be -- to interfere with the metabolic or metabolism in the mitochondria and you get some sludging of blood. But in severe cases you get mitochondria that are not very functional. You develop a NOXI (phonetic) as a result of this and also you get sludging in the blood stream and potential complications from that phenomena.

So to summarize, the epidemiology of this disease, it's worldwide in distribution, it’s increasing
in both its distribution and incidence. Clinical manifestations are usually a viral-like illness. It may be asymptomatic in which case one could then have transmission through the blood supply. As a result, it may be fulminant or fatal. It is more likely to be severe in immunocompromised individuals.

In terms of host immunity, innate immunity, especially the spleen and macrophage activity appears to be very important but so does adaptive immunity including both the B and T lymphocyte activity. And cytokines are thought to help control infection but also may lead to severe disease.

I did want to -- there are many people to thank and I would just, I guess, echo Sam’s slide in terms of thanks to Andy Spielman who was my mentor for many years, and certainly Sam and the group at the Harvard School of Public Health, in addition to many other individuals, David Persing, many other people that have worked with me over the years. And I am very grateful to their help -- for their help. Thank you.

(Applause)

MS. HERWALDT: We are going to try keep on
schedule so that we can all have a break. I appreciate that, okay. Everyone needs a break and so this discussion period will be short. We will be here throughout the day, and can discuss questions that come up.

Sunday should we open it for any burning questions, will that be the best way? We have some explicit questions that we were going to discuss that all of us have touched on to some degree during our talks and Sanjai has posted them here. So, we can go either two rounds, and Sanjai, do you prefer one round versus the other?

MR. KUMAR: Well, I think -- but each time is short that we will hear any question I think just explain.

MS. HERWALDT: Especially any questions for Sam or Peter?

MR. CLINE: What is known about how the parasite gets into the red cell?

MS. HERWALDT: And by the way, if everyone asking a question could say who they are and --

MR. CLINE: Harvey Cline, NIH.

MS. HERWALDT: And if you could repeat the question, whosoever is answering it?
MR. CLINE: What is known about how the parasite gets into the red cell?

MR. KRAUSE: Okay. Yeah, the questions is, is there any information on how the parasite gets into the red cell.

I will say that there is a specific apparatus called the rotri (phonetic) apparatus that is involved with -- well, essentially the -- and I don’t do this. So I am going to give you a primitive explanation, but the organism that adheres to the blood cell and this -- the rotri apparatus is operative so that you get actually a -- there is some -- I think there is some lyses of the cell, but ultimately this -- the organism is enveloped within the red cell through these specialized organelle that I think helps define what in fact Babesia are.

So that is a very inadequate explanation, but people have studied this and there are others that could speak to this better than I. Certainly, I don’t know, Sam are you -- can you give us a little bit of --

MR. TELFORD: There is some indication that complement is used as a --

SPEAKER: Yeah, yeah, it is on.
MR. TELFORD: Okay, it's on, right? There is some indication that complement is required for parasite entry into the erythrocyte, but those studies were done with the Babesia rodhaini, which is a fairly different parasite than the Babesia microti for example, and it may differ from species to species.

We certainly know there is species specificity with respect to the host cell. For example, we can't grow Babesia microti in any cell and some people have grown Babesia divergence in a range of cells from human to ox, to even rabbit, but then there are some others which completely resist in vitro studies of -- in vitro cultivation, which means you can't really study the invasion mechanisms. And so it's very poorly understood.

MR. NIKOSI: Barbara, over here.

This is Hira Nikosi, FDA. This is directed to you, Barbara. We heard from your talk that the USA is the major foci compared to other countries, you know, because you had written with bold USA. So the questions is, is that species of Babesia such that the efficiency of transmission is much higher in this part of the world than the rest of the Europe?
MS. HERWALDT: The question relates to what seems to be a predominance of babesiosis cases in the U.S.

MR. NIKOSI: Yes.

MS. HERWALDT: And first of all, we have to keep in mind the important caveat that what we are talking about is what has been documented and reported. And, for example, just to bring up the issue of Africa and babesiosis, certainly in Africa, one can imagine that there could be a lot of difficulty differentiating the Babesia species and malaria or plasmodium species in humans.

Now babesiosis -- the Babesia infection has been documented in a fair number of animal species and there have been some case reports of possible cases of babesiosis in people. And some of the case reports have not been very convincing in terms of the evidence and there has also been some sort of surveys that haven't necessarily been very convincing. But my point is that we really just for example don’t know how much human babesiosis or Babesia infection and what species may be present in various parts of Africa.

The same applies to -- with, you know, some of course different caveats for, you know, Central and South
America. You know, there have been some sort of surveys and, you know, occasional case reports. But for comparison of, say, the U.S. and Europe, definitely it has seemed to be in the past that there's been many more cases reported in the U.S. than in Europe. But, again, there is the issue of identification of cases and what -- there always seems to be a story, and a series of serendipities in terms of how cases come to people's attention and initially, for example, there seemed to be this artifactual -- what now seems artifactual predominance of the relatively few cases being in the British Isles and in France.

And that probably was to some degree because of awareness there. Now, even so, a smaller number has been in the U.S. And -- but now as people are looking more and have more tools at their disposal, let alone the issues of what may be happening in terms of how this is going to spread, people are finding various Babesia species in various parts of Europe.

It is a long answer to your question. My point is that I'm famous for my caveats and infamous for my caveats. The bottom line is that, again, the big picture is USA -- particular parts of USA can be microti, but I
don’t want to come across as implying that that is truly reality. And to some extent it may be reality, but to some extent I think we are experiencing a shift in baseline as we discover more and more about animal Babesia species, and we are discovering more and more about which ones can cross the line and become zoonotic.

And do you want to add anything to that?

MR. TELFORD: Yeah, I just like to say that what we see here in the U.S. is a direct result of our land use patterns, our development habits of two acre zoning and protection of wild life, deer in particular, have led to tremendous increases in the density of an aggressive human-biting vector and we are starting to see that kind of developmental pattern emerge in some of the EU countries where it's always been the case and the land use pattern has been pretty stable in the U.K. Now we have places where people are going to look at the pattern of suburbanization in the U.S. and model their development in a similar fashion and will probably have the same issues that we have related to wildlife around their houses.

And so, in the next 10 years, I think babesiosis, the more rare infections associated with things -- with the
sheep tick, *Ixodes ricinus*, we will start to see those emerge because the thickness of these will emerge as a result of changing land use patterns.

And so things are just going to get worse with the thing that we know the most about, and then there are all these other miscellaneous infections, which may amplify depending upon how good the vector is at biting humans. Most of these things we don’t even have an idea of what the vector is.

WA-1, for example, *Babesia duncani*, we've looked for a long time, did experiments, and we're still sort of scratching our heads.

MR. KRAUSE: I did want to also emphasize what Barbara had said about diagnosis, and in Connecticut ten years ago if any -- if someone had a case of babesiosis, maybe 15 years ago, people would turn to the lab at UCONN and ask them to do these studies. Now they are available through commercial resources and that was not the case earlier.

So, I think that the -- you know, the fact that physicians become more familiar and that the testing is available is very important and it's especially so with
Babesia because unlike Lyme disease, there is no path pneumonic EM rash. I mean, you have a flu-like illness or flare-like illness will be confused with many other illnesses.

So, the availability of laboratory testing is crucial and the difference between United States and Europe may be that we just happen to have, right now, you know a greater availability of testing whereas the actual amount of diseases is similar. We don't know, but that's a very important factor I think.

MS. HERWALDT: I would like to also emphasize that malaria continues to be the default, even in places where there are expert clinicians. Over and over and over and over again, when people see ring forms, the default is malaria. It doesn’t matter what the epidemiologic context is; people think malaria.

Now, this is even assuming people look at a slide and a lot of people aren't aware -- a lot of people in this room are, but a lot of people aren't aware and Sam alluded to this that manual review of blood smears is not routine.

When people get CBCs, blood smears are manually reviewed only under certain circumstances, if the colds are
counter flag something or if a hospital has a policy to check blood smears for people who have "x" or "y". So -- but even if something is seen, the default is typically malaria.

And then, another point is I think, not just are the people who have mild symptom missed, but even people who are severally ill and even people in vast facilities like NIH or MGH, it often can take a long time before someone looks at a blood smear and see something, and people can be very sick by the way and have very low parasitemia levels. But you can even have high parasitemia levels and miss it.

And some of these people who are immunosuppressed, people think, oh you are asplenic or you immunosuppressed or some other reason, you are going to have rip-roaring infection and everyone is going to diagnose it.

Frankly, all bets are off when you are immunosuppressed. Some of these people aren’t diagnosed for long periods, sometimes because their symptoms are masked or misdiagnosed. But there are a lot of complexities here. And it gets back to this issue of, you
know, how does it tie into your question; it ties into the question because frankly we are not even sure what's going on here. But we do know what we do know and that is that there is a fair bit of Babesia microti. But we can't speak to what isn't there because of these sorts of issues.

MR. KRAUSE: One other comment is that the initial parasitemia may be relatively low and many patients will have like, you know, one percent or less. And so if you have a lab tech who looks at a 100 red cells, he may very well -- that patient may be infected; they don't recognize that. So that is another problem as well.

MS. HERWALDT: Dr. Matt Kuehnert from CDC, do you have a question?

MR. KUEHNERT: Thank you for introducing me. Everyone can see my name.

MS. HERWALDT: Or Sam or Peter.

MR. KUEHNERT: I had a question for Dr. Krause. I believe, you had a slide on persistent babesiosis and some of the underlying factors associated with the patient. What I was wondering was, you had description of a malignancy and asplenia, and I was wondering how much overlap there was between those. Did you have patients
that were otherwise healthy, but just were asplenic? And I am thinking just in terms of blood donors.

There may be blood donors that may be asplenic, but obviously not have other chronic conditions and if you looked at those patients and any other factors that were associated with -- to solitary asplenia?

MR. KRAUSE: Well, I can say that in general the greater the immunosuppression, the worse the parasitemia, I mean generally speaking. And so patients who are asplenic have a generally more severe course and that really varies. Some, as I say, will have a normal course, but most will not. Most will be more severely ill and some of them in fact are -- have fulminant illness and die.

On the other hand, if you have -- and as you saw in our slide, we have a number of people who had both malignancy and asplenia, and those folks generally fear worse. I mean, they are certainly at even greater risk for fulminant disease, but these patients, the patients that we described, 11 of them survived and of those, a lot of those folks were recognized fairly early on.

The problem was there was just a problem in terms of getting rid of the organism. It just continued to
persist despite therapy. So, I think there are differences between someone who is asplenic only and someone who has asplenia and malignancy. Those folks are probably going to be worse off especially B-cell lymphoma.

MR. KUEHNERT: Is there a group that has a persistent parasitemia, but not severe symptoms? I think, you know, if we are --

MR. KRAUSE: Well, absolutely. Yeah, in fact -- and I may be didn't point this out clearly enough, but in that initial study that we did in '98 where we followed patients who were treated/not treated, all of those -- you know, that long -- that Kaplan-Meyer curve showing the decay over time. Those folks, as I say, were -- they were sick for a week or so or maybe two at the outside because almost all of those folks were immunocompetent.

They felt fine after a week, but they had this very prolonged parasitemia, at least as defined by PCR, and that's another issue. But I think that has been -- that study has been held up in terms of looking at smears as well. Barbara and Dave Libey have done studies looking at that.

MS. HERWALDT: Yes and Dave may be talking about
that to some degree in his talk later today. And then also just the example I gave in terms of the cluster in Minnesota, where four consecutive donations were associated with transmission by all means.

And Sanjai, back to you.

MR. KUMAR: Well, I guess it would be nice to have one more question. There is some question behind the new questions something the --

SPEAKER: It's just -- this gentleman had to stand up --

MS. HERWALDT: And do we need to take questions from the radio audience as well or --

MR. KUMAR: Let's just move on now.

MS. HERWALDT: Well, then maybe you can pick the person because I don’t -- okay. So, the most burning question --

MR. CATTS: Yeah, Louie Catts from Iowa. I am interested in Peter telling us how we define a cure of babesiosis. This will become important as we begin to talk about blood donors.

MR. KRAUSE: Yeah, the questions is how does one define a cure of babesiosis. That is -- we are doing a
call on and you know it's a good question because -- I mean, I guess a cure will be defined as no parasites in the blood and no symptoms.

How does one know that there are no parasites in the blood? That's the problem. And if you use smear, you will get one answer. If you use PCR, you will probably get another answer.

I think, you know -- and how long does one have to wait until you know that that patient is cleared? Now, as I think every or most people in this audience know and maybe I've made the guidelines, if a person on a screening questionnaire who wants to give blood has stated that they have had babesiosis, they are not allowed then to give blood for the rest of their lives. Does that make sense?

I think that right now perhaps that does makes sense. You want to err in the side of safety, but the truth of matter is I think it's likely that people truly are cured that they don't carry this for the rest of their lives. But it is an excellent question and we don't really truly know the answer to that; just how long a person may be -- may remain parasitemic.

Again in that study that I showed, we showed no
more -- you know, after -- we followed patients until they were PCR negative on -- actually on two occasions. But that still is like a three maybe six-month at the outside analysis. It may be that they got under the radar of PCR. In fact they were, they still had a few parasites and that who knows, a year later they are now parasitemic.

So, very excellent question. I would say no parasites, no symptoms, but how does one really -- how can one really be certain that a patient has no parasitemia.

MR. TELFORD: There is one way and that is hamster inoculation. Remember, when you are doing PCR, you are taking a 100 micro liters of blood, extracting it for DNA and then using maybe a tenth or utmost a half of that template for your PCR reaction. And so a hamster -- you can check a hamster with 2 or 3 milliliters of blood. Even so, you know, what would happen if you checked up 20 hamsters for a total of 60 milliliters of blood?

So, theoretically, one liter -- you can have one parasite per liter and still have a risk of transfusion problems. And so -- but I have been consulted a number of times by people with these relapsing cases and we just do hamster inoculation, month after month, until the hamster
becomes negative.

MS. HERWALDT: And a low parasitemia issue also is relevant to the look-back issue where you can have one recipient become infected and one not. And, of course, there can be all sorts of issues in terms of host vectors and viability of parasites, but if you're a low level parasitemia, you may happen to have parasites in a particular unit but not in another.

SPEAKER: I only have one question.

MS. HERWALDT: Sanjai is the boss, so.

MR. KUMAR: No, I'm not the boss, it's your session. But I think we need to move on because it's so good and the questions are so good.

MS. HERWALDT: Yeah, we can have another room for the rest of the day.

MR. NIKOSI: Just a reminder that at the end of the status session, we have a complete open discussion again. So I think we can bring those questions back again.

MR. KUMAR: You know you want these discussions go on, but let's come back in 15 minutes.

(Recess)
ASSESSING THE RISK OF TRANSFUSION-TRANSMITTED BABESIOSIS

MR. LEIBY: It's always a problem speaking after a break, and everyone's still out on break when we start. Where's Barbara?

MS. BARBARA: I'm here.

MR. LEIBY: All right. Barbara's here.

Barbara commented on the fact that this workshop should be considered the first Babesia workshop by the FDA which is true, but I was reminded by Sam that some 10 years ago the Red Cross put together a workshop in the Connecticut region in which many of the same players were there, Ritch Cable, Sam, Peter, Roger Dodd, myself, and a few others, if I missed you, I apologize.

MS. BARBARA: (Off mike) -- 1999, there was a meeting in Atlanta.

MR. LEIBY: Yes, the DOD and the FDA as well. So there's been a number of cases. Anyway, I was asked by Sanjai to put together a talk assessing the risk of transfusion-transmitted babesiosis, and I think we are starting to move into many of the questions we actually closed with in the last session. So hopefully I can shed
some light on those.

The way I approach this talk, I thought, perhaps the best way was kind of to take a Cox postulate kind of idea and try to frame around the requirements for agents for transfusion and transmission.

First of all, when you think about this and you think about this for any agent, this includes babesiosis, the agent first of all must successfully infect donors. If it's not in blood donors, it's not going to be transmitted by blood transfusion. So first of all it must get in blood donors.

Second, it must be present in the peripheral blood, if it in fact is found in tissues only or perhaps (off mike) stage in (inaudible) cells will not be transmitted by a drug transfusion because it will not be taken out during the blood collection process.

Thirdly, the agent must survive blood collection in the storage processes. So when we pull a unit of blood off the shelf that's been in the refrigerator for three weeks, if the agent is nonviable, it cannot be transmitted to a blood recipient. And it's important that the viability and the ability to reproduce and cause disease
once they are transmitted is maintained through the storage process.

And lastly, the agent must infect the blood recipient following transfusion. In fact, in some cases -- most cases, causing disease. So we are concerned about that as well. And so I'm going to frame my talk around these four agent requirements and how do they actually apply to babesiosis.

Now, first of all, how often is Babesia found in blood donors? This is a list of seroprevalence studies, and I don't by any means mean this to be exhaustive. Seroprevalence studies Babesia microti extending from 1980, most recently in 2005 are some of our studies.

And you can see here, there is a great range in the N values and the percentages. I think the highest is actually one by Peter Krause. He showed on Block Island, 9 percent. And I think Peter talked about that in his talk just a little while ago. There's also lower rates. Some of ours and non-endemic areas of Connecticut, Wisconsin and some other areas of Connecticut as well.

I've also marked on this slide the studies that have been done in blood donors. Perhaps, the first study
to my knowledge is actually done by Mark Popovsky on Cape Cod and published in 1987, which reported 3.7 percent were infected. Gene Linden (phonetic) from New York did a study on Shelter Island which he found 4.3 percent to be positive. And then we've done a various series of studies mostly in Connecticut.

In fact, we've been looking at the rate of antibody positivity or seropositivity in Connecticut blood donors since 1999. So we are into our tenth year of actually testing blood donors. And we are testing by IFA - the picture over here of some blood cells with -- have gone through the IFA process. Clearly, you can see the positives. The number tested varies each year, but generally about 2,000 to 3,000 per year, blood donors in Connecticut.

And our rates have been pretty consistent, and approximately about 1 percent each year in Connecticut are seropositives. We have been deferring blood donors. Each time they are found seropositive, they are deferred indefinitely, and that's something we are going to -- was raised last -- before the break, and we can talk about that more as we proceed. So these donors are deferred
indefinitely, so we are not counting the same donors over and over.

Now if we look at where we've tested over this time -- and this is actually courtesy of Stephanie Johnson of Connecticut who works -- for me on these studies. And this looks at the coverage of our donor's test in the Connecticut. This is our primary area of research at the Red Cross.

And as you can see, we've covered fairly well the entire state of Connecticut. Our studies were initially focused on the highly endemic areas in Middlesex and New London counties. So this is also highly endemic for Lyme disease -- for the Lyme agent. I shouldn't say disease -- for the Lyme agent. And you can see by the dark color green are those in which we've tested higher rates per 10,000 donations. We also have other areas throughout the state in which we've tested fairly high rates.

If we look at the rate of seroprevalence, by zip code again per 10,000 donations, you can see it's well concentrated in this section of Middlesex and New London counties with some other areas as well. And we find infections throughout Connecticut, just not at the same
high rate or high levels. And I think that was the point Barbara made before that Babesia can be widely distributed, but there can be concentrations in highly endemic areas.

And it was this observation to us that there was indeed highly endemic areas, and that we wish to compare highly endemic areas of Connecticut with areas which we consider not to be quite as endemic. And this comes from a paper which we published in 2005 where we tested 1,700 donors from the endemic area as well as the same number from the non-endemic area of Connecticut. We tested them initially by an ELISA that was developed by Corupsy (phonetic) Corporation -- Corupsy no longer actually exist -- using some synthetic peptides, initially screeniothet (phonetic) and then confirmed by IFA. And we could actually not surprisingly demonstrate a significant difference between the endemic areas and the nonendemic areas.

Again to bring up Barbara's point, that doesn't mean this is the only area we need to be concerned about. One must consider the whole state of Connecticut to be at risk for babesiosis. So we clearly established, I think, in these studies and in other studies that the agent is
actually able to infect blood donors. We have found the evidence of infection based on antibodies.

What about the agent itself? Is it found in the peripheral blood? That's the second kind of postular or a requirement for this transmission. So at this time we called back some of these donors who were willing to come back. We called back 16 of these 24, and we called back three of these six and performed a nested PCR to determine if they were in fact parasitemic. And as you can see, 8 out of 16 and 2 out of 3 or overall 10 out of 19, 53 percent of these donors were in fact parasitemic based on measurements by PCR.

So now the donors in many cases are seropositive having antibodies, that they also have the parasite themselves. Now it's difficult in this kind of situation to determine when they became infected, how long the infection has persisted, because we are actually finding these donors retrospectively as being positive. We are not identifying them prospectively. So we don't really know the course of infection, when they were affected, when the acute stage occurs. And as I'll show you a little bit later how the parasitemia often comes up and then
dissipates.

But we've carried these studies on since then, and in the natural history study which I'll show you in a moment, this is the same graph you saw before, years, number tested and antibody. And over those years we've had through our natural history study, we've tested donors by PCR as well. And you can see now we've tested well over 150 donors, and these are all in the natural history study which I'll get into in a moment.

And you can see that there is different rates of parasitemia as high as 53 and 56 percent early on. We've had some lower rates more recently in 2006 which has been 16 percent. An obvious question we get with this, and this will come up later when I talk about lookback investigations, is why has there been such a precipitous drop in positivity with PCR. And that's really a difficult question, the answer, and one we can all argue about for quite some time. I'm sure Peter will have an idea, Barbara will have an idea, Sam and everyone else speaking -- Victor as well.

One of the things we think is as we have been deferring some of our donors throughout this time period,
we are actually pulling out of the donor pool those who are chronically infected and most likely to transmit infection. So I think as some of the more greatest at-risk donors are out, we are only picking up some of the newer infections, may be some of the acute infections. So there may be some changes in the donor population. We may be, in fact, pulling out of those donors who are -- I guess I already said this -- but most likely to transmit infection.

Now I've alluded to this already, but I think it's appropriate that I spend quite a bit of time talking about our natural history study which we have been conducting in Connecticut since 2000. Initially, and still at some levels, we are in collaboration with the CDC who provided funding, mostly working with Barbara Herwaldt who spoke earlier. And this is really a long-term study which is ongoing at Connecticut and Massachusetts, and we are actually enrolling the seropositive donors which I showed you earlier that we found through IFA testing.

Add this will get at some of the questions, I think Matt Kuehnert was raising and others about how long infections last, what's the course of the serologic patterns as well as parasitemia. Because we bring these
donors in every 30 to 60 days that are tested by serology which would be IFA blood smear, PCR and in some cases, we are still collaborating with the CDC hamster inoculation.

The donors are also asked to fill out a rather lengthy risk factor questionnaire so we can understand perhaps where they have been exposed, what is the initial risk. And we also have another questionnaire, a shorter one we ask each time they come in to potentially determine if they have been re-exposed to tick infections.

And lastly, as I re-alluded to, what we are really looking at is, is there any kind of relationship between serology and parasitemia patterns over long periods of time. And as initially envisioned, the individuals enrolled in the study were actually enrolled in the study for a three-year period unless they were negative over three successive withdraws at which time they were released. We've suddenly changed that criteria since then, but most of the information I'll you are people who've been in the study for up to three years.

As other speakers have done, I'm actually going to give you the conclusions first, and then I'm going to come back and show you some of the data. And what we see
in these enrolled participants, and there's close to 150 to 175 at this point, we really see three broad infection clearance patterns.

First of all we see quite a number of enrolled donors have elevated IFA titers with or without demonstrable parasitemia. We see clearance of this measurable parasitemia and we see a return to serologic baseline values. For all practical purposes, they appear to have cleared infection. This is probably three-fourths of the donors in the study. And as I showed you there, these are the kind of donors who presently now are deferred from future blood donation, and perhaps in contrast to what Peter suggested, I think these are some of the donors that perhaps we could get back into the donor pool.

So unlike what I stated yesterday with malaria where I thought there should be a permanent deferral for malaria -- past infection of malaria, I think in some cases we can get some of these donors back in. Not every donor, but many of them.

We'll also talk about donors with long-term elevation of IFA titers. These are the ones I think are of greatest concern, and the ones probably likely transmitting
an infection. Many of these are with or without parasitemia, and I think displaced into the chronic carrier state that Peter also already talked about.

Then we see some donors who have some less consistent patterns but they may be involved with infection clearance and re-infections. You got to keep in mind that some of these people are living in endemic areas, so they are constantly being re-exposed to the agent. On a seasonal basis, perhaps even more often than that. And in many cases these donors have no knowledge of tick re-exposure.

And I don't think that was mentioned by anyone in the first session yet. They are much like Lyme disease. Most people who were infected by babesiosis do not recall an associated tick bite. And as I think Peter said, there is no erathemia, there's no bull's-eye ring that lead you in to know that you have been infected. The ticks are rather small, they are (inaudible) stages, and actually the locations of the tick are sometimes very difficult to ascertain.

Now as a series of examples, this is very typical, this is what we see in probably three fourths of
our 150 some donors enrolled in the natural history study. This would be subject# 1426, first identified in July of 2000, had an IFA titer at that time of 1:512, not a booming titer, but still an elevated titer. A month later they were enrolled in the natural history study. The titer had dropped a little bit, but they were positive by nested PCR and positive by hamster inoculation. And I think Sam mentioned and described hamster inoculation quite well.

The donor came back in another month, gave another sample, the titer had dropped a little bit. We could still identify the infection by PCR but not by hamster. That doesn't mean that you can no longer do it by hamster, perhaps the same alluded if we injected 10 hamsters, may be one of the hamsters would have come out positive.

Then later that year in December, no longer could we measure parasitemia in the subject. The titer is still at 128, and then several months later in April in ensuing samples from this donor, they had dropped to less than 164 which is considered negative. So by all measures all practical purposes that we can do at least by both serology and by PCR and hamster inoculation, this donor appears to
have cleared infection, returned to serologic baseline.

So these are the type of donors I would propose that perhaps could be acceptable for donating once again. These are actually Western blots. I don't know if Victor remembers doing these for me, but Victor Berardi (inaudible) will speak a little later, did some Western blots on these same patients which I think he really gives a nice description of these patients. These are Western blots for B. microti IgM, these are IgG. As you can see early on in the samples, there is -- this donor was IgM positive and then the IgM disappeared, maintained IgG for a while and then it dissipated. We'll see some different Western blots in a moment.

About a quarter of those enrolled in our national history study are those who seem to have long-term antibody titers like this donor. Here again subject# 2348 first identified in August of 2000, elevated IFA titer also positive in this case once by PCR twice by hamster. Here we have the reversed phenomena. Probably if we had done another 10 PCRs, we probably would have found them as being positive. But never the less they were clearly positive by both methodologies.
Based on this, the physician decided to treat, 10-day treatment for babesiosis. And as Peter also alluded to, he would now suggest treatment of anyone who has (inaudible) babesiosis. We found early on in the study, the physicians in fact were very reluctant to treat unless the patient had outward symptoms of babesiosis. That may be changing, and maybe that's something we can discuss about.

But anyway, after treatment, this patient successive times over another couple of years, we never were able to demonstrate any kind of parasitemia, but throughout this study, the entire time and all the samples even the ones not shown by the arrows, the IFA titer remained rather elevated 1:512. To me, that suggests there's something's going on in that patient, some kind of stimulus wherever the parasite may be. Perhaps the parasite' not in the peripheral blood. Maybe this suggests that there really is an exoerythrocytic stage that we can't measure. But in any case, these are the type of donors who I would be concerned about, would not want back in the donor pool, and actually we need to watch more closely.

Here's the Western blot that Victor did again.
Very little IgM. We probably caught this one later in the infection. And you can see throughout all the samples a very robust IgG titers. Here's a typical -- a typical, I should say, patterns -- this is donor# 1078. Here's the one we followed for three years once again. And if you look early on again, parasitemic and the parasitemia then appears to wane. This was the case of one that was not treated by the physician. They did not see the need. But what you'll see here in this pattern is you'll see quite a bit of fluctuation of the IFA titers, 256 down to 164. We thought this donor was going to revert, and we were going to actually release them from the study. Then they popped back up to 256, went back down and back up.

What's interesting if you look at this pattern is actually look at the dates when the samples were collected. Early on August in the summer, dropped down in February, came back up in what would be May, the early part of the tick season again, reverted again over winter, then in May again of '02, elevated titer again, dropped down again. So we see there's reoccurrence problem, perhaps this donor is someone who is being re-infected each season or re-exposed, or perhaps the antibody titers were being re-stimulated.
Again on Western blots, this was the initial infection which appears to be IgM. We never saw that again. And then we see some differences in the bands -- and Victor would probably be better to comment on those than I, but we see some fluctuations in the banding pattern for IgG.

As we thought about this more, and as we began to realize that more of these donors were coming in with consistently high IFA titers over long periods of time, we began to question what the results really meant. This is donor# 03-0367. This is a 79-year-old male, and throughout our natural history study, it's the only one that we've ever had positive by blood smear.

And as we've already heard, blood smear is relatively insensitive, so perhaps it's not surprising. But he was picked up by a blood smear very early on. He was also treated for babesiosis, had a very high levels by PCR and hamster and very high titers of 1024. And the fact that he is 79-years-old puts him at greater risk because he is elderly, and I think we see many cases, and as Peter already alluded to, in older individuals.

As we filed his pattern, we see once again
relatively consistent high IFA titers. We saw only the one hamster positive, and we were doing nested PCR at the time. And even after treatment, we had one positive that came back up. And we began to think more and more about these IFA titers and what they mean and how we could really demonstrate if this was indeed a chronic infection. Until in our lab we went forward and developed our real-time PCR presumably more sensitive.

And when we tested these same samples by a more sensitive real-time PCR, we noticed that we found many more positives. And so this will begin to suggest and correlate that many times if these elevated titers among these donors, they in fact are parasitemic. So this lends support perhaps to the idea or the hypothesis that these long-term antibody titers are really indicative of chronic and persistent infections. Going back again to the idea that these are the donors we need to be worried about.

Let me shift now to our third topic or third requirement for transfusion-transmission and that is survival and storage of the agent in blood products. First of all, I want to point out that leukoreduction really has a negligible impact. First of all it's inside the red
cells. Leukoreductions is not primarily worked through the collection of red cells and the filters, and perhaps more importantly is the fact that transmission cases are routinely reported from places in Connecticut where the blood is almost entirely leukoreduced. So leukoreduction does not prevent the transmission of babesiosis.

I point out that the parasite is found in red cells and platelet products. It's not in the platelet products per se probably, although there is a potential, as Barbara pointed out, to have stages outside when the cells lyse. But most platelet products, particularly the random donor platelets contain contaminating red cells, and so we've seen a number of transfusion cases I'll talk about through red cell contamination of platelets.

The parasite is killed in frozen plasma. We've done some studies in the lab to clearly show that, but I must point out the parasite can survive in cryopreserved red cells. It's a very different phenomena. Much like putting red cells without cryopreservation, basically lyses the red cells and makes them unusable. I mean, Babesia is basically a one-celled organism. If you would just freeze that, you would destroy the integrity of the parasite. But
through cryopreservation, the parasite survives quite well and is at risk for transmitting infection.

The parasite also survives quite well in red cells maintained at 4 degrees. The viability is also maintained. There's been studies that have shown it survives for 21 days experimentally, and perhaps more importantly, there's been a transfusion case using 35-day-old red cells that transmitted the infection. So in the red cell, in that safe environment, the parasite survives quite well.

Now lastly, the last one we have to come to those four requirements for transfusion is it really must be able to infect recipients. And there has been a large number of transfusion cases, and I think perhaps in many respects, that's why we are here today, it's because we are seeing more transfusion cases in increasing numbers.

The numbers that have been bantered around tend to change, but probably there has been at least more than 70 known cases worldwide from '79 to present. There's been one case in Japan, Barbara alluded to that, and that was actually with a locally acquired Babesia microti-like agent. So that is a Japanese case. Our Canadian friends
have had one case, but that was because a Canadian came and visited the United States, became infected, traveled back to Canada, donated blood and infected a recipient.

The rest of the cases have been in U.S., and of recent years has been approximately 10 per year, and I think the speaker from the FDA may comment on this after me. Barbara alluded to this too. There's been one possible case in New York, but it's not really clear if it is a naturally acquired case or a transfusion case. I think there is some argument over that.

This is a reference if you would like to pull out that paper and take a closer look at it. The recipients have been neonegs (phonetic) to those 79 years of old, rather broad range. Fatalities are increasingly reported. As already mentioned, red cells and platelets are implicated, and there is no licensed tests. So even if we want to test today, there is no test in place. Same old song and dance we seem to have about many of these infectious emerging agents.

I want to share with you some of the American Red Cross hemovigilance data from the last 2 years, and this goes into how we've been seeing increasing cases not only
across the U.S. but also in the Red Cross system. In that last 2-year period from 2005-2007, we've seen 17 cases of transfusion-transmitted B.microti in the Red Cross system. There have been five fatalities. I would say that not directly as Babesia always have been implicative, but it is part of the cause of death of the individuals. And these involve 16 red cell units and one apheresis platelet unit as well.

Out of these 17 cases there has been 16 donors implicated, in one case a unit was split and given to two recipients. All of the donors were IFA positive, only one was PCR positive. So that gets back to a little bit about testing, and particularly when you do an investigation like this and you start pulling back in the donors to test them, at some point they may no longer be parasitemic. So that, in many cases, is a non-surprising phenomena.

Fourteen of them were residents or travelers to endemic areas. In fact, I have a later slide, I should have brought, but actually 10 of them were actually residents, 3 in New Jersey, 7 in Connecticut. And then four of them were actually travelers, unusually enough, two were travelers from Indiana, one was a traveler from Ohio,
and I think the last traveler was from Maryland, and they went to endemic areas, became infected, returned to their states, transmitted the infection through blood transfusion. Again, none of them recalled symptoms, and only one of them reported a tick bite.

The recipients, often elderly, 11 were 61-84 years old, 2 were less than two years old. That goes along with the pattern that those at risk are the elderly, the asplenic, and the very young as well as the immunocompromised. Four were asplenic, two had sickle cell disease. In fact one of the sicklers was also asplenic. And there's actually a very interesting case that we presented -- Harvey Klein would remember this at the NIH symposia last year of an NIH donor. This also involved sickle cell patients.

You know, what's interesting here, and I've learned this is that sickle cell patients are in fact basically asplenic, and so -- they are not asplenic, but they are functionally asplenic, I should say. And so they are also more likely to get infected as well.

Now the last slide I'll go with is actually one -- I was looking at transmission data, and this is actually
lookback data. And this is sometimes the data that blood bankers seem to be the most interested in. And unlike, perhaps, Chagus where we sometimes struggle to show positive lookbacks, the data we show of Babesia are really rather striking. And this comes out of the Connecticut region and largely -- I have to hand it to Ritch Cable, and Stephanie Johnson and others in Connecticut have done this work. This is from 1999 to 2005. Again the donor's test that you saw before, IFA positive, PCR positive, these (inaudible) are all the same.

But if we did our lookback investigations and we do them during 2-year periods, during 1999 and 2000 when our rates were very high as far as PCR positives, we tested 17 recipients of blood from seropositive donors and 5 out of the 17 were in fact positive. Keep in mind the underlying rate in Connecticut is only 1 percent. So before anyone asks me how do I know that those aren't natural infections, statistically, would seem unlikely. Ensuing years 2001-2002, we tested 13 recipients. Three of 13 where positive, 2003-2004 we tested 21, we found zero -- there was also 12 and zero at the same time.

So we were quite successful, 8 out of 63, 13
percent of demonstrating transmission through lookback investigations although it changed through time. Keep in mind that doesn't mean that transfusion cases were not occurring in Connecticut. That's been well documented. It's just at this time we did not demonstrate anymore through lookback.

So in summary, going back to what I talked about earlier in those four requirements, there is clear evidence of B.microti infection in blood donors, particularly in highly endemic areas. PCR does indicate that B.microti is present in peripheral blood, and this is subsequently been supported by transmission cases, also by what you just saw with lookback investigations.

B.microti survives or remains viable in collected and stored blood products, red cell platelets and cryopreserved products, transmission recipients as demonstrated by reported cases and lookback investigations. So taking all that together, I think, it's pretty clear that B.microti poses a significant blood safety risk.

To get all these studies done it really takes quite a large number of people. At the home laboratory, Laura Tonnetti and Megan Nguyen were involved with some of
these studies. And I should also mention Melanie Proctor too, although she does mostly anaplasma. She was involved and continues to be involved in these studies as well. In the Connecticut region, Ritch Cable, Stephanie Johnson, Eric Van Tassell, Russell Melmed, and Jonathan Trouern-Trend, CDC Barbara and all of her cohorts including Marianna Wilson who is here, and also Victor Berardi from Imugen who will speak a little bit later.

Thank you.

(Applause)

MR. KUMAR: Thank you David. And let's move on to the next talk that will be given by Sharon O'Callaghan from FDA.

MS. O'CALLAGHAN: Thank you, Sanjai.

It's going to be hopefully short and sweet and not very technical like the previous talks were. I'm going to go over the overview of the FDA reporting requirements, the surveillance tools that we have to capture events associated with Babesia.

First I want to talk about the biological product deviation reporting mechanism that we have, and then go into the fatality reporting. And with both reporting
regulations, I'm going to talk about the regulations as well as the summary of the data.

So we'll start with the BPD reporting. Now the requirement for reporting biological product deviations is at 21 CFR 606.171, and it requires the reporting from the licensed manufacturers, unlicensed registered blood establishments, and transfusion services. So we have reporting required for all manufacturers of blood products.

What's required to be reported this is an event associated with manufacturing of either licensed or unlicensed products that either represents a deviation from current good manufacturing practices, regulations, standards or specifications that may affect the safety, purity or potency or represents an unexpected or an unforeseeable event that may affect the safety, purity, or potency, and occurs in the facility or at a facility under the control of the manufacturer, and involves a distributed blood or blood component.

We've issued a guidance document in October of 2006 for the blood and plasma establishments that further clarifies what's required to be reported and what's not required to be reported. So when we look at the reports
that we receive associated with Babesia, we've categorized these into five different groups. There's the possible Transfusion Transmitted Disease group which is where a recipient of a blood product tested positive and a donor was either implicated or could not be ruled out.

Most of the reports that we get in this category, there has been evidence that it's been linked to a specific donor. But in the event where there maybe four or five donors and they can get back three of them and can't follow up with testing with the other two that would require a report even if those other three were ruled out. So it would be reportable even if the donor cannot be ruled out.

Post Donation Illness. This is after the donation; the donor reports an illness or diagnosis that occurred after donation. So we have a fair number of reports where the donor has successfully donated, has said that they felt fine, but then maybe a day or two or maybe up to a week or two later, they have either come down with an illness that has been identified as Babesia, or they've been tested and actually confirmed as positive Babesia.

History of Disease. This again involves after the donation the donor subsequently reports that they had a
history of either testing positive or being diagnosed with Babesia.

The Ab+ Prior to Donation. This is where the donor would report after the donation that they just had a positive test. They may not have the disease, but they have tested positive maybe at another blood center, through a research protocol, some other place that they have tested positive.

The Ab+ Post Donation. This is information that's been obtained through a research protocol where the donors are being tested subsequent to the donation. So they have a donation today that's now testing positive, but they had previous donations that may or may not have been tested. Okay. This would be kind of the lookback scenario.

So to show you the number of reports, we've -- in most of the categories we've seen some increase in the reports, mostly because it's been the more awareness of reporting in general and then also the awareness of the Babesia infections to begin with. So for the possible transfusion-transmitted diseases, we had two in '98 and only one in three and five in '01-'03, and then we jump
from 6 to 10 to 15 to 10 for '08 for a total of 52.

The post donation illness, that's kind of been fluctuating over the years, you know, we had 10 in 2002 and then another 10 in 2007. History of disease, we've seen more of an increase towards the last several years. Antibody positive prior to donation, we only had a one report in 2005, and then the antibody positive post donation, that's basically from the Red Cross which Dr. Leiby just presented, is most of those reports. And you can see -- so these would all be reportable because the previous donation had been distributed where the donor is now testing positive.

Okay. With the fatality reporting, the requirement for reporting fatalities is the 21 CFR 606.170(b). And what's required to be reported is the complication of either collection or transfusion if it's confirmed to be fatal. CBER is to be notified as soon as possible, and then a follow-up report within seven days. The facility that's required to report, if it's a donor fatality, then it's the collection facility. If it's a transfusion fatality, then it's a facility that performed the compatibility testing that's required to report.
As I said we did issue some guidance on reporting fatalities that we identified that the initial report has to be submitted as soon as possible, and then follow up with the seven-day report to include things like an autopsy report, transfusion committee findings, and things like that. So the number of reports that we've received for fatality as related to Babesia has increased from one in '98 to zero in the next six to eight years, and then we had two in '06, three in '07, and three so far in '08, so for a total of nine reports of Babesia fatalities.

The summary data from these nine fatalities is that the recipient age was between 43 and 88 years old, the latency interval was 29 to 48 days, and the interval to death was 34 to 50 days from the time that they received the transfusion till they died. The IFA titers of the implicated donors ranged from 1:128 to 1:1024. All but one implicated product was either -- I believe, mostly were leukocyte reduced red cells or just a, you know, standard red cell.

But there was one that was a frozen deglycerolized red cell that actually caused the transmission. And some of this -- this detailed data from
this has been proposed for a paper. So that's kind of the overview of BPD and fatality reporting.

I wanted to thank Sue Cannon for providing me with the fatality reporting data, and I look forward to any questions.

Thank you.

(Applause)
MR. KUMAR: Well I think we are back on time now. So that's good news. So if we can have all of the speakers up here as part of panel discussion. So here are the questions that we had put together, but I think since we are back on time it would be nice to go through these questions quickly if we can, and then also we encourage open questions from the key speakers.

(Off mike) Barbara please.

(Off mike) Barbara is coming here. Before we start the fifth panel -- David, we have you up here. We are going to ask you.

If you could just tell us a little bit about -- just educate us from a blood banker's perspective, in endemic area, how do you identify at Babesia risk donor? How do you defer them? How long do you defer them? And how do you bring them back? I mean, that includes donor questionnaire, how do you test them, how do you do the testing?

MR. LEIBY: I'm not a blood banker first of all. I'm just a scientist.

MR. KUMAR: We work with them too much. So --
MR. LEIBY: How do we identify them?

MR. KUMAR: Yeah.

MR. LEIBY: At present we are in -- perhaps Stephanie can correct me if I blow this. At present, we select drives that we think are suitable for identifying donors who potentially are infected. Sometimes we are looking at areas that are more endemic than others. Sometimes we provide an information sheet at the drives telling donors that they may be tested for babesiosis, that if they are positive they will be notified. The testing is then done in Connecticut by IFA. They are notified in fact if they are positive. When they are notified, they are notified and counseled, and they are indefinitely deferred. That's the procedure we've been following for some time.

They are indefinitely deferred based on an IFA alone. Initially, we are only doing it on a PCR positive. But as we followed donors and became more concerned that some of our IFA positives may in fact have underline parasitemia, we became more conservative, let's say. So we are deferring them based on an IFA positive alone.

Did I miss anything?

SPEAKER: (Off mike) -- Did you mean in general
or did you mean -- research?

MR. KUMAR: In general, in general. No, no. My question is more -- goes out of your research study.

MR. LEIBY: Oh, out of research?

MR. KUMAR: Yeah.

MR. LEIBY: To my knowledge and other blood bankers -- the real blood bankers can actually answer this question -- the only way to identify anyone at risk is the question that I think Peter alluded to earlier was on the UDHQ or the BDR, they are asked if you've had babesiosis, probably many people don't know what that term means, just like they don't know what Chagas is or other diseases that are on the list. So if they don't answer yes, they are -- based on the criteria at least, they are acceptable for blood donation.

And I don't know the rates of people who are ready to respond to those questions, but they must be extremely low. So other than --

MS. HERWALDT: And the other thing (inaudible).

MR. LEIBY: Yeah, but those are maybe surrogates. Those aren't really, necessarily markers of Babesia. So the only one we have is the question of Babesia.
MR. KUMAR: Thank you. If any of you -- any of these questions -- these are for the panel members -- if any of you think you are more suited to answer these questions, and then we can quickly open it to the audience.

MR. LEIBY: I think trying to define what the incidence rate at this point is very difficult because we don't really have any type of reporting mechanism -- that's going to one of your other questions, I think -- reporting mechanisms in place, physicians may in fact miss many of the cases. We are only, I think, the knowledge base in physicians, looking for the agent, the infection, and the symptoms is relatively low levels. And so while we may sit here and say we're in with the FDA's data as well, that there is 10 or more cases per year.

Would you agree Barbara? Probably 15 perhaps -- and we likely think that number has been increasing, but that may be due to recognition alone as opposed to more cases. But I just think the information is not out there.

Ritch.

Then we'll go to you, Peter.

MR. CABLE: Ritch Cable here. There have been three studies done all in Connecticut on risk. One was
done before we started all this by a fellow named Michael Gerber who was a pediatric fellow who got interested in Lyme disease and was interested in studying whether the Lyme disease was transfusion-transmissible through and Babesia at the last minutes. And this was a study done on 150 or so cardiac surgery patients at Harvard Hospital. They were tested before surgery, and 6 months after surgery for both Babesia and Lyme. The testing in fact was done at the University of Connecticut labs just as most of Peter's data, for example.

The 1 out of 601 red cells that were followed, one of the recipients had seroconvertible Babesia. None of the patients seroconverted for Lyme disease, which we'll be talking about in another conference. That patient clearly had a hemolytic episode at the right timing after transfusion. All the testing was done months later, you know, in a stored way. But he clearly had post transfusion babesiosis. By looking at his chart we couldn't prove it with serum samples and we couldn't exclude entirely that he didn't acquire Babesia other than from transfusion. He was a post open-heart surgery patient, and there was no interviews (phonetic) at the time. So that's one in 600,
and that estimates in general infectious disease, and it's the main risk estimate in the literature.

Later on, when all these PCR results were -- we discovered these, I did publish an abstract and put in a couple of review articles -- couple of estimates based on a couple of assumptions about PCR, basically saying PCR units were infectious to certain rates. And I got estimates in the 1 in 2000 range. One in 2000 red cells -- these are Connecticut red cell units -- were infectious.

Finally, we did a study with Shimmian Zoo (phonetic) -- we are still doing a study looking at the data from Shimmian Zoo where we did a cooperative study with Yale where we followed chronic transfusion recipients, and we tested them for several pathogens including B.microti, and again the rate seems to be in the order of 1 in 1,000, because we did detect one seroconversion for B.microti, and -- don't you know what a sickle's disease patient who got a gazillion red cells during the period of the study. So I mean, I believe in Connecticut that seroconversion recipients occurs on the order of 1 in 1,000. That's pretty high.

What are these in other states? I don't have a
clue. Nobody else has a clue. But at least in Connecticut where the seroprevalence is as stated, and where the disease is endemic, it's a truly mind-boggling infection rate. Of course, everyone who is infected doesn't get sick, and everyone who is sick doesn't get diagnosed as we have already discussed. And so Connecticut only gets two or three Babesia infections reported to us by hospitals through the TTD reporting system a year.

MR. KUMAR: Thank you, Dr. Cable.

So let's go to Peter, and then question -- back there.

Dr. Krause.

MR. KRAUSE: I was just going to cite that Shapiro-Gerber paper, I was involved with that as well. I think Ritch was as well. But he's already stated the figures, so --

MR. CABLE: You were on that paper, Peter?

MR. KRAUSE: I was, yes.

MR. CABLE: I'm sorry.

MR. KRAUSE: No problem. I mean, it was -- you know, it was an ingenious study that really Gene Shapiro and Mike Gerber were the ones that thought about it. They
had the presence of mind to realize that they could look at cardiac transplant or cardiac patients, patients getting cardiac surgery, realizing they get a lot of blood, it would be an ideal group to study, and to look at them before and after surgery. So they were interested in Lyme disease to see if there was transmission that way. But I was interested in Babesia. So -- and the person who really did the testing was Ray Ryan. So anyway. Well I was just going to mention that, but Ritch has done it.

MS. HERWALDT: Could I ask a question in reverse? Ritch, I don't know how much this changes per year. I'm not a blood banker, obviously. But can you give me a sense of exportation patterns from say places like, you know, Connecticut, and I know Rhode Island. Someone is here from Rhode Island blood center. How much exportation is there from the Northeast to other parts of the country?

MR. CABLE: Most of the blood goes the other way because the Northeast is big cities, and big cities are short off blood. Connecticut has in the past done a reasonable amount of exportation. I believe the Rhode Island blood center exports -- well, they've got to speak for themselves. I know they export a number of platelets,
I don't know about red cells. Many of the exports these days are of platelet concentrates and not of red cells, but platelets could transmit --

MS. HERWALDT: When you said Connecticut does a reasonable amount --

MR. CABLE: Not anymore, no. Well, Connecticut is not an importer, but that doesn't mean anything. If you are talking about the number of units -- I mean, we are shipping units out in inventory balancing modes and then getting more units back in. So yes, those units could go -- I don't have those numbers. I'm guessing -- I'd be guessing there would be under 5000 units a year that go out of Connecticut to other -- and a lot of them go to upstate New York and Massachusetts where it would be hard, you know, to figure out -- you'll have to go back to all the donors and figure out who was the guilty donor.

To my knowledge we've not had a case reported to us in which exported blood from Connecticut was implicated. We certainly would have cooperated and worked them up aggressively as we do our own cases. We have not gotten those -- so I don't think that's happened.

MS. HERWALDT: We've had -- we (inaudible) in
other situations, not in association with Connecticut, but other blood centers in other states. And I was just wondering out of ignorance whether there is some of general patterns.

MR. CABLE: Well, I think it's much more likely that the exportation of Babesia would come from the upper Midwest where blood supplies are often in excess and we are exporting -- many of those blood centers are exporting -- blood centers. So you want to look where their blood goes would probably be a more fertile area to look for exported babesiosis. I mean, they are endemic and they not exporters for sure, but many of them are.

MS. HERWALDT: These are just anecdotal, but some of the exporter -- you know, exported products came from various northeastern states. But --

MR. CABLE: And then the New York blood center doesn't export now.

MR. KUMAR: I think we have a question back there.

Thank you Dr. Cable.

We have a question back there. Yeah.

MS. PERSAR: Yeah, thank you. My name is Candy
Persar (phonetic) and their work for EPA. And I have a quick question. Do you know if there's going to be any research initiated for identifying borrelia in the bloodstream, and are you concerned about it in the blood supply, and transplacental reasons?

MR. KUMAR: I don't know who the person -- maybe Barbara, you maybe the best one --

SPEAKER: You need to repeat the question.

MS. PERSAR: Okay. Basically I'm trying to figure out what other tick-borne diseases you are trying to measure in the blood beside Babesia, and I was wondering for borrelia burgdorferi?

MR. LEIBY: Well, as I think Sam alluded to earlier, there is many tick-borne agents that are potentials. To all our knowledge -- and there's several people here who could probably correct me that we have never seen a transfusion-transmitted case of Lyme borreliosis. Ritch cable and many others have looked. There's been some cases they thought were potential, on being investigated have turned out not to be true. Certainly with a number of Lyme cases in the United States, and others can speak to this better than I, if it was
frequently transmitted by blood, I think we would have seen it by now. We certainly are vigilant and concerned about it, but we have not seen it.

Another agent -- I'll go to FDA then. Another agent, anaplasma, that causes human granulocytic ehrlichiosis has one documented cases, and there's at least two other I know of in the pipeline. So there may be other tick-borne agents that we might have to be concerned about.

MR. NAKHASI: No, I was not responding to that question. I have a question of my own, so.

MR. LEIBY: Does anyone else want to jump in on the Lyme question?

Go ahead Sam.

SPEAKER: I was just going to mention that the spirochtemia during the acute phase is fairly transient. It's not that there aren't any spirochetes in the blood, but they are very, very low levels and they're not there for very long.

MR. LEIBY: And I think there's always also been a question about how long the spirochetes survive under storage conditions. At least that was my understanding.

MR. NAKHASI: This question is for David
Now David, how much -- you know, you presented an excellent data on both serology as well as the PCR and the hamster injection and other things. So my question is how much do we know about these assays, both serological assays and PCR assays that would account for these variations in the antibody titers and the negativity of the PCRs?

The reason I'm asking that question listening to both Sam and Peter, there could be -- and Barbara, that there could be other species which could be either serologically cross-reactive or PCR primer such that you don't pick up those things. So I just wanted to know how much is the -- we know about these assays?

MR. LEIBY: Well, I'd first answer your question by saying our studies are done in Connecticut. To my knowledge, B.microti is the only one present in Connecticut. Now when you get into PCR, and this applies to all the parasitic agents, whether we are talking about T. cruzi and even Babesia, by and large the infections tend to be very low level, they tend to be intermittent, and as Sam mentioned as before, you are taking 100 micro liters of blood and you are testing that by PCR.
Now if the agent is not in that 100 micro liter sample you take, it's going to be negative. It doesn't mean that the donor or the recipient does not have the parasite in peripheral blood, it just means that in that sample you took and tested, it wasn't present. You may have to take multiple samples, you may have to -- as Sam alluded to, you might have to inject, you know, 3000 hamsters to show that it's in there.

And it's not the sensitivity so much that the PCR really gets down to quantities of blood you can actually test. And I know I've had this discussion endlessly with Barbara, so maybe she wants to comment on this as well. But I think that's really the explanation.

MS. HERWALDT: And where you also asking about the fluctuations in the IFA titers?

SPEAKER: Yes.

MS. HERWALDT: The testing -- and David can speak to it -- David's done -- by the Red Cross, it doesn't change the bottom line of what David said. But the specimens for the most part weren't tested in parallel, and the testing was serial, twofold rather than fourfold dilutions. And so it just gets to the issue that some of
the ups and downs would be smoothed out. Again, it doesn't take away from David's basic points, and it certainly doesn't take away from the fact that some of the people had chronically, you know, high, relatively high seropositivity.

MR. LEIBY: And I also think in the afternoon session, both Marianna and Victor will probably address those issues more directly and with more information about the reliability of IFA, Western blot testing, PCR and so forth.

MR. KUMAR: Yeah, we have questioned their, and then Dr. Krause again.

SPEAKER: In American Red Cross, for most of the suspected transfusion-transmitted cases, it's clear. But what criteria do you use when a recipient -- when a patient lives in an endemic state and transfusion-transmission is suspected, and that both have positive -- what criteria do you use to conclude that it was transfusion-transmitted and not acquired otherwise in an endemic state when, you know, the titer might not be -- what criteria would you use?

MR. LEIBY: That's why I pointed out the fact that the underlying rate in Connecticut that we've seen is
only 1 percent. Now if overall those lookback investigations we looked at, the rate was 13 percent. Statistically it would seem unlikely. It does not rule out, of course, that some recipient may have been infected, but it seems to me that it is more likely that they acquired it through blood transfusion. They are at greater risk through the transfusion.

MS. HERWALDT: Are you focusing more on the index case patient or the lookback?

SPEAKER: I actually wasn't questioning the lookback, but for most of your lookback you had serial tests on the donors. My question was just when a hospital is investigating suspected transmission and, you know, only test the donor once, and the PCR is negative, and there's a low titer in the donor, how do you -- what confidence would you have in assigning transfusion is the cause when the recipient has the risk of just living in an endemic state?

MR. LEIBY: It gets to then, we have multiple donors who potentially were involved in the case, then we test all the donors if possible, and there is only one donor who turns up positive. And so that's potentially the one at risk for transmitting infection. In most cases as
you know they are not PCR positive because, you know, investigations are done months later. And so with that positive donor and that positive recipient we go on the likelihood that it was transfusion-transmitted to the best of our abilities.

Now it does not -- as I said, it does not rule out that the recipient acquired it. Now we also have -- we require medical history and information about the recipient themselves, you know, are they 85-year-old who has not left the house in the last three years and went in for surgery and came down with babesiosis. They would be unlikely to have acquired it in any other fashion than through blood transfusion. So there has to be a component of epidemiology looking at the recipient and the potential risk factors beyond just blood transfusion.

MS. HERWALDT: Yeah, I was just going to make the same point that David made that it's not as if one just looks at a bunch of lab results in isolation, both for the recipient and the donor other issues are taken into an account. The epidemiologic context --

SPEAKER: No, I understand how cases are investigated as I review them. But my question was just
there are cases where it isn't clear, and what would anything -- of course, all of those factors have to be taken into consideration, but there are some that aren't clearly, and I think often may be assigned to transfusion when it is maybe more likely that it was acquired in the community.

So is there -- after you take everything, the donor's history, their risk of acquiring it in the community, is there anything else that we should be considering? Is there any, you know --

MS. HERWALDT: Well, I guess, the bottom line is I agree with you that there definitely are situations in which it's not absolutely clear and it gets to the strength of the evidence issue in terms of, you know, is a definite confirm possible. And then it comes down to what is at stake and is the issue, you know, should be counted in case tallies, is the issue -- what are the public health implications, is the issue whether to do lookbacks. So and depending on what the question is, the answer varies. You know, for example if there is any question that could be transfusion acquired, then I think it's very important -- I'm saying this in broad strokes of course it depends on
the particularities.

But if there's a possibility of it being transfusion acquired, it's very important to do a transfusion investigation because of the possibility that there might be other people out there who are infected and don't know why, and -- but in terms of the case tallies in keeping track of how many cases might have been transfusion acquired, things like strength of evidence are included.

MR. KUMAR: May I interject here? I think just again for the interest of time, when we were putting this program together and we were probing around the need for this workshop, one question or issue that came up again and again, weaknesses in the reporting system for babesiosis. This is the question three here. If you can quickly get to this question here and get some thoughts and comments not only from the panel here, from anybody in the audience. I think that's one of the main thrust for this session actually.

So if you can just look at the question. Again and again we hear that these patients don't get reported properly to FDA or to each other. So what we can do really, actually to you know, strengthen that, what are the
-- what we can do, what you know, field can do?

MR. LEIBY: I'll start that. First I want to finish the other one. The flipside also occurs quite frequently too where you have a recipient who has, let's say, who is immunocompromised, has a rip-roaring Babesia infection, apparently doesn't have any other risk factors. We go back and test all five donors who were implicated, all five donors implicated -- potentially implicated are negative. Probably it was transfusion, but the donors have cleared the infection and they now appear to be negative. And so that does not qualify as a transfusion case because we can't pin it down.

Getting to your reporting question, that's long been a conversation that Barbara and I have had that there is really a lack of reporting. There's actually even a lack of publication of most cases these days because there's been quite a few cases re-published, but most new cases people don't consider them novel are unusual, and so they are not getting into the literature.

So there's this kind of anecdotal sharing of cases that, you know, many of us do among ourselves and then we factor these numbers and we put them on slides and
show them to groups like this. And there's no central reporting system to my knowledge. I know the FDA is interested in this, I believe, probably the reason for your question, having some centralized place where these cases can actually go in from physicians or from the public health service in, you know, the regions of other states.

But they may even go to the greater need that it's not a notifiable disease.

MR. KUMAR: Well, is it time to do it now. I mean, CDC does such a wonderful job. I mean, I use that as an example how reporting should be done for transfusion-transmitted malaria. And for the last 45 years, almost every single case is documented, very well investigated, you can go back and look. Something like that. Is it time to do that for babesiosis now?

MS. HERWALDT: And again it depends, when people say, you know, something reportable or something notifiable, there are different implications of those words for someone, say at CDC or in the State Health Department, than sometimes what you may be thinking of at FDA.

Now the question of whether in general cases of babesiosis should be nationally notifiable, that is
something, you know, I'd be happy to talk about, but it's not just something that CDC decides. As Peter alluded to before, this is something we've wanted and have talked about for years. But whenever something is made nationally notifiable, it's a big deal for State Health Department. And by the way, making something nationally notifiable does not mean that all cases, even if they are recognized, are reported to CDC. It requires a state having it reportable.

Okay. So you are still dependent on states having it reportable, and then when you make it nationally notifiable, then the states in which it's reportable, then are "required to report to CDC." Now that -- and you have to propose to something called the Council of State and Territorial Epidemiologists to make a proposal that "x" disease warrants being notifiable.

For example, Chagas disease, is not nationally notifiable. There are lots of things that for various reasons we'd like to have reportable, but it's a big deal because you don't -- states don't like to add, you know, lots and lots of diseases. But anyway, to cut to the chase, we would like to make it nationally notifiable, but that will not necessarily correct the problem of cases not
being identified in the first place.

And in terms of the issue of reporting to FDA, there are tricky issues that goes beyond babesiosis in terms of what are the implications of say David at Red Cross hearing about something, or myself at CDC on being aware of something, and our reporting it to FDA. Because we are not a regulatory agency. Matt Kuehnert may want to weigh in on this. This goes way beyond babesiosis. But we are all on the same page in terms of the issue at hand that we want to make sure that everyone's aware of the cases. So it's not quibbling about that, it's just the technicalities of the regulatory factors in terms of official notification versus communication being opened.

MR. KUMAR: Well, thank you, and short of that, I mean, maybe we can do something to -- we can extend. I think Dr. Krause has -- (inaudible) for a long time now. And then probably we'll take two or three more questions. By one o'clock we must --

SPEAKER: In terms of question three, one could theoretically answer that question by looking at the data that Ritch talked about. So there, you know, study -- there are at least, Ritch has cited three studies looking
at where people have looked prospectively at what the percentage transfusion-transmitted babesiosis is. One can look at that -- what then one should have theoretically in terms of transfusion compared to what you actually observe. And I would suspect that there is a disparity there, that what you would get from those studies suggests a certain rate that you should be seeing and the actual rate is less. And I would certainly think it's less than -- you know, that there is going to be a difference there and it's going to be less than what you actually observe than what you should observe based on careful epidemiologic studies.

MR. LEIBY: Debbie.

SPEAKER: I just wanted to say that in New York and in Connecticut and I would imagine in Rhode Island, it is required to report it to the state, and since those are the heavily endemic areas, perhaps CDC can start to gather that data and at least get a good accounting for what the transfusion-transmitted rate is.

MS. HERWALDT: Actually I'm glad you made that point. There are multiple states including all the really key states in terms of the Northeast, for example. And by the Northeast, I use that term loosely. You know, going
down to New Jersey in which it is reportable. And actually we have worked with a number of the state health departments in terms of even trying to come up with case definitions. And actually it's another important point not belabor it, but when people talk about, oh "x" number of cases have been reported in a certain state, the case definitions vary from state to state.

Let alone the issue of lab complexities that Mariana and others will get into, not all labs that do testing for Babesia are necessarily -- how to put this in a nice way -- the results aren't necessarily valid or consistent or whatever. But -- and by the way, I'm not at all demeaning New York which has been phenomenal, Connecticut has been phenomenal, Rhode Island, I mean, just fabulous in terms of the work on babesiosis, fabulous epidemiologists, and we have great working relationships with your states. But I'm just making the point that it gets back -- and this is by the way just cases of babesiosis that coming up with a case definition is something that we've been working on, and Peter knows this. We've shared case definitions for years.

MR. KUMAR: So let's -- last one quick question
and a quick response.

SPEAKER: It's a quick comment following up -- (inaudible) America's Blood Centers, but was in New York many, many years. And there was an attempt -- they -- probably it's not just having a collection of the data, the problem is getting physicians to recognize it and report it. And the New York State Department of Health in the 80s we worked together to create brochures for physicians, for the recognition of Babesia in hospitals and all that, and that's I think it where the major problem lies is that the infection is not recognized. And I don't know -- in order to get something that is meaningful, that's where we will have to put a lot of energy.

MS. HERWALDT: So you have to recognize the case and then you have to consider the possibility of transfusion. And once that's done, there are relatively few places and labs that do the testing. For example, in the Red Cross system, David is aware of these sorts of investigations. Non-Red Cross systems, we work with a lot of folks in Rhode Island blood centers, we're going to be talking later, and people in other health departments and other blood centers, New York, Connecticut -- well
Connecticut through Red Cross, but New York through various labs and blood centers does testing.

So you know, for some of the, you know, well validated cases it's, you know, several people getting in a room together and you know, combining what we know. It's not as if -- for the most part it's someone out in some state doing some testing and, you know, finding some transfusion-transmitted. It's not like there are -- we don't think there are a lot of those in which they've documented transfusion-transmission and we -- one of us doesn't know about it to some degree.

But there are a lot that are hearsay or not well documented on not investigated at all, let alone not diagnosed.

MR. KUMAR: Thank you, Barbara.

With that we thank all of the speakers here, and thank the audience, and let's break for lunch now. There's a cafeteria downstairs here. There's another one, Nature Building (phonetic), just across the street. Yeah, we'll come back at 1:00 o'clock sharp, but the cafeteria across this street, Nature Building, much larger, they have more choice, but let's just try to come back at 1:00 o'clock.
Thank you.

(Whereupon, a luncheon recess was taken.)
Afternoon Session

Laboratory Tests to Detect Babesia Infections

Mr. Leiby: -- which is Laboratory Tests that Effect Babesian Infections. Our first speaker will be Sanjai Kumar who has actually put a lot of effort into today's session. So it's only fair that he gets to say a few words. But he will be talking about biological and clinical features that influence the detection of human Babesia infections.

Mr. Kumar: Thank you, David. And as you said, it's just saying a few words. I'm no babesian expert by any measure, but I've been forced to think about these issues now. So, what I would be doing here mostly putting questions here for David to answer later on. So it's really talk. It's more questions. I hope you heard that, David.

So -- and again, so I'm a malariologist, so I always think in malaria term. And so this -- the parasite itself give me a window to do that actually. So intraerythrocytic parasite protozone belong to phylum
apicomplexa, okay. And that's what I'm going to talk about the rest of my presentation.

So first of all, parasite is in the inside red cell. It's not in the system like bacteria or viruses. So you can detect only, but you can find it. You can find if -- as many others said before me, who brought up this issue, the five infected red cells with Babesia parasites, how do you go and find them. Antibodies are another way to look at it, antibodies in the system.

But how useful it is for donor identification and diagnostic purposes? So what are the other members of this phylum, plasmodium, theileria, eimeria, and toxoplasma. So it's kind of a very distinguished group of parasites to be in. You know, some of the pathogens is most famous cousin, plasmodium, we all know about this. These parasites have great public health and economic importance around the world.

So the reason I raised the issue is there is other resources available other than looking at Babesia itself. There's more research done for plasmodium, the genome information is available for three or four species. For toxoplasma, I think, genome is completed and theileria
is of great economic importance as well.

So how do we learn from that? The similarities in morphological features and antigenic determinants between plasmodia and Babesia, and we can exploit that while we are developing new tests. I think because we still -- with all these research, we can still -- we are in still a stage where we can -- designing a stage so we start from here also.

So Babitha Mahajan and my lab and myself, we started, this last few days we started to see -- what is -- look at building phylogenic relationship between other Apicomplexa only. So it's a phylogeny just specifically about apicomplexans here, okay? So you see all the divergence here. The European parasites listed together. So the original starts separated somewhere early on here. So then you see plasmodium sitting right here. And then another split came to Babesia microti.

But it is surprising here. If you look at the microti species across the continents, they do split apart from each other. All plasmodium is sticking together. That shows the modern working. Divergences together. All theileria come together here. But there isn't -- there is
not that much evolutionary distance here between microti here and plasmodium here.

But the other thing we need to worry about if these -- this is basically based on only -- not basically, it is based on only 18S ribosomal RNA gene sequence. So it's based on one gene sequence only. But still we need to worry about it if we have detection methods. Those are based on -- one strain of a species may not work for others also. It depends on what the test is really. So that's something we need to be cognizant about.

And this question of divergens is so farther apart. So -- and this was again brought by Dr. Krause and his staff. Apicomplexan, what is their biggest property. They have these unique organelles called rhoptries and micronemes at the apicalin (phonetic) and as they call apicomplexan. They use these antigens to participate in invasion of erythrocytes.

So these are the content proteins. They just come there at the time when they come in contact with red cells, (inaudible). This release though is deposited in the proteins and red cells as the initial contact and recognition. So analogous pathway for invasion has been
identified in Babesia also between plasmodium although we
don't know the very minute things here, but it seems like
some of the common red cell receptors are used here as
well.

So this is what I say here. So there must be
then if one does -- uses careful bioinformatics here, one
might find cross-reactive epitopes that one could use to
develop a common antibody test. Or perhaps better, one
could -- many of these molecules, these rhoptries are
there, microti molecules are there, and so are many of the
merozoite surface molecules are common.

So you can sum, all this is not that great. That
many sequences is not available for plasmodium. But
nonetheless one could find for a common approach or one can
simply make recombinant fusion proteins. This is something
among us from the kit developers here to think about. So
while you're developing test for malaria, just through in
Babesia there also.

So how do we use that information later on,
that's something we have to figure out. But perhaps, it
may not be that difficult to use common closely related
molecules to make a fusion protein or develop a pan-genus
species. That's something if we do this smartly should be possible.

And this is review article that was published in 1996 in Parasitology Today. It was called Parasitology Today. And I apologize because we forgot to give the credit who's paper is this year, but they -- what they call (inaudible), this is theileria, Babesia or plasmodium. So this is another old work here now.

But look at the common features in this club-shaped organelle here. These are the rhoptries here. The microneme is here and the food vacuole and everything is so common here. So this is the Rhop-1 molecule here from Babesia here. This is the Rhop -- there's an entire family of rhoptry antigens here in plasmodium. And after the initial contact, the invasion occurs. But this is when the differences started to happen here. Babesia loses its parasitophorous vacuole probably, but the plasmodium maintains it throughout their life.

But many of the antigens are common here and we can utilize that in developing the test. That's all the point I'm trying to make here. So there -- another thing
we can think about while developing these tests, whether for diagnostics or for our purposes, more for donor screening, donor identification, Babesia parasites can persist as low-grade, asymptomatic infection for months or probably a year or more.

The same thing happens in malaria parasite also. And this is more important if you think about developing direct parasite detection methods because nothing is better than that. Somebody is parasite positive, person is differed. You are parasite negative, you come and donate blood. There is no need to worry about whether it's current infection or previous infection. And one day hopefully we will get there.

Parasite burden in asymptomatic carriers is not known. We don't know what is the minimum infectious dose for Babesia. Same problem with malaria also. There is no recommendation to treat asymptomatic Babesia infections. I mean that's another thing. If those treated get adequately treated, so we don't have to worry about a potential carrier are from babesiosis.

And asymptomatic carriers are assumed to be the primary source of transfusion-transmitted babesiosis. And
I'm sure we have heard enough about that and we'll keep hearing about that because that's our major target group here.

What are the detection methods here? Direct parasite detection, microscopy can be done similar to malaria, thin film, thick black film. Although I've never seen any, like, for plasmodium we know it is five parasites per micro-liter. That's the sensitivity limit for microscopy. I haven't seen such reports for Babesia.

Inoculation of blood samples in sensitive animal models. We heard about that. We'll talk about that a bit more. DNA detection. Direct -- indirect evidence of parasite exposure; antibody testing, IFAT, ELISA, all that is done and people have made a lot of advances there and we will hear three subsequent talks, but only they will talk about that.

No approved laboratory test available to detect Babesia infections in blood donors are for any reason. Antigenic variations between different Babesia species are known to affect sensitivity of parasite detection. That's something I mentioned, and I think there's some data also that divergens cannot be detected by using a test developed
So microscopy, there is no need to dwell on that more. Inoculation of blood either in gerbils or in rodents for microti or divergens, that's accomplished. But -- I mean, it's just impossible to use this method for donor screening. How many gerbils or mice we will need? This is going to be a bit difficult, I guess.

(Laughter)

MR. KUMAR: DNA test. PCR tests of high sensitivity have been developed and I will show some database from my own lab. So this was something that was done. This is the only piece of data that was done in my lab. We started and we explored this a year ago. So I'm still not very familiar with this, still trying to learn.

So this is the Babesia microti we got from ATCC. It's called Peabody strain.

And we went back and looked a bit more about the history of this. This parasite isolated in 1973 from a woman who got infected in Nantucket, Thailand somewhere. So the parasites were isolated. And initially, they were quite refractory in mice but after 40 (inaudible) or so, they were very well adopted. So, you know, what, on our
hands we can achieve up to 50 to 20 percent parasitemia; here are the famous piroforms.

I could not distinguish them from falciparum with my own expertise also. But then again the presence of extra-cellular parasites probably might give it away. But it's the simple microscopy Geimsa stained film. So we infected BALB/c mice at 10 percent parasitemia. By the way they were represented parasites. We have a stock of these parasites now at 10 percent parasitemia. We prepared a DNA by Qiagen method. Nothing magical here. And we spiked the different parasite concentrations is one ml of O positive human blood.

So we use a two-step PCR using 18S Ribosomal RNA sequence. It's specific of Babesia microti. So after one-step PCR, we barely see anything larger fragment here, 288 base pair. Then we would use internal PCR primers and this time we see 112 base pair fragment here. This is something we see every time here. You don't have any DNA template here. So see all the non-specific brands. You add DNA here, the non-specificity disappears here. But this is something very reproducible.

So it doesn't seem like primer-dimer. That
should be somewhere running down here. But we just can't tell. But its specificity issue does disappear on itself. We took out the DNA fragment and sequenced this and this is indeed a ribosomal RNA gene. So we're not amplifying anything else. So this is -- nothing magical about this, what we have done, but what we struck me here, very easily we can detect a single parasite in an ml of blood. But still that will leave.

And perhaps if we try harder, we can go lower sensitivity too. Probably, we can inoculate into four two-ml, four five-ml of blood. But still that will leave if in a single parasite detection limit in a unit of blood, there are still 450 parasites in a unit of blood. But now the question is we do not know what the infectious dose is here.

But if you had a better idea about that, maybe okay, if you can detect one parasite per ml for 500 parasites, if it needs more parasites to call permanent infection, you may be okay with this kind of assay. So that's the limitation here. That's what we're up against. Assay sensitivity here would not be the only issue. The issue may be the other clinical features we don't have full
So talking about antibody testing, I mean, we are all grappling with the same issues in malaria also. Those sensitive issues are available for malaria. But how do we use them. So there's some reports. I see that IgM antibodies may be suggestive of acute or recent infection. IgG titers may persist for months or even years. For donor testing, assay must be of high specificity.

Reported anti-Babesia antibody positivity in U.S. is shown to range between 0.3 percent that David show from Connecticut to less I have seen before, 17.8 percent. That's somewhere in Northern California and Washington State. So suggesting either low assay specificity there or high rate of asymptomatic infections. But that also in the same report this particular area with high antibody positivity is not endemic area. So probably we don't have that many asymptomatic infections there.

So the biggest challenge from a donor's screening perspective is to distinguish between a current infection and a previous exposure. So donor loss from false positive reactions or positive reactions from previous exposures, already resolved infections, could be substantial. So
that's what I came across recently.

I went to a seminar. I mean, I've been thinking about this, and -- if there's something we can do or we can bring some ideas in the field, we can help to move the field forward. I went to a seminar by Philip Felgner recently. And he presented this data in his seminar that struck me.

And there are a lot of similarities. I mean, they are completely different pathogens borrelia, but they have lot of similarities. Same ixode tick used by beating Babesia and lyme disease borrelia and also it's the same mouse. Peromyscus, a white-footed wild mouse. That's the reservoir hosted. So there are similarities here.

So what they have done here is, their goal was to identify new immune targets, antigen discovery about antibody. So they're using the entire array, genomic array. So instead of DNA array, this is totally protein array now. So what they did here, I do not recall what is the total genome size for borrelia, but at least they had 1,292 open reading frames. So that covers 80 percent of the genomes.

So there's the answer right there. DNA
fragments. They clone the genes. They made protein in self-extract system and then they spot them as same as one who extract -- spot a DNA on this chip here, all 1,292 of those. And then they used the antibody activity to identify antibody targets as part of the antigen discovery program. So there are two things.

So the use sera from patients with early or later lyme borreliosis and compared to control sera and also the same time they compared the recognition of the natural infection as their sera with the mouse that infected, the natural infection in mouse, in white-footed mouse, peromyscus, and they compare the reactivity.

So first I will just talk about this and then come back here. So there are a lot of common antigens acquired during natural infection in mouse and in humans, which is not surprising. But then there are unique recognitions too, the sort of hosts determining those responses against those antigens there, okay.

So now coming to the sera antibody reactivity, from the natural infection what they find about 15 percent of genome reacted with antibodies from natural infection in humans, okay. So here are the 12 sera here. These are
individual reactivity. And you can see this is the log scale here for the fluorescent they are measuring. And these are the panel of the antigens they recognize.

I mean, that doesn't anything in itself, but this is what I suggest now here. If you look at the antigens, those are used for ELISA for plasmodium. Most of those are merozoite surface protein one, two, three and so forth. So those are common antigens, major vaccine targets. The way those antigens were recognized by major antigen discovery research program about 20 years ago in Australian groups. What they did there was simply they prepared (inaudible) library in the very beginning when the cloning was in infancy.

Those took those antigens there, and they took immune sera from the field. So the most dominant expression antigens and the most common antibody responses got recognized. That does not mean that the antigens -- here we see only 15 percent of the activity. The rest of the 85 percent, that means they are not important.

And there are different reactivities, but the reason we don't recognize them is because the exposure in the host is not equal, but the power -- this technology are
far severe. You are not based on the amount of protein that's based on genome. What you can do is you load equal amount of these proteins in the chips really.

So the amount of antigens is not a limiting factor here. And the second thing that happens here if you look at the antigen reactivity in plasmodium, there are certain antigens we know those are expressed during the early infection and that during the acute infection. And when the antibodies, those are against the major antigens, when the clinical immunity is achieved, those antigens are different.

So I think if somebody runs an obviously required investment, commitment and funding and same approach may work for plasmodium, Babesia and perhaps other antigens, the number of antigens one can put here, I mean, we are fussing about whether we can -- and ELISA has one antigen and two antigen, you ask for somebody more than antigens, it's not possible.

Here you can put probably 5,000 antigens here for many pathogens, different stages, and if that's a good research program, you can identify the antigens expressed during the window period before the parasites become appear
are clinical symptoms. During the antigen -- if you can
begin to statistically distinguish those antigens, one can
develop a panel where you can have an assay that could be
used to distinguish between acute infection, chronic
infection, and previous exposure.

And I think it's quite possible that this is
going to require major commitments and investments. And I
believe this chip could be used actually make a multi-
pathogen chip, which will work for several pathogens.

So in summary, in the role of donor testing in
U.S., donor deferral and reentry for Babesia infections
deserves further consideration. Direct parasite detection
methods would require the additional technological
sophistication to capture those few Babesia parasites that
may be present there and then we detect them so the method
will be useful then in that situation.

And there's screening for anti-Babesia antibodies
as a strategy for donor testing could be beneficial, but
then first we need to figure out what to do with those
which are positive, what to retain them, how long to defer
them, how to reenter them, all those questions. Those who
are working in the field or in the lab about this, they
know a lot about this, but I thought what question to bring up so we can talk about this.

And for diseases such as Babesia that have a limited but persistent transmission, a multiplexing approach where several pathogens can be detected simultaneously would be a useful strategy because every time if we asked whether another pathogen in your screening program, I think it's not going to fly very well.

I think that's all I got to say. These are the people in my lab who did the work here and I must acknowledge them. And thank you. I'm right on time, 20 minutes.

(Laughter)

(Applause)

MR. LEIBY: We would expect you as a leader to be on time. Second speaker in this section is Marianna Wilson from the CDC. I thought you retired.

MS. WILSON: I have.

MR. LEIBY: Oh, okay.

(Laughter)

MR. LEIBY: Review of available laboratory methodologies and usefulness for detecting Babesia
infections in humans. Marianna?

MS. WILSON: Well, we're going to go from interesting potential test to the actual test that we are actually doing and so some of the basic data for these tests that have apparently accumulated through the years. Well, the diagnostic tools for human infections. You have heard about them all today, but I'm going to go through them individually.

Blood film examination was the first test available. Easy to do, easy to make a blood film and state it but not so easy to read the blood film. Animal inoculation. I'll talk a little bit more about that. Antibody detection and DNA detection.

You have seen pictures of the organisms before, but I just like to remind you again. The organisms may be confused with ring forms of plasmodium species. You can say that if you see a tetrad of the organism, the four organism multi-crossed appearance, that this is indicative of Babesia species. But you don't know what species it's indicative of.

Initially we thought that we only had one Babesia, the microti, and so everything was called
B. microti whenever it came through. However, obviously now we know we have a number of different species of Babesia which infect humans and you cannot -- you can no longer use more of a logical identification of the species.

Blood film exam requires a trained microscopist. As far as the sensitivity of it goes, during early and later stages of infection, organisms may be present in low numbers but not visually detectable visually.

Specificity? You can confuse parasites very easily with plasmodium species if you're not much of a parasitologist. Animal inoculation has been used in a number of -- in a lot of cases. It's been very useful to help elucidate problems, problem patients. We used to often -- we would often do animal inoculation whenever we received the sample to just, number one, try to culture the parasite because, number one, there's no culture system as you've heard before, for B. microti, and most other Babesia organisms are difficult to culture also.

So animals have always been used as a culture system. It's useful to expand undetectable levels of parasites, bringing it up to detectable levels to so you can confirm infection. And you've seen some data -- using
that today. It's also useful in investigations of non-
B.microti infections in which we'd able to produce
parasites for molecular identification and antigens.

However, these parasites are not -- I mean, they
are difficult to put into animals. Something might --
could go well, like microti goes well into the normal
hamsters, but the other species won't go. So there have
been multiple times I'm trying to put parasites into other
animals trying to propagate them, but it hasn't worked. We
haven't found the right combination yet.

And certainly, animal inoculation is not
practical for routine samples. So antibody detection has
been the main test of choice. And the first test that was
developed was the indirect fluorescent antibody test. The
imminent blood assay came along sometime afterwards and
there have been some attempts at enzyme immunoassays or
ELISAs also. So I'll talk about these in pieces.

For the indirect fluorescent antibody test, you
saw a picture, I forgot which speaker, but you saw a
picture of an IFA test. You have got to have intact red
blood cell which contain -- that are infected with Babesia.
That's your basic antigen for the test. For immunoblot and
ELISA assays, you can use (inaudible) parasite antigens, which means you have to smash them out and get some sort of (inaudible) antigens, which -- for parasites you have like a mesh.

Now, for the B. microti, and I'm speaking strictly about the B. microti at this point, IFA test. In general, we've not tried to use specific IgG or IgM -- we have used IgM, but not specific IgG conjugate. We have always used an anti-immunoglobulin conjugate, which sees all the classes of immunoglobulins.

The initial evaluation of this test was done by Chisholm et al., published in 1978. The sensitivity, which was based at the time on the first 12 patients identified in Nantucket was a 100 percent. All of these patients were slight positive and the test reacted with all of those. So it's like, great, 100 percent.

Specificity was looked at over some time and finally published from CDC in 1986 also by Chisholm et al., and the specificity was really pretty good. The only major problem was about five percent of malaria patient's sera reacts with B. microti antigens.

Now, this has never been the test, there
certainly is no commercial kit for this test for IFA. But Dr. Krause in 1994 or earlier put together a battery of samples and did -- we had a comparison of four laboratories who were performing the IFA test at that time. Three of the labs were doing human patients -- testing human patients, one lab was a veterinary labs. It dealt with other babesias.

And it was -- in some respects it was nice to see a sensitivity between the four labs ranged on the same battery of samples, ranged from 88 percent to 96 percent. Specificity was a 90 percent to a 100 percent. So there were difference between the labs slight difference in our test procedures and slight difference in our antigens. And there were different people performing the tests. So as long as you have a test which is not well defined and well controlled, you're liable to have problems.

Now, in terms of detecting antibodies early on, really Ruebush et al., again, from CDC in 1981 found that titers of 1:1024 or greater may indicate recent infection. These were really based on patient who were slight positive and were clinical cases. Dr. Krause published in 1996 a paper on, IgM, the detection of IgM antibodies antibodies
and found 91 percent in parasitemic patients.

And as far as antibody persistence goes, again Ruebush in 1981 found that the IFA test may remain positive at low level 1:16 and 1:256 for greater than 12 months post-treatment. Now, this was prior to the time of the PCR. So the only way that we had of telling whether these patients -- of assuming that these patients had been cured was the fact that they were no longer parasitemic by slide exam. So certainly this may -- this time limit may actually be different from this.

For IgM antibodies, I have personally seen no published data on persistence of IgM antibodies past a couple of months. I hope that perhaps some of the -- Victor might have some data on that or maybe Peter might have some other data? But IgM antibodies in parasitic diseases often last a lot longer than they do in viral diseases. For instance, attacks of plasma, you can detect IgM antibodies up to 18 months after onset of symptoms when you know you have an initial infection.

So I would caution people to think about the fact that just because you have an IgM positive titer, that does not necessarily mean that you're looking at an acute
infection. You have to take a number of things into account.

Now, with Babesia microti IFA test, we have found that there is little cross-reactivity between the other human species with Babesia microti. Initially I think the first -- the WA1 case was the first case in which we tested this guy with B.microti antigens and he was totally negative. The patient was totally negative. And we saw organisms on his blood film.

So it's like what's going on? Well, that was just the start because for the majority of the other cases we've had. They do not have the other different species. They do not cross-react with B.microti. Consequently, at least for the IFA test, the specific Babesia species organisms must be used as antigens.

Now, we were able to obtain the B.duncani or WA1 and prepare IFA antigens and we were given antigens for -- given parasites B.divergens from the Texas group. The problem with -- and we offer these -- we don't offer these tests, we use these tests in case we think that there may be something other than B.microti.

The problem with these tests is we don't know
what the sensitivity of these assays are. There have been so few cases of these diseases, we can't say, you know, all right, so we've had four cases of WA1 and they would all react. But who knows what the next one is going to do? So the only thing we can do is to determine specificity with these assays.

And in general, the specificity is 99 percent, but we've had to -- you have to play a bit with the cut off because certainly the B.duncani or the WA1 is more reactive than the others. And so we have to use a higher cutoff for that particular antigen.

Now, just to give you an idea, these are the antigens that we use, microti, duncani and divergens. These are the cutoff titers for the test and these are patients with these different -- infected with these different species. Now, infection, of course, is totally negative with all three. B.microti patient, strongly positive with B.microti, essentially, negative with the other two. B.duncani patient? Strongly positive with B.duncani, but negative with the other two species. And B.divergens patient? Again, reasonably positive, but negative with the other two.
So you're talking about trying to develop a test for Babesia. I'd like to again suggest as Barbara did, we also have to consider that there are other babesias out there. And we are probably going to be seeing more of those as time goes on. So you need to have -- you need to keep in mind that you need to be able to potentially detect these other babesias also.

Now, talking about the B. microti immunoblot assay, there was a commercial kit on the market for several years, but was taken off the market. But it's now considering bringing it back on the market. The antigen is the infected red blood cell lysate.

And there was a paper published by Ryan et al., the Connecticut group in 2001, and shared that the sensitivity was 96 percent and the specificity was 99 percent. And those are pretty good figures. Now, there also was a commercial laboratory that performs these assays and our next speaker, Dr. Victor Berardi, I'm sure will share his experience with this assay. So I'll not talk about that.

Now, there have been several reports of the use of lysate assay. The first was based on -- the antigen was
based on B.microti recombinant protein two -- or recombinant proteins. Dr. Leiby et al., screened the Connecticut blood donors with ELISA, found 203 of 3,490 blood donor are a little -- are up almost six percent ELISA positive.

Now, they confirm the ELISA positive with the IFA test and found that 30 of the 203 or 15 percent were IFA positive. So in this case, the ELISA was considered sensitive, probably sensitive, but it lacks some specificity. And in order to try to take care of the specificity problem, (inaudible) worked on and developed a combination peptide of these two recombinants. And in their evaluation found that sensitivity was 92.6 percent, specificity was 93.3 percent.

But the nice part was that of the 60 samples, which had been positive in the previous study with ELISA were now negative when the combination peptide was used. There was also a commercial laboratory which has published on an ELISA assay using a whole red blood cell extract and their publication indicated that the sensitivity was 95.5 percent. But that was based on IFA positive sera and that was the only definition of those samples. And specificity
was 94.1 percent.

And now, PCR. PCR came along. It was like this is a huge help, helping us see how wrong parasitenial lasts because, certainly, PCR is more sensitive than the blood film exam. But you have to take -- when you talk about sensitivity, you have to think about what types of samples you're talking about when you say a figure for sensitivity. So for the moment I was going to say, sensitivity of PCR has been indicated in published papers to be 95 to a 100 percent. Specificity is 100 percent.

Recently the Red Cross group has an abstract talking of their Real-time PCR and there was another abstract by the Mayo Clinic Group several years ago on Real-time PCR. And certainly it looks like the Real-time PCR is probably more sensitive than the two-step nested PCR. And as you saw from Dr. Leiby's data on the natural history of the patient, certainly the Real-time PCR picked up a lot more positive than the same two-step nested PCR.

Now, when we are involved in the transfusion transmitted investigations, it was never made a huge point although we often do certainly if it's a patient who doesn't -- who has a non-B.microti infection, but we have
often not been able to confirm B. microti infection in the recipient.

And I would suggest to you that it might be helpful to do this because if you are dealing with a non-B. microti infection, you could end up with a B. microti IFA test of negative on your recipient who is obviously infected from a blood film exam. And then if you try testing donors, you are going to have all negative donors because of the lack of cross-reactivity in the IFA test.

So we would suggest that you should always confirm infection in the recipient by doing a blood film exam, the B. microti IFA, the B. microti PCR to confirm the species. And then if the B. microti PCR is negative, then attempt to identify the species by other molecular techniques.

Then you start screening the donors. And I don't know whether for laboratory testing the suspect donors has always been initially screened with IFA. That is the most sensitive assay for chronic or for older or fairly recent infection with B. microti. We then did PCR or the IFA positive and then because CDC has a Parasitology lab, we would like to do blood film exams on everybody. So we'll
do blood film examination on IFA positives also.

Now, when you have a series of cases of transfusion-transmitted cases, which have occurred during 1997 through 2008, I've selected these. There are 29 that were selected because of the fact that they do have an IFA positive donor. There were 272 or more donors involved. There were 225 donor specimens, which were submitted for testing. So we didn't have all the specimens, but we certainly had the big majority.

And as I said, an IFA positive donor was identified in each of these cases and PCR was performed on that positive IFA donor. There were 30 B. microti positive donors in 29 cases. One cases had two positive donors out of three. Out of these 30 IFA positive donors, 10 of the 30 were B. microti positive. 33 percent. That's not too good. And you certainly have heard before that PCR is not as sensitive as you wished.

Now, we took a look at the influence of the donor's specimen collection time on the PCR results. We had 10 donors for whom we had segments from the actual donation. Of those 10, 7 or 70 percent were positive and only 3 were negative. There were no donors in the 1 to 30
days post-donation classification. There were 31 patients -- sorry, there were three people whose specimens were collected 31 to 60 days post-donation. All three of those were negative. And then we had nine collected during the 61 to 30 days post-donation. Three of those nine were positive. And then greater than 91 days post-donation, we had eight, and those were all negative. So as you would expect because of the strange way the parasitemia, works in this system, you may have patients who have parasitemia for a long time or not. The other point is we have no idea when these donors were infected. This is just from days post-donation.

So I really don't know where on the spectrum of how long they have had the disease that these samples were collected. Obviously in this case we had three negative donors who were obviously positive, they were infected, and their infection was transmitted, but it certainly was below the sensitivity of the PCR. And this is using the test diagnostic PCR. And hopefully with the use of Real-time PCR, these figures will be improved. But at this option we don't have the opportunity to try to compare the two on these types of samples.
Now, when you evaluate and compare diagnostic test, usually you start out with a patient sample in which you know the time of infection and you know how long they have been infected. But with Babesia, we are not able to determine the exact time of infection. You may have clinical cases who can say, sure, I had a tick bite two weeks ago and now I'm sick, but the majority of cases you really don't -- you are unable to determine the exact time of infection.

That makes it difficult when you try to determine how good a test is or not. And also there is a lack of a standard definition of infection for the samples used to evaluate these tests. Everybody's had to make due with what they had. It is a problem obtaining samples that are clinically defined and positive and blood film examined and PCR. But -- so the test -- the numbers I've given you have been relatively soft numbers because of these problems in trying to compare these tests.

There was one paper that Dr. Krause published in '96, in which he looked at the same 19 patients who had clinical or acute Babesia and part of the definition of their disease was that they were IFA positive. So 100 were
positive. 95 percent were B.microti PCR positive. 84, blood film positive, and 74 percent hamster inoculation positive.

So that gives you -- that's the one paper I know of that gives you a relative comparison of the test on the same patients. And certainly it goes along with what we all know. So in summary, our currently available diagnostic tools are helpful for determining patient infection with B.microti, but are either not adaptable IFA or blood film exam or not yet sufficiently reliable, EIA or PCR for mass screening. That's it. Thank you.

(Applause)

MR. LEIBY: Thank you, Marianna. Our next speaker will be Victor Berardi from IMUGEN Incorporated and which is titled as Performance of Serologic and Molecular Babesia Diagnostic Techniques.

MR. BERARDI: I'd like to start off with probably the most important -- some disclosures here. I am from a privately held corporation. So, I guess, that immediately makes me evil along with my coworkers. In some respects, we're certainly not pure. Most of what we provide are either -- services related to methods development, or
validation for new methods. And we also do have a diagnostic testing component that largely deals with vector-borne infections or blood-borne infections. Blood-borne being work related to HCV, HIV, in terms of genotyping, drug resistance. And most of the vector-borne work is related to Ixodes, tick-borne diseases, being Lyme disease, Babesia and anaplasma infection.

We also provide, and actually run a CAP -- College of American Pathologists proficiency testing program for tick-borne diseases. We do contract work for blood banks. We do have an intellectual property interest in some peptides related to Babesia, some data we saw here previously. And I'll probably convince you that even though economically it would be very nice if they were suitable for use in the blood supply, I certainly think they are not. And all this work presented here has been internally funded by our own organization, does not involve money from other commercial interests or from federal funding.

I'd also like to acknowledge some people who have really helped me with my understanding of Babesia, Tim Lepore at the Nantucket Cottage Hospital, Allyson
Silverthorne, who's been very helpful obtaining follow-up specimens. Nantucket has been a great source of material to evaluate for Babesia infection. Some of the material I've seen from David Leiby, I have never seen in my life, having done this for 20 years.

Some of his patients are very interesting, his follow-up has certainly made me think of things that I haven't thought of before. David Persing's worked with us as a director for many years. Sam Telford's helped us and Tom Mather at the University of Rhode Island has been very helpful to us with many things related to Babesia. You’ve seen this slide many times before from other people here. I'll spend no time with it. My time's very limited, which is probably very fortunate for you.

Early on, when we were involved with trying to develop alternative assays for Babesia, we needed a starting point. So one starting point was to take a standard test method and compare it to what was a standard detection method being blood smear. And we had access to 87 serum samples from patients on Long Island in the late 1990s, in which we simply performed immunofluorescence testing. They were the actual sample -- or it came from
the patient at the time their blood specimen was positive. And we had a very high concordance of finding antibodies at least by immunofluorescence in patients who were smear positive.

And I was surprised. I think everyone here has presented the data. And we could conclude one of two things. I concluded the incorrect thing at that time by thinking cavalierly that virtually all Babesia-infected patients would have therefore a detectable antibody by IFA. And using smear as criteria that was true. However, in actuality, I think the conclusion is there’s a high concordance of detectable antibody in smear positive patients, but only in smear positive patients.

At that time, we were doing some work related to evaluating recombinant antigens, also with the evaluation of some peptide antigens, and we’re also looking at immunoblotting as a method. I'm not going to spend any time with this other than -- if one successfully extracts the right antigens from cells of Babesia, one can come up with immunoblots that look like this. There are several proteins, several BMs (phonetic), we can use to look at patients. This is an IgM blot, that’s an IgG blot. There
are a lot of things we could look at.

These are some samples from different patients with different levels of IFA antibody, we can see quite an array of reactivity, going from minimally reactive to strongly reactive. So it is a potential test to be used for detecting antibody to Babesia. These are some IgM blots, these were random samples, some of -- actually, these may've come from Dr. Leiby. These were specimens with various dates of collection after their smear positivity. And in the case of IgM here, we can see in essence, as time goes on, in different patients, less and less reactivity. But we can see reactivity for a few months, routinely.

IgG is a bit more complicated. We can see all different kinds of antibody patterns, because we don’t really know when the patients were infected, they were smear positive, but we don’t know when they were really infected. So we had available -- after immunofluorescence, we had Western blot testing available, and we also had some peptide antigens available to use in an EIA assay, and the data presented here are data using a combination to peptides in an EIA assay comparing immunofluorescence,
Western blotting, to EIA using those peptides.

And when we look at smear positive patients, we see a similar finding, relatively similar finding with all these tests, they appear to have that very high sensitivity, there are percentage numbers here, but the population is very low, 32 is certainly not enough patients to draw any conclusions on. I think of these things -- of -- as falling within a range, 90 to a 100 percent, we’re in a range. If we’re below 85 percent, then we’re within another range. These 32 patients that -- were selected specifically, because we had pre infection sera, and we also had post infection going out many months to many years in these patients, and we used these patients specifically to do this evaluation.

And I wanted to show you what happens with serology in patients -- at least from the time they’re smear positive as we go out into time, looking at different test methods, because there are some differences here in terms of how long we’re able to detect antibody. And I tried to be representative and not skew what’s up here. If we'd look shortly after infection, we see very high titers with IFA, not much is happening. Curiously, a couple of
patients sera revert by Western blot IgM. The IgGs are positive and our peptide -- looks like some of the follow-up samples are going down in terms of antibody concentration.

When we look at a time period between approximately of 3 to 6 months, we see, well, some of the IFAs are dropping, they’re still positive, we’re losing a few more IgM Western blots, and also in the EIA assay we’re beginning to see some sera reversions in the first few months of infection. I do not have good treatment data on this sample set. We know some of the patients were treated, and we know some of them weren't, because some of them had virtually no symptomatology. They were initially diagnosed by accident.

Half -- a half a year to 3 years, now we’re beginning to see some IFAs, or some seroreversions at least in the IFA test we use, similar to the CDC one. It is not antibody class specific, we use a conjugate directed against heavy and -- the heavy and light chains of IgG. Now, we’re seeing IgM Western blots sera reverting, and we’re losing the ability to detect antibody by IgG Western blot in a very few patients. And in the peptide assay
we’re not detecting any antibody anymore.

Now, this could be very useful in a screening test, in one sense, because gee these people had Babesia. And now several months or a few years later, we’re no longer detecting antibodies, so maybe we wouldn't be deferring donors unnecessarily. But we really don’t know about their infection status. Out to 5 years, we’re still seeing antibody by Western blot IgG. And maybe that’s one of the most useful characteristics in a long term sense. I think we’re able to detect antibody longer using that test method than we are by immunofluorescence or certainly using the peptide assay we were using.

And whenever we look at data, there are always exceptions and these were a couple of patients who did not obey what -- they were disobedient in that we would’ve liked to see them just become antibody negative, they would not cooperate. And one of the patients here we see out here almost 4 years with relatively high IFA titer, no IgM, but very suspicious -- they have antibody in the peptide assay, which makes us suspicious of -- I don’t know what -- persistent infection, chronic infection, reinfection.

During this time, we’re also looking, as was
everyone else, at using molecular detection methods for Babesia, and we had been using a PCR assay, very simple PCR, basic one step with gel detection methods. We had the opportunity in -- of one year, 2003, to look at 111 patients who were tested by smear, and also tested by PCR for not only Babesia -- by anaplasma in our laboratory. And on the basis of this study, we began to see, I think, what PCR really had to offer.

There were 26 smear positives in this population. But on the basis of PCR, we came up with 31 positives. Having enough laboratory background, in one sense, I was pleased, but in another sense I was terrified, because we all know the potential problems of amplicon contamination and PCR assays. And even though we used inactivants (phonetic) it's always a fear and -- whenever something unexpected happens in the laboratory, I am the first person to doubt the finding until it can be proven, because of the potential problems.

We were fortunate in that we could get follow-up serum samples. And we could do antibody testing on the PCR positives that were smear negative. And what we found, the two of them already had -- they had antibody. So we were a
little less concerned with amplicon contamination. We were able to get follow-ups on two more patients who seroconverted, and that was -- this was probably the first time we were really able to get a sense that not everyone was antibody positive at the time they had Babesia infection. And in this case, one patient was simply lost to follow-up.

We could generate a simple number here, we could argue about the percentage, I don’t know what the error bars would show, but it looked like smear was certainly less sensitive than PCR. We looked at antibody findings in 31 of those patients who were smear and PCR negative. And these coming from -- I didn’t want to say, but from Nantucket Island. Thirty-five percent of the PCR negatives had antibody. And curiously, there were four that also had IgM antibody, and that worried us a bit, because our thinking was there was at least a casual relationship between the presence of IgM by immunoblotting and the presence of parasites.

So we were concerned that we were -- we maybe were missing Babesia that was present. So we developed further methods, and went onto real time with a brief
interval of using an ELISA digoxigenin detection system, and -- what I would like to present now is some very recent work. When David Leiby asked me to come here, I felt it was time to update some of our data, so we did just that. We went into our files, and we located the last 100 PCR positive Babesia patients that we’ve seen in our laboratory. These are all from 2008, so that -- I don’t know what the cutoff date was, mid-July, and -- where we had acquired 100 cases of PCR positive babesiosis.

And we went back and we did antibody testing on all these cases. We did blots for IgM IgG, we did immunofluorescence, and we did peptide ELISA, but in this case using a single peptide based upon some work we thought that it could have the same performance. And what we see here is if we believe that the PCR is specific, that we don’t have amplicon contamination, although this is real time, and we’re not opening tubes of amplified product. And as time goes on, we will have the opportunity to see how many of these patients seroconvert.

Based upon data not presented here, we find about 98 percent seroconversion using the current CT cut-off that we use in the PCR assay on the basis of PCR positives. So
it's very high. Why not a 100 percent, some -- these are not blood donors, these are from -- these are patient specimens, so that could be someone who's totally well, who needs a test, because they’re worried about ticks, or it could be someone who may have Lyme disease may have anaplasma, or may have a Babesia infection. These could come from patients with erythema (phonetic) migrants, on which case the physician is utilizing our assay complex, a multiplexed assay to look -- at the same time for Babesia co-infection.

And some of these patients could be cancer patients, because we have certainly seen in the case of lymphoma patients. Some of them can be PCR positive, they can be smear positive, and they will not generate antibody. If we look at concordance of the test methods in this particular population, we’re seeing that’s -- if we look at IFA blot and EIA, most of the samples -- the tests are -- all three tests are positive for the most part. We had very good agreement with the test methods.

So this is suggesting to me something about the performance of the test -- the tests together. If we look at IFA and blot, we have a better concordance -- with --
peptide antigens, did not perform quite as well, and IFA did agree with EIA in a few cases. But in general, the results, I think, are highly concordant. However, when we look at total sensitivity, we’re in the range of somewhere between 83 and 87 percent in terms of serologic assays detecting antibody in PCR positive patients.

This may conflict with data from others, but these are clinical specimens. The patients are not necessarily being evaluated for Babesia, they may be Lyme patients. To try to -- I guess, before we look at that -- in that -- patient population, 21 percent, we estimate had active B.burgdorferi infection. I say, "active," because not only do we have to detect antibody, but we have to classify the patients in terms of where they may be in their clinical illness in a temporal sense. So a lot of these patients had B.burgdorferi infection as well; they were co-infected. We see a population that was not past treated.

So what do we see in patients who we tested with all these -- the same methods who were found to be PCR negative. This is the same patient population from 2008 of this year, we simply took blots of specimens that encompass
the same intervals of time, and we did antibody testing on these patients. I guess, there is some caution here at this -- this is not a sera survey, these are highly -- the samples -- there are certainly a -- a bias in terms of pre-selection of these samples. So it really can't be used to estimate seroprevalence or incidence or anything like this.

But in the 100 PCR negative patients, surprisingly to me, we're not seeing very much antibody left. In other words, we're not seeing a lot of patients with antibody. And what caught my eye as we're seeing zero who have IgM reactivity, that's a bit different than what we've seen when we've used PCR before as a primary test method. The most we're seeing is about 5 out of a 100, who have reactivity with these antibody methods. The single peptide EIA, as it turned out, in three of the cases, they were not concordant with the other test methods.

So they're -- we had three false positives. What I call false positives that were PCR negative and the patients did not have antibody by blot or EIA -- I'm sorry, IFA. We could use this to estimate specificity if we wanted to say we needed concordance of test methods in -- and we can see here that at least with immunoblot or IFA,
we have a fairly high specificity, but you’ve heard that from others here already. These patients, really were no different than the PCR positive patients for Babesia, about 11 percent of them had active Lyme infection and there were two anaplasmas (phonetic) in this group. So I wanted to draw some conclusions.

In my opinion, DNA detection, PCR is more sensitive than say fixed (phonetic) smear or antibody detection methods. And I'm sorry to put this in, I'm going to say, active infection. I think Dr. Leiby's data would disagree with my conclusion, and he's certainly well be -- may be correct. We haven't had the opportunity to look at as many patients over a period of time as he has. So maybe, I can say in acute or early infection, we certainly can think this is true.

Almost all PCR positive patients will have or will develop a detectable immune response. And that response may persist for many months or many years depending upon treatment and also depending upon the test method used for looking for antibody. Immunoblot, IFA and in this case, the peptide assay had similar test sensitivities in PCR positive patients. And unfortunately,
the peptide assay was relatively non-specific in this patient population. If 97 percent is good enough -- I doubt that it is in a blood screening environment, and I think I would like to stop there, because of time.

(Applause)

MR. LEIBY: Thank you, Victor. The last speaker in this session is Darlene Folan. Hope I got that right.

MS. FOLAN: Yes.

MR. LEIBY: And she’s from the Rhode Island Blood Center, and she'll be speaking on Babesia testing plant for Rhode Island Blood Center. Darlene.

MS. FOLAN: Thank you. Thank you, Victor, for the time. I know that he had to give up some of his time so that I could speak. And I'm actually presenting Dr. Young -- Dr. Carolyn Young's data for the Rhode Island Blood Center. It's not necessarily data, but more -- our plans going forward with what we want to do with Babesia. I can skip this slide, because you guys are all the experts and you know that.

There’s -- currently, there is no licensed test for the clinical diagnosis, as you all know. But what we do is when we get reports of a transfusion-transmitted
babesiosis case, we do send down to the CDC, Marianna Wilson was there the first few cases that we had sent down. And she was wondering why we had less, if we had done something different. And I told her, no, but we’re happy, and maybe if we can do it, we want to do it on plan, then maybe she can stay retired.

Rhode Island Blood Center -- we’ll send it down to the CDC if we get a case. And we are saving our samples now for a longer period of time so that we don’t have to send back and call the donors in and ask them for more sample. So we’re hoping to have index samples available to the CDC as soon as we get the report. Our present screening involves questioning the donors and asking them whether they’ve ever had Babesia. And if they say yes, then there is an indefinite deferral.

The donors who are implicated in transfusion cases who are also -- wholly asymptomatic at the time. All the products that we’ve had involved in our cases were red cells. From 2004 through -- 2005 through 2007, 14 donors had antibody to B. microti, found through the investigation of the suspected transfusion-transmitted babesiosis cases. Three of the 14 were PCR positive, 2 had B. microti DNA on a
segment from the implicated donation. The third donor was PCR positive on the sample that was obtained 4 months after the suspected donation.

The third donor's result, Dr. Young feels may've been due to a reinfection or a persistent parasitemia. And it gives supports for testing every one of the samples from the -- original donations. Rhode Island Blood Center, we currently retain our samples for 3 to 4 months depending on the space available. Dr. Young took Rhode Island Blood Center data from 2005 through 2007. In the frequency of donation, she came up with a 1 in 12,000 risk of Babesia based on the suspected transfusion cases that we’ve already had reported.

Babesiosis is a reportable disease. We report to the Department of Health and the Department of Health reported 5.7 per 100,000 population in the year 2006. 2007, we had a donor that was implicated in three neonate cases, and it was the same unit that had been split, and so that was -- the impetus for Dr. Young to thinking about PCR testing for the neonates. In 2008, we had one donor implicated in a pediatric sickle cell patient with Babesia. The pediatric sickle cell patients, in a maintenance
program use about a 100 RBCs annually.

So going forward, we’re going to be using that information to plan and test some of our units before we send them to the hospitals. The hypothesis that she's working on is that PCR testing above 3,000 units annually will reduce the transmission of babesiosis by transfusion in selected immunocompromised patients most at risk for complications from Babesia infection. So this is all going along with her thinking on what we should be doing.

So we plan to test 3,000 donations for the -- per year for the purpose of providing Babesia tested units for selected patients. And these patients would be neonates, pediatric sickle cell patients and pediatric oncology patients. Most of our donors that we choose for this program will be O donors. Many of the donors will be pre-tested for D, C, E, and Kell negative antigens so that they are already in the sickle cell program. So that’s one of the reasons we’ve kind of narrowed down what we’re doing.

Most of the units will be screened rather -- most of the units that will be screened may not always be used, but they will be set up to have them there available if there’s a -- an emerging case that comes up. So we want to
do this proactively rather than waiting and testing them after the transfusions. The inventory and management is going to be the most tricky part, we need to make sure that we have enough on the shelf, but not have too many tested that then are being held away from other donations. So we need to work closely with the hospitals to kind of know their population so that we can anticipate their needs and have stuff on the shelf for them.

The testing plan is actually to have PCR testing based on testing by Imugen and their sensitivity, Dr. Young feels, will be 1 to 10 copies. The donors with positive tests will have antibody testing performed. Donors will be deferred indefinitely if it's PCR positive. The PCR donors -- positive donors will also be referred to infectious disease consultants for follow-up. We will also follow through with the donor notification. This is her plan at this point. We are -- we have a fact sheet that's been developed regarding Babesia, which will be given to the donors. We will ask for informed consent from them.

We are working -- or Dr. Young is working with Dr. Krause to work on an IRB approval. And recently added to the list of what we need to do to get this in place is
to submit for an IND (phonetic). And once all of those things as well as the computer system that we need to make sure we can update these test results is in place, we will begin to stop this, after we meet all of these requirements. So there is a plan in place, we’ve done a couple of the steps. This is the research team that Dr. Young is working with in order to bring this to Rhode Island, and that’s it. So thank you for your time.

(Applause)

MR. LEIBY: If I could have the four speakers up here to join me. Sanjai, come.

SPEAKER: (Off mike.)

MR. LEIBY: I'm sure they’ll put some questions up in a moment. But first, if there’s any questions for the speakers.

SPEAKER: (Off mike.)

THE CHAIR: Roger?

MR. DODD: Yeah, I'd like to ask Ms. Folan, what is the rationale for just doing a PCR rather than doing an antibody test given some of the data that we’ve seen today?

MS. FOLAN: In working with Dr. Young, we’ve looked at some of the donors in some of the implicated
cases, and she’s looked at the antibody responses, because we have that done as well from the CDC. And she feels that that’s not always indicative of them being the one that was infected. So she feels PCR's better.

MR. DODD: (Off mike) Thank you.

MR. MIED: Yeah, David, Paul Mied, FDA. I have a question for Dr. Berardi. You said you used a single peptide in your EIA, presumably that was -- that peptide was selected because it has an immunodominant epitope region. Do we know enough about other antigens from Babesia microti, so that we could add other peptides to that test presumably to increase sensitivity and specificity?

MR. BERARDI: I don’t think we know everything that we could know. The peptides used actually were developed by Corixa as an organization. And we’ve looked at several recombinant antigens first before those peptides -- those particular -- until we -- we looked at several recombinant antigens. We found reactive antigens -- in the case of the recombinants, the specificities were very poor. Peptides were made from two of them that were very good candidates. And then a combination peptide was used.
For the sake of presenting data here at this time, we evaluated once again, but, one of the peptides simply because it was easier to synthesize; the combo peptide's a very long peptide, the yield's very low. And we simply didn't have time to do that. We have looked in the past, using two peptides, and we had very similar sensitivities; there was no improvement in sensitivity. We're virtually already there. We got the same results as immunofluorescence, same results as immunoblot. So unless there's a substantial improvement in terms of hidden epitopes, non-expressed epitopes, in terms of native protein -- using native proteins, I suspect that we're not necessarily going to improve sensitivity.

MR. MIED: Yeah, I wanted to ask you if you tried some of your immunoblot antigens in an EIA.

MR. BERARDI: We have tried to do EIA using native proteins, and we've had very poor success, but that just could be us. We just might not be very good at it. Very high levels of background reactivity. It took quite a while to learn how to extract the Babesia from red cells and obtain as high a purity as possible. It seems as you said, you just break open the red cells and there are the
Babesia. But in the process of lysing red cells, oftentimes, one disrupts the organism and does not get full expression of what’s present in the Babesia.

So it actually took us several years of experimentation in terms of how to break the red cells and how to retain as much of the Babesia intact as possible to then put in an immunoblot test. And actually what it took was a paint shaker. We use -- it has partially to do with tonicity, but literally a paint shaker to mechanically agitate the cells, then collect the supernate, and then do ultracentrifugation on that supernate, and repeat this process over and over and over again. And that has yielded the -- we’ve had the highest yield, and the best luck using that technique. I know it sounds very low-tech.

MR. MIED: This is a question, maybe, for Marianne. Do the peptides that you’re talking about, Victor, do we know enough about the antigens, the commonality of antigens with B.duncani and B.divergens to think that it would work for all three?

SPEAKER: (Off mike) It will not.

SPEAKER: (Off mike.)

MR. BERARDI: No, it will not. B-specific --
this has been published. And -- in terms of specificity studies has been published in the past. But they are not reactive, they should -- let me correct that they should not be reactive based upon past studies. I would not rely on them being reactive.

MR. LEIBY: Jay, you had a question?

MR. EPSTEIN: Jay Epstein, FDA. As you yourself suggested, your results appear to show a higher sensitivity of PCR than has been seen in other studies. And I was just wondering if you would comment on any fundamental methodological differences that could account for that? You suggested that perhaps it's because you're looking at more acute subjects. But other studies that have been cross sectional should've picked up acute subjects as well.

MR. BERARDI: I think what we're observing with Babesia was not intended in the sense that -- the data that was up there, all the recent data, all those patients also received an anaplasma PCR test as well. And to develop a extraction method that worked well with both Babesia and anaplasma, and to have a common real time PCR procedure, we had to look -- we had to pay a lot of attention, first of all, to the extraction system, the particular chemistry we
were using for extraction that -- we have certainly changed that from what we used prior to performing real time assays, and we know we got -- look -- we got approximately one log of additional sensitivity by just simply changing extraction methods.

We did not desire to do that for the purpose of Babesia, we felt we had a very sensitive assay, and we were getting good agreement with what was published. It was really an accident based upon wanting to increase the sensitivity of the -- our anaplasma PCR assay. So I think there are some methodologic difference -- certainly, the primers will make a difference. It took us some time in switching the PCR to find primers that did work well, and to find sequences in which there -- we could predict something based upon past usage.

MR. LEIBY: We’ll go to Hira next and then Peter.

MR. NAKHASI: Okay. The -- this question is for all the panel members here, because what we heard from all of you, I guess, is different assays, IFA, PCR, Western blot. I didn’t get a good sense which one is sensitive and which one is not, and I'll accept the PCR we heard just now, that may be a little bit sensitive. So the question
is two-part. One is among the three tests, which would be the ideal -- not ideal, which will be much more sensitive and specific, or is there a combination of these -- one could put together, and then have an -- some level of sensitivity and specificity?

MS. WILSON: I would suggest that you -- it depends on your patient population that you’re testing. If you’re testing patients as we normally do, we’re looking at acute infections. So for acute infections, certainly PCR is more sensitive than blood film exam, and often -- not often, but occasionally more sensitive in serology. So my first test in that point would be PCR. If you are looking at patients who have old infections or chronic infections, my first -- as in blood donors, because they -- you assume they have older infections -- certainly, some of them can have brand new infections -- but you assume most of them have had their infection for some time. Our first choice is IFA. It is more sensitive than PCR.

MR. LEIBY: Let’s go to Peter, he was first, so we’ll go over here, Peter.

MR. KRAUSE: I just wanted the panel's opinion on the significance of a positive PCR in terms of simply dead
DNA floating around, living organisms that are not replicating or replicating organisms. People have said that the presence of PCR in a patient, over time, could simply represent DNA that’s in the bloodstream, but the organisms are essentially dead. There are DNases within blood and probably the DNA is broken down fairly quickly, but I'm wondering if the panel has any thoughts about that, whether the presence of a positive PCR would always indicate that there's a living organism present or not. I guess, that’s the easier way to say it.

MR. KUMAR: I guess -- maybe something I can comment from general perspective. I mean, in Babesia microti, I have seen reports -- I don’t know what happens in -- actually in hospital -- you know that better. One to 10 percent parasitemia during a acute infection. So there are prospects of genomic DNA circulating around during acute infection. But usually, I mean, once the acute infection is gone, the spleen takes care of clearing these things very fast.

So for example, in malaria -- you don’t see that high parasitemias in plasmodium infection generally. Even in endemic areas, they get treated. But circulating DNA as
a problem for PCR detection has not been reported. But here situation may be different. If you have 10 percent parasitemia, (inaudible) will be rupturing falling -- DNA will be falling apart, the spleen probably cannot clear the mess fast, actually. So it could be some issue there.

MR. BERARDI: I think when we look at patients who have been treated -- Babesia PCR patients, some of them surprisingly will become PCR negative within a week. If we -- maybe a better example -- although it's not direct, would be anaplasma. If you treat a patient for anaplasma with doxycycline, at least in our laboratory, they will become negative within 2 days. If they've been in treatment for 1 day, it becomes very difficult to come up with a PCR positive.

Lyme patients, say a patient with Lyme arthritis, if there is a positive finding in synovial (phonetic) and they are treated, within 30 days, we don't see them PCR positive characteristically. And you would certainly have a lot of experience with the follow-ups in Babesia patients as to -- I don't think the DNA persists for long after the infection is cleared. It has to for some time, but doesn't seem to be evidence of it persisting for a long time.
MR. LEIBY: I seem to recall there were some analogous studies done with T. cruzi in which T. cruzi DNA -- non viable parasites was injected in animals and was monitored, and it did not last very long as far as detection.

MR. LEIBY: Yeah.

SPEAKER: (Off mike.)

THE CHAIR: No, no, behind you --

SPEAKER: (Off mike) Well --

MR. LEIBY: You’re next --

MR. ATREYA: This is C.D. Atreya from FDA. My question -- or more of a clarification for Dr. Folan. In your pediatric patients when you saw the blood was given contaminated, one, did you follow-up to see whether the patients really developed any disease, because in the earlier discussion in the morning, looks like young patients or young people are not affected by this disease?

MS. FOLAN: I'm sorry, can you repeat that question?

MR. ATREYA: No, in your -- one of the slides you showed that one of the contaminated bag was given to three pediatric patients?
MS. FOLAN: Yes.

MR. ATREYA: So did you have any follow-up done on those patients to see whether they developed any disease?

MS. FOLAN: To my knowledge, no. What we had was the sample from the unit to be sent down to the CDC. If there were samples after that, then I wouldn't know that those donors were the ones that were called back in.

MR. LEIBY: Okay. To stay on time, I think our last question will be by Rich, sorry Mike.

SPEAKER: (Off mike) Marianne said that three-tenths of a percent low positive rate IFA either was a post-rate or had been done -- people in non-endemic areas, or that there was an active Babesia in the non-endemic area. I think those were based on studies in Connecticut. And as David showed, of six people positive -- and none of them are here -- if three were tested for PCR, two were positive. So although they -- non-endemic in our article, that wouldn't be a false positive.

I don't believe anyone has done any substantial number -- Peter Krause -- talking about -- 100 donors that's in -- by IFA, right Peter.
MR. KRAUSE: Right.

SPEAKER: (Off mike) I'm unaware of any subsequent tests of any -- clearly negative donors to tell us at all what the IFA negative rate -- certainly, nothing we do in Connecticut can be considered a false negative from our study. And I don't know who's interested. That would be a really good thing to do is provide some clearly negative sera to people who're trying to do this and solve problem -- what if you did find the positive -- what do you have to do -- blood donor -- I mean, I don't know. That's why we have been having trouble -- trouble -- I bring this up, because I don't think we know -- and we start using this in Kansas -- or even if we start using it in a wide scale in marginal areas perhaps New Jersey or eastern Pennsylvania -- Maryland, that you know, we run into big problems -- but I don't know that. I wonder if David or any of the others want to --

MS. FOLAN: I just -- could you clarify -- you're talking about the ELISA assay or the IFA assay?

SPEAKER: (Off mike) The IFA assay.

MS. FOLAN: The IFA -- and --

SPEAKER: (Off mike) Well, the lowest rate you
showed was three-tenths of a percent. That would be a rate that’s a little high -- test --

MS. FOLAN: The original -- the data that was put together at CDC, back -- early on in the '70s, I found how many patients were -- how many samples were tested, they were not blood donors. They were people from -- people who had Rocky Mountain Spotted Fever, other tick diseases, at that time Lyme disease was unknown also. But it was samples like that as well as malaria samples. And the malaria samples are where the problem was as far as non-specificity goes, so --

SPEAKER: (Off mike) Well, it would be nice to see a large-scale donor

SPEAKER: (Off mike.)

SPEAKER: (Off mike) well, we don’t know -- and I don’t think it's --

MR. BERARDI: Well, where would those samples come from, because my sense of Babesia, my experience has been one of incremental approximation. It would be nice to say, oh, yes, it's easy, we have a test it's 99 percent specific, and -- or 99.9 for the blood supply. But there has been no gold standard, what is a case of Babesia. If

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it's smear positive, then that’s good, but if it's PCR positive, is that absolute, not in my mind. We have to eliminate false positives. And as much as we would like to admit -- not admit they occur, they do occur with some frequency. It may be very low, or it could be very high, depending upon the laboratory and a whole lot of other things. So what is a case of Babesia --

SPEAKER: (Off mike) Well, I -- you know, I --

SPEAKER: -- when it's not obvious.

SPEAKER: (Off mike) well, you know, somebody who's never left -- the rate --

SPEAKER: (Off mike) In terms of --

SPEAKER: (Off mike.)

MR. KUMAR: I guess, one solution could be depending on how many PCRs you do every month or every year. At some places you can get a small fragment sequence for $5 nowadays. So getting a DNA sequencing done, I mean, it seems like you go a long way to get your diagnosis done anyway. So getting your template sequence is one solution, at least for diagnostic purposes. If you’re concerned about what you see, whether it's real or not, you can depend your diagnosis based on that.
SPEAKER: I think -- I'm sorry.

SPEAKER: (Off mike.)

MR. LEIBY: I think in a -- for the sake of time, we’re going to have to stop there. I mean, I think these are the problems we’ve addressed and continue to address for Chagas' disease. I think it’s the same problems with malaria. I mean, these are common issues with many of these emerging pathogens. And there’s no -- not always a very clear answer. And I think we struggle with them. So maybe we can discuss that further at the end. But I think at this point -- sorry, no break, you have to stay here. Paul Mied's taking over.

MR. MIED: Okay, for our last session, we’ll talk about possible approaches to minimize the risk of transfusion-transmitted babesiosis. We’ll have free talks, one to address testing of donors, and the other two to talk about pathogen reduction technology. So the first speaker in this session will be Dr. Ritchard Cable from American Red Cross. Ritch will talk to us about universal versus regional exposure based testing for Babesia microti and possible algorithms for donor reentry.

MR. CABLE: It’s on? Okay. I wanted to thank
the FDA for having the workshop. I've been working with this in a while, and as you can see from the data, Connecticut has a -- and Rhode Island now, have rather unique problems with babesiosis, and we were feeling quite alone in trying to struggle with this. So much so that I've threatened to name this disease a Nantucket hemolytic fever to get some more, you know, attention from the federal government. But I'm pleased that we're now getting the attention it deserves. I -- my goal today was to -- assume we have a decent test which we don't have. So let's put that aside and to address the questions on how would you apply such a test to testing the blood supply.

Carolyn Young's presentation from Rhode Island did suggest that one alternative, which I'm not going to discuss, which is a CMV-like approach, test some units, and let the clinicians define patients at risk, and then order for the patients at risk. Parenthetically, we did try that out with our hospitals, and they really hated the idea, at least the blood bank directors did, because that causes all kinds of problems in management. And what is a person at risk for Babesia, anyways that would require more definition. How do I advance this, push the enter button.
SPEAKER: (Off mike) Push the --

MR. CABLE: Yeah. So putting that approach aside, we’re going to be testing a lot of blood for B. microti with this new method. Well, we could test during certain times of the year. I think you’ve already heard that there’s a significant seroprevalence year round. I’m going to show you some data in Connecticut to that point in the next slide. And just to state that during our natural history study in Connecticut, we found a PCR or a hamster positive donor in every month of the year.

So that -- as -- you would expect from what you heard, testing during certain times of the year would not make a lot of sense from that experience. Another thing would be would we test only red cell products, or would we have to test platelet and plasma for recess products (phonetic). Clearly, we aren’t a 100 percent sure how red cells get -- I mean, how platelets transmit Babesia, we know they do. It’s not clear whether it's Babesia outside the platelets in the red cell, Babesia in the platelets, although they’ve never been seen there, or red cell contamination of the platelets.

But at this point, I would assume you have to
test platelets as well. And presumably -- testing is not required for plasma donors, because even if the periplasm is outside the cell, almost all plasma is freeze-thawed, and that should take care of the agent. And we’re going to talk about pathogen testing next, so I won't touch on it beyond mentioning it. Now, this is data from Connecticut on the most recent 2 years -- the reason you only have the most recent 2 years, it was only the last 2 years that we went to a plan of looking for an -- donors for the natural history study year round, and we test relatively uniformly year round now.

And using the last 2 years' seroprevalence data you can see that although it varies, there’s no obvious theme; it might be a little higher in the late spring, July-August area. But it's also high in March, which is a little high -- hard to explain, based on what we’ve heard. So I -- my guess is that you’re not going to get a consistent pattern of monthly variance in seroprevalence, at least not a very substantial difference.

These differences are running from about a 1/2 percent to about 1-1/2 percent, seroprevalence in the testing we’re doing in Connecticut with -- that you heard
from David Leiby. Now, assuming, we want to do -- apply a
test for B.microti in blood, then should we do it in
selected geographic areas? And if we did that would we do
it by collection site or by residence? Well, if we had to
do it by residence, what about people that show up far away
from home to give blood, so we might want to do it by
collection site.

But then what about travelers? And I'm going to
spend a fair amount of time a little later talking about
what the potential is for traveler movement around the
country. Barbara already sort of gave my talk for me, and
I think you know what the answer will be. I'm not going to
talk about the other Babesia species in this talk just to
remind people this is strictly B.microti. Well, one option
is, is to test only in endemic areas for B.microti. I
think that's the natural tendency of someone who wants to
do something, but doesn't want to tackle the whole U.S.
blood supply.

The -- but there are problems, one -- well, how
do we define an endemic area, do we do it by large -- is it
all New England, is it just Connecticut, is it just
Southeastern Connecticut, is it just New London County and
so on. Or is it all of Massachusetts, just Southeast from Massachusetts, Cape Cod and Martha's Vineyard, we don’t go to Nantucket -- and so on. In New York State, is it upstate, downstate, just Long Island, just Eastern Long Island, just east of the Shinnecock Canal in the summer, which is the current approach of the New York Blood Center I know.

How the -- how does one do it? There are simply no seroprevalence studies to really define an endemic area. And as I'll show you in Connecticut, it -- what we'd looked at doesn't support it at least in Connecticut. We don’t have any method for seroprevalence studies and we also don’t have any cut-off for what -- I mean, amount of seroprevalence that would render a non-endemic area endemic. And I -- I'm guessing that this is going to be all or nothing if you did a U.S. seroprevalence study, you’d find in immediate areas, and what do you do with them.

And more importantly, this is a moving target, this disease, and how would you keep these endemic areas current if you did a large, seroprevalence study, which you’d have to do every couple of years. Here's a couple of
reports of expansion of B. microti. One is up the Hudson River valley and this is from Barbara Herwaldt in New Jersey, so that New Jersey's clearly not an endemic area when it wasn't, you know, a few years ago.

So I think we can say that we would need to keep that -- these endemic areas up-to-date. Well, if we are going to use smaller geographic -- if we’re going to use geographic areas to define endemicity, and there are -- I told you about the problems, would we use small areas or large areas. In other words, would we define the areas rather broadly or rather narrowly. Well, smaller areas, we have some significant problems I'm going to show you about now that -- there is significant travel in small areas between the areas. There’s people who live in Hartford and work at Electric Boat in the endemic area down in Southeastern Connecticut. There’s people in New Haven who go to Foxwoods to gamble, and Foxwoods is in the endemic area.

And how would you deal with that kind of small-bore travel, you know, 30-mile trip in a car, or -- even a 100-mile trip in a car. Again, no way to update the data. And I point out that although the data on babesiosis'
clinical prevalence is not well characterized across the country, it is in New England, in the endemic states of New England, and I believe it could be in all the endemic states if we made a big push. And that data would be collected on a regular basis, and would allow us to update the data, the problem is then that defines an endemic area as the whole state.

But a study in Connecticut I want to show you about suggested the -- using small endemic areas will not be terribly effective. First of all, just looking at the six -- this is a table from the map that David showed you earlier. These are the counties in Connecticut. These are the two endemic areas he showed you in red down in southeastern Connecticut, and we have seroprevalence rates in the last 2 years of 153 and 179 per 10,000. But you know, all the other counties have -- about half that or maybe 40 percent of that.

And the lowest rate in the last 2 years has been in Windham County, which is right next door to New London County and probably is more dangerous than Fairfield County. I don't know why it end up -- ended up that way. We, of course, don't know whether a donor donating at a
blood drive in Fairfield County acquired the disease in Fairfield County or at Foxwoods. And in part to address that, we did a little -- a tiny case control study, and that’s -- it's ongoing, but we’re going to present this data at AABB in a poster, and I thought it appropriate to share here.

We basically looked at 767 seronegative blood donors in Connecticut, 58 seropositive blood donors in Connecticut. We showed them a map of the endemic areas of Connecticut, and we asked them if they'd gone there in the last year. We asked them if they did any outdoor -- recreational activities, and we had two questions, one on the beach, and two, other than on the beach. Because this is right on the shoreline. We asked them if they stayed overnight, we asked them if they worked there, figuring if they worked there, they were there more than just that -- just one day, they were there on many days.

And what we saw was, overall, that both groups had 35 percent -37 percent responds to, "Yes, I've been in those areas under these conditions in the last year." So there’s a -- you know, people in Connecticut get in the car and travel around a lot. That’s not a terrible --
surprising. And for each of the underlying causes, you can see a substantial percentage for each, other than work. The work is a 5 percent and 7 percent, still meaningful. And there was no statistical difference, although it's a small study between those that were seropositive and those that were seronegative.

So this doesn't prove that the people who were seropositive, collected at a blood drive in a non-endemic area didn't acquire it from travel to the endemic area, but it suggests that perhaps they acquired these closer to home. And just as we found Babesia at Tufts a veterinary school out in the backyard, they found Babesia in the reservoir in my backyard in West Hartford, Connecticut. So -- you know, Babesia has been found throughout the state of Connecticut in ticks, and why wouldn't we expect people to get it while gardening in West Hartford, in the Hartford County at central park -- central park, Connecticut as opposed to the so-called endemic area.

So my belief is those county seroprevalence data I showed you, probably most of the people, first of all, were truly infected, and second of all, probably acquired it close to home as most people with Babesia do. So our
conclusions of the local scene was that more than a third of donors have meaningful travel and exposure. There’s no difference in the travel histories of seropositive and seronegative donors, and either more intensive prolonged exposure to endemic area is necessary, we don’t know how much that would be, or I think more likely they acquired B. microti closer to home.

Okay, and then going on to larger endemic areas than just parts of a state, I'm going to propose that for the purpose of this discussion, we use state borders to define the endemic areas. First of all, if we were going to do that and we had to ask travel questions, a point I'm going to get to in the non-endemic areas, it's going to be a lot easier to ask donors, have you been to Connecticut in the last year than have you been to south -- to New London County. I'm -- I couldn't believe when I had to come from the malaria workshop to this presentation, unhappy as those travel questions have been for malaria. But I think if you had to ask those questions and I'm not advocating it, it would be a lot easier if you use states of the union, which I think people in the United States probably understand better than states in Mexico, at least I do. I can name
the 50 states and probably roughly where they are. And well, maybe, not all of them.

(Laughter)

MR. CABLE: Also, we can use the Department of Public Health reporting to define endemcity, and they do annual reports and we don’t have to do all the work. And also it would help coordinate whatever initiatives come out of federal state cooperation that should be going on for this disease and -- you know, we talked about earlier. I would propose and I'm -- here's where it starts to get troublesome. I would propose that there are seven states for this activity, I'm going to model a plan for you using these seven states that will show you the difficulties of even doing this.

The seven states would be, Connecticut, Rhode Island, Massachusetts, here's trouble, New York, because upstate doesn't have much Babesia, if any, New Jersey, Minnesota and Wisconsin. So the people that live in upstate New York are going to hate me for this. But using those seven states as the, "endemic area of the United States," what would be the impact if we started to test in those seven states? Well, it has 16 percent of U.S.
population, so I'm guessing, although I don't have the exact data, about the same percentage of the U.S. blood donors.

I think the -- people do a pretty good job of recruiting in these states, I don't -- I've no information that the -- that overall in those states we've much less recruitment or much more recruitment, so let's say 16 percent. But as -- Barbara already told you and David told you there've been several reports of traveler babesiosis with transfusion transmission. Well, what are the number of travelers that come from the excluded area to the included area. In other words, go from Indiana to New York.

Let's assume a 1-year deferral for someone from Indiana going to New York that assumes if they give blood in Indiana, someone's got to ask them have you been in New York in the last year. Let's assume they do a 1-year deferral, or alternatively, they do a test just on those donors in Indiana. Well, first of all, we could define exposure to New York State in a similar fashion as in the Connecticut questionnaire, outdoor recreation, overnight stay or employment. Overnight stay is probably the easiest
way to look at it, since not too many people work in different states than they live.

And fortunately, there is a data source similar to the data source that we discussed for the malaria travel at the workshop. I mean, the DPAC (phonetic) which is something called the domestic market travel report, which is done at great cost by the travel industry association, and that somewhat great cost can be made available to blood banks. So I got myself one of those with David's help and started to look at it. And this is what I found. That if you -- they only look at travel by adults.

But that’s good, because, you know, blood donors are mostly adults. There are one billion overnight trips by adults per year in the United States. In other words, one billion times a person in the United States goes away on a trip of one or more nights before they come back home, and if they leave again, that’s two trips, so we don’t know how many trips per traveler. But we do know how many trips were taken. 240 million of these are between census regions. Now, fortunately, the census regions include the mid-Atlantic and New England regions, which include all of the endemic states, they unfortunately include New
Hampshire, Maine and Vermont, but there’s not a lot of people living there. And they also include --

(Laughter)

MR. CABLE: -- Pennsylvania, but it's only a matter of time before Eastern Pennsylvania is endemic, because New Jersey's not far away. So I thought, okay, we can boil this down to let’s just look at travel between the census regions, because it's -- that’s the only way the data was collected. We did make an inquiry what it would take to get data by states and they said we can start with $40,000 and we said, never mind.

(Laughter)

MR. CABLE: For $300, I bought this, and this is what you’re going to get at this workshop. Using that data, we estimate about 35 million overnight trips to endemic states from non-endemic states annually. Assuming that one-and-a-half of these trips per traveler per year occur -- and I have no idea if that’s correct -- one per year is clearly an under-estimate, because some people make more than one, somehow three or four trips between census regions for the average American traveler seems like a lot. Most people travel that far just once a year, sometimes
twice a year. So I said, okay, let's say one-and-a-half.

Then we'd estimate that 23 million U.S. adults would give a travel history of travel from a non-endemic area to an endemic area. In other words, 23 million people are spread around the United States, including that guy in Indiana, you’re asking if -- had they come to New York. And that was about 10 percent of the U.S. adult population living in the non-endemic areas. So assuming blood donors have similar travel patterns, and my guess is, if anything, they travel more than non-donors, because they tend to be more affluent, 10 percent of non-endemic states' donors would be projected to have overnight endemic stay -- state exposure.

Now, if you said, well, we’re not worried about overnight travel, we’re only worried about there for a week, or only worried if you went -- if you took your clothes off and wandered in the woods. Then obviously, it could be much lower, but I don’t have any statistics to give you on that. So -- just as a number, 10 percent. Okay, so in conclusions, for B.microti donor testing strategies, and not talking about what test it might be, it would be needed year round, endemic areas will be difficult
to identify, and seroprevalence data is limited.

The only feasible solution I saw to limited testing was use the 7-state testing strategy, which would require testing 16 percent of U.S. donors, asking all other U.S. donors' travel history, and testing 10 percent of them or deferring 10 percent of them for a year. That’s my conclusion. So my next conclusion is that a limited strategy testing would require arbitrary deference to endemicity, and would not address the traveler problem.

So what we might want to do is once an automated test comes online actually do a little IND across the whole country to get actual data on seroprevalence, which might then allow us to make some reasonable decisions, where we have no basis for decision making at all today.

So I think my recommendation would be get a test implemented in the endemic areas, and implement it in the non-endemic areas together with seroprevalence data, perhaps to allow them to stop doing it, or doing it in a much targeted fashion after data is collected. Obviously, the other approach is to say you all test all the time, which is what -- what’s now virus testing has devolved to, and Chagas' testing is slowly evolving to. But a universal
testing strategy would obviously considerably be more complex, and might well be limited in -- by the false positive rate, which I -- as I said we don't know.

Okay, that’s one talk and give me two talks for the price of one. The next talk is well, what do you do when you find a positive donor in this testing? Well, we know that -- we defer donors with babesiosis, and we don’t know the applicability of the standard for seropositive donors in research studies. Although, as David said, we’re deferring them. We’ve identified in the natural history study donors with very prolonged parasitemia and also donors with very short term parasitemia. And Richard Benjamin encouraged us after getting a rip-roaring call from an angry donor who was deferred forever, that couldn't understand why -- you know those donors. He told us that we really had to look into reentry, so our plan is to at least see what the FDA has to say about this idea, and then tell the donor, I'm sorry, you still can't donate.

(Laughter)

MR. CABLE: Because that’s how those things go. But we thought a way to look at -- so I did this study in support, actually, before I was asked, for this conference,
in support of the concept of -- is there any way we could reenter the research donors. But obviously, it might be applicable to other donors that -- identified with TTD or with clinical babesiosis and so on. So we -- I looked at the natural history data from 2000-2006, I'll only look that long, because those are the only donors with long term follow-up that would allow a reentry assessment, and I tried to look at some candidate reentry schemes.

I call them schemes, because you'll see they really are schemes. But they're -- they try to be based on other reentry schemes that the FDA's put out that are based on initial and 1-year testing. And I've proposed let's use the 1-year and -- initial and 1-year IFA and PCR results. We had them in the natural history study, why not use them, and therefore the algorithm looks somewhat similar to other TTD reentry schemes. We applied them to donors in the natural history study, we did allow some flexibility in the timing of samples, zero time was up to 3 months, 12 months was any time from 11 months to 13 months and so on, because we couldn't have gotten everybody to fit in retrospectively.

And then when we found people who were assessed
for reentry, we then followed up after the reentry decision for whether those people who were reentered had a subsequent PCR test. If people who would’ve been reentered -- we didn’t actually reenter anybody, but if people who would’ve been reentered by the scheme then came back on a subsequent visit and were PCR positive, we called that a reentry failure. That would be someone that in real time you would’ve reentered in the donor pool, they would’ve come back, donated and either tested positive or infected somebody; bad outcomes.

Now, the important thing is that this is such a long process, that this may well represent reinfection rather than persistent infection, but as you start thinking about reentry for Babesia and you think, well, these people live in endemic areas, it almost doesn't matter if these people are going to get infected again, why bother to reenter them and it really -- do we really care whether they -- the infection's gone and the tick got them again, or do we care -- do we just care that they stay, you know, tick infection negative. I think, the latter, so -- although you -- we don’t know from the study whether that’s reinfection or persistent infection, to me it didn’t matter
in thinking this through.

Well, okay, a little bit -- this is a little bit arcane, but I gave the four schemes some numbers. The initial IFA was all greater than 1 to 164, that’s our cut-off for entry into the natural history study. The initial PCR was either negative, or positive, or not available. Some people didn’t give a PCR within the 3-month requirement, so they were not available. The IFA at 1 year had to be for the reentry for all schemes less than or equal to 128. That’s not negative, that’s less than or equal to 128. If it was negative, there weren’t going to be many donors reentered, not very many.

PCR at 1 year could -- would -- was also negative in all these schemes, and the difference was that in one case we required all PCRs to be negative, and then in the a case and then the b case, we would take any result on intermittent PCRs, but the 1-year PCR had to be negative. I know that’s a little confusing, but that’s seen to be the way the data wanted to be analyzed. I have to admit we kind of -- we were kind of -- cook this up after we looked at the data.

Looking at this, we had 118 -- 116 or 139 people
originally eligible for reentry. In other words, their initial sample was sufficient for reentry. And of these 55 and 76 were followed for 1 year. Obviously, if they weren't followed for 1 year, we can't make any assessment of them. Unfortunately, that also includes those donors David told you about, who quickly were removed from the study, because they came -- became seronegative. Since we had no follow-up on them I didn’t include them, they might well be able to be reentered, but I have no way of telling what would happen when they came back, so I did not include them. So we’re under-estimating the success rate of reentry, because those donors are not included.

As to the number of those who were followed for a year, that were re-enterable by the criteria -- by the four criteria, you can see that all four approaches gave you pretty good efficiency. In other words, the rate of reentry was high enough, that blood banks would bother to do this, that it would be worth our time and so on. However, no matter what the scheme -- no matter what scheme we chose, two or three of these reentered donors came down with a positive PCR subsequently and were failures. So that didn’t look too good to me.
We looked at some alternative entry schemes like requiring all intermediate IFA titers to be less than 1 to 128, what -- whereas that worked well for getting these donors reentered, all 43 of these donors could be reentered in one year, still two of them would’ve been reentry failures, were among the three reentry failures. And if you said, well, we’re going to require intermediate bleeds to be negative, then you’re obligated in operational reentry to bleed the donors more than once, which I don’t think is -- we wanted to propose.

So the other option was well, let’s just wait a longer time after the reentry before we assess its effectiveness, or reenter them after 2 years of negative -- you know, of qualifying results. The problem is there’s not enough follow-up in the natural history study to answer this question. So our plan is to call back people who’ve left the natural history study, ask them to give annual follow-up samples for a couple of 3 years and gather some longer term data that might allow a 2-year wait for reentry, because a 1-year wait seems problematic.

So reentry conclusions is it's feasible. We could reenter a significant number of donors, but there was
a small, but we thought unacceptable failure rate, at the present. Sampling beyond a year may be required to develop an acceptable scheme. The possibility of reinfection in a high-risk population over a couple of years may make meaningful reentry impossible. But if we had the reentry scheme, it would be useful for donor management not only in our study, but if Babesia screening was implemented, and it might also over time be a -- be shown to be useful for patients with clinical babesiosis or -- or implicated as involved in TTD infections. I wanted to acknowledge all those folks who have worked on the natural history project and thank you very much.

(Applause)

MR. MIED: Ritch, thank you very much. We'd like to have Laurence Corash from Cerus Corporation come and talk to us about inactivation of protozoan pathogens in labile blood components. Larry.

MR. CORASH: Thank you for the opportunity to discuss this topic. I am an employee of Cerus Corporation, and we are developing and have commercialized in some geographic areas products for pathogen inactivation. I'm also a faculty member of the Department
of Laboratory Medicine in San Francisco, where I've actually seen the case of Babesia some years ago on a blood smear of one of our patients.

Actually, I should tell you one of my initial scientific exposures to Babesia was in the early 1990s when a representative of the Australian Meat Board came to see me about using our inactivation technology to develop a vaccine to inoculate cattle in Australia, because of microti in the cattle population.

And vaccine had to be incredibly cheap and you had to be able to shot it into the cow from the air, from a helicopter. The alternative to doing this was that they would build the fence across Australia to keep the infected cattle out of the uninfected cattle. I don't know what they did, but that’s the magnitude of the problem in Australia.

These are topics that I like to cover with you. The objective of this technology as we've developed is to inactivate infectious pathogen including protozoans and leucocytes. And we use a targeted process towards nucleic acid, and it’s a photochemical process for platelets and plasma. But, as I’ll discuss in a movement, for red cells
we don't use photochemistry, it's a thermal process.

The aim, of course, is to prevent transfusion-transmitted diseases. We think this also can improve the availability of blood components and safety, and play a role on reducing donor restrictions.

We have some data already that we can improve clinical outcomes from other aspects of this technology and the technology, of course, does have to be operationally feasible and cost efficient.

We started working with psoralen compounds for platelets and plasma, because we had a long research history in psoralen photochemistry, and psoralen compounds aren't nucleic acid targeted. We've synthesized a very large number of compounds. The compound which has been commercialized is called S-59, but now goes by the official name of amotosalen.

It has been optimized for its inactivation efficiency, and also for its safety profile, and preservation of function of platelet and plasma. It has two reactive ends, and goes into viruses, and bacteria, and protozoan, and across cell membranes very efficiently.

S-303 is the compound that we use for red blood
cells. It also has a -- an acridine ring on somewhat similar to the psoralen structure which helps target it to nucleic acid. And then it has an alkylator function which inactivates nucleic acids. But different from the psoralen compound it has a central ester bond called the frangible, so that this compound breaks down completely, and that’s one of the ways in which one gets safety, so you don't transfuse any of this compound.

Mechanism of action is that both of these compounds intercalate into helical regions of DNA or RNA. Fortunately, blood components of course don't require nucleic acids or replication of their nucleic acids for their therapeutic function.

For the psoralens in the absence of light there is intercalation but no covalent chemistry. When one illuminates with ultraviolet light then one gets permanent cross linking or covalent monoadducts, both of which are effective in blocking replication, and thus in blocking infection.

Now, the platelet and the plasma systems have been commercialized in European and other geographic regions of the world. They consist of a series of
functionally closed containers into which the blood compound, the platelet, or the plasma can be introduced through a small red pouch which contains the amotosalen. The cassette -- the three bag cassette then goes into an illumination device. It’s a bench top device, and in a 3 to 4 minute process the photochemistry is completed.

In both of these systems we use a compound absorption device to remove the residual compound and three photo products. And then the treated platelets or plasma go into traditional storage containers, plastic storage containers for storage of platelets for 5 to 7 days depending upon the regional regulations, and for plasma into the freezer for 1 to 2 years.

One of the aspects of developing this process was to work in plastics containers, and to use technical procedures such that they would be easily adoptable into blood bank component rooms, and that the technology would be relatively transparent to the technologist. And there are a series of control processes along the way, in terms of barcodes and now a UVA indicator label similar to the Radsure label. People are probably familiar with the document, Illumination of the Product.
The red cell system, as I said before, is not a photochemical system. It uses a thermal reaction which takes place at room temperature. The compound S-303 and a quencher, glutathione, is added simultaneously, or now we are adding the glutathione first and the S-303 secondly to a unit of red cells in an additive solution and then the container is mixed and stored at room temperature for a period of time during which pathogen inactivation takes place, and one gets complete decomposition of a compound.

Pathogen inactivation is completed in 2 to 3 hours. We hold the unit at room temperature for another 10 to 12 hours to ensure complete break down of the compound. We have used this process within an indwelling compound absorption device in the final storage container; although, now we have a system that no longer requires a compound absorption device.

In previous some studies which have been extensively published, and I won't focus on all of these studies today, we've shown that for the platelet and plasma photochemical treatment process that all of the blood-borne viruses that are tested for today are very well inactivated by this technology.
We've looked at a number of the emerging pathogens that you see here indicated in red. And I've had some very interesting experiences recently in a region of the South Indian Ocean where the technology has been used actually make platelet components available during an epidemic of Chikungunya virus. And this technology is also now being used in the Caribbean where dengue and T. cruzi are endemic in some regions.

It's effective against a spectrum of non-enveloped viruses. It is not effective against the hepatitis A virus, because that has an extremely tight capsid. It's effective against gram-negative and gram-positive bacteria. Not against spores, but spore formers that go into the vegetative phase are sensitive.

And the data that I'm going to dwell on today are the protozoan inactivation studies which we have done to show that it is effective against these pathogens. And I should add that these studies have been done with both cell-associated, cell-free, and for retroviruses where you have host genomic integrated retroviral sequences. It's also very effective against leukocytes, and I'll make a few brief comments about that later.
So for inactivation studies looking at the Babesia and Plasmodia in platelet and plasma components, infected red cells were added into the component and then these components were treated with a 150 micromolar amotosalen, in 3 joules per centimeter square of ultraviolet light. That’s the standard in the commercial treatment process. These assays are all done as infectivity assays for the microti, it's a murine infectivity model and for falciparum. It's an in vitro culture model.

The level of inactivation that one can detect depends, of course, upon the titer of the input pathogen that you have available to you, and then the limit of detection of your assay. One can expand the limits of detection by culturing or testing large volumes. There are obviously feasibility limits in some experiments as to the volumes that you can test. But where you see a greater sign, and that means that we've gone beyond the limit of detection, and where it's greater than equal than the experiment that has been carried out to the limit of detection in the assay system. And for both Babesia in platelets and plasma and plasmodia, falciparum in
platelets and plasma the levels of inactivation are quiet robust.

The same is true with the Babesia and Plasmodia and red blood cell concentrates now using the S-303 process. These are full unit experiments with red cell concentrates with red cell additive solution prepared under blood bank conditions at hematocrits of 55 to 60 percent. And inactivation again is quiet robust. These studies are all published, and you have the full references in your handout so you can look at the detailed methodology.

For Trypanosoma, we've studied T. cruzi, because that’s an important pathogen in many parts of the world. And Leishmania, because it's endemic in some places in Europe, and also obviously in some parts of the Middle East.

And T. cruzi is also highly insensitive using animal infectivity models and also in vitro culture systems in both platelets and plasma. And we've studies Leishmania in platelets components, but have not yet done studies in plasma.

Likewise, in red blood cell components T. cruzi
is highly sensitive to inactivation. We have not done studies with Leishmania yet in red cell blood components, but those studies will be done this coming year.

Now, the processes for platelets and plasma have actually gone through the European CE mark registration process where they've been registered as Class III drug device combinations, and the labeling on these products are that they can be used under conditions that are not clinically different from the conventional components.

There are no patient population exclusions based on the clinical trials that were conducted, and in regions where 7-day platelet storage is allowed with either bacterial detection or with a pathogen inactivation procedure, they are being used for storage after 7 days.

They've also undergone secondary regulatory approvals in France by French medicinal agency, AFSSAPS, for platelets and plasma. And for platelets in Germany by the Paul Ehrlich Institute which regulates the blood centers for biologic products there.

In addition, the Central Blood Bank of Kuwait which has implemented the technology has received now variances from AABB under Rule 5151 that this technology
meets the requirements in terms of limiting or dealing with bacterial contamination of platelet components.

There has been substantial experience in routine use now in Europe, and I should also say in the Caribbean, and all the way into the Indian Ocean on the island of La Réunion for platelet components, more than hundred thousand doses have been transfused in multiple centers in these countries.

Interestingly, I think it applies to some of things that we're considering today. This technology has been used to replace bacterial detection methodology and now of course under the AABB standard that accreditation has also been granted.

It's replaced gamma irradiation in all of these centers prevention of transfusing-associated graft-versus-host disease, because it's actually more efficient than gamma irradiation for inactivation of T-cells. And perhaps somewhat analogues to the situation for Babesia where you have a rather prevalent pathogen, and you may want to think about using it selectively for certain patient populations. It's actually replaced CMV serology in these European countries for patients at risk for CMV
infection.

A number of post-marketing hemovigilance studies, which are active hemovigilance studies have been conducted, and those results have been published. I'm not going to discuss those today, but including the study in the island of La Réunion during the epidemic showing that the technology at least protected recipient patient from transfusion-transmitted Chikungunya virus infections, which is a very strongly platelet-associated virus.

So in summary, this technology which is a photochemical technology, and also the red cell technology does inactivate a broad spectrum of pathogens and leukocytes. It inactivates protozoans efficiently. We have large experience for platelets and plasma, and including routine use in regions with endemic protozoan infections, particularly I think for T. cruzi and for Leishmania. And the experience thus far has shown us an acceptable safety profile that includes a reduction in acute transfusion reactions due to very complete inhibition of cytokine synthesis by residual leukocytes and also substitution of 65 percent of the plasma and platelet components with the platelet additive solution.
Thank you for your attention.

(Applause)

SPEAKER: Thank you --

MR. MIED: Thank you, Larry.

The last talking in this session will be by Dr. Raymond Goodrich from CaridianBCT Biotechnologies. He'll talk about the potential for pathogen reduction technologies to address blood safety concerns due to existing or emerging parasitic agent.

MR. GOODRICH: Okay. I'd like to thank the organizers. Dr. Kumar, for the invitation to present here today. In terms of a disclaimer, I'm an employee of CaridianBCT Biotechnologies, formerly known as Navigant Biotechnologies. They did ask me about the name change, only unfortunate it was after they decided already. But I think perhaps one of the reasons for making the change is that this clearly rolls off the tongue much more rapidly than saying Navigant.

I wanted to start off with by talking about what some of the issues are associated with each the approaches that you've heard a little bit about today regarding the ways to interdict, or prevent transfusion of some of these
agents, and in particular today the topic being for Babesia.

This is actually a slide that was put together and presented at a conference earlier this year, I believe it was, that I thought really summarize what some of the issues that are associated with this. I asked permission of the individual who did not want his name on this to use this slide, because I did think that it was a very good discussion of both the pros and cons associated with each of the types of approaches that have been used or are being used for the prevention of disease transmission by transfusion products.

Clearly donor exclusion has advantages in terms of its -- the rapid rate at which it may be implemented when and if a problem is identified. It may deal with unknown or unexpected agents sort of in a indirect way, by addressing the use of certain questions or exclusion criteria you may prevent the transmission of agents unexpectedly, because I think it was described in some cases earlier by some of examinations of different types of parasites, but in the same vein addressing Babesia transmissions.
Limitations, of course, for donors with unknown risk, the definition of the risk group, I think you’ve heard all of these today, reliability of the application, the availability of donors. Testing clearly has advantages if they can be sensitive and specific, reliable quarantine measures can be put in place as a result of the results from these tests.

The limitations of course is that the test has to be able to detect the agent that you’re looking for. You have to know what that agent is. You have to have some idea about its characteristic. And clearly there concerns about limits of detection.

Pathogen inactivation or pathogen reduction technologies or other removal technologies also have potential advantages. They can be effective against many unknown or unexpected pathogens. They can inactive many bacteria and protozoa, things that you might not screen for today, and they can reliable in implementation. The limitations however are present. I don't know of any technology that does not have downsides to it. And this technology also has downsides to it. They include the fact that there is damage that occurs to these products as
the result of doing these treatments or manipulations.

There may be resistant pathogens such as non-envelope viruses, certain types of non-envelope viruses that resist inactivation. And then clearly there has to be the question if we have the issues on the detection side for the lower limits at which we are able to detect these agents on the inactivation or pathogen reduction side we have issues about whether or not the technology has a capacity to deal with very highly viraemic or highly parasitemic donations.

So I think there is a balance here of the risk and benefit that comes with this technology with pathogen reduction technologies in general. We can all discuss and identify, I think intuitively what some of possible benefits are to deals with issues of pathogen risk bacteria, viruses, parasites, again a very a broad spectrum of agents, potentially to reduce the risk that are associated with donor white cells in blood.

But there are also concerns that have to be dealt with. And those include toxicology. We're adding agents to blood that may not be normally there, or that may not be there in the concentrations that we're adding
to blood. We're doing things, manipulations to these products.

There is the issue, as I mentioned, of cell or protein quality reduction, might include issues about quality of the products during storage over extended storage times, and the transfusion requirements that might result. Clearly, if we reduce the effectiveness of these agents we have to increase the amount that we might use, and that's not something obviously that's desired, and of course there is the cost.

It's not only the financial cost, or what the technology costs, but it's the logistical cost. How practical is it to be able to do this. I think today, and again this is my opinion, that we have a hypothetical benefit of disease prevention with these technologies that can't be easily or readily proven versus a clear measure of change in product quality.

Now, tomorrow where might we be? And if I didn't believe this I wouldn't be here today talking about it. I think tomorrow we can have a clear benefit of improved blood product quality as measured with improvements in multiple patient outcomes.
That issue, of course, is proving the benefit is very hard to do. It’s very expensive, and they require large sample sizes, large clinical trials. That’s more of our problems than it is of yours.

Why bother? This question I ask myself. I think I part that it's because -- and this really paraphrasing something by Henry David Thoreau, "This is a technology that can strike at the roots."

Now is a phraseology, if forever -- for every solution that flails at the leaves of the problem there is often one that strikes at the roots. And I believe that pathogen reduction technologies can be one that strikes at the root of this fundamental problem.

We have been developing a technology called Mirasol Pathogen Reduction Technology. These are some -- I used to call these designed goals. I think in some ways I'd call these design achievements now, because the products is approved now for use under a CE mark in Europe for platelets and most recently for plasma.

We've tried, of course, to make something which is safe, effective, which maintains the clinical efficacy of the components. It’s in an open platform system that
is suited to treat various components, whether manually or apheresis collected. Simply process, it's easy to use, and implement, and something that would be effective for all three components in one technology platform.

We base this on the use of riboflavin or vitamin B2. I was actually very intrigued by some of the literature that I saw on this compound, and its ability to be able to interact with nucleic acids that carry out photochemistry with nucleic acids. And what intrigued me the most about it was the also the history that’s known about in terms of its existences in blood, in the human body, even its photochemistry has been very well studied in many ways.

And so one of the things that we felt gave an advantage to using this particular approach in using riboflavin in combination with UV light was that it was a good foundation upon which to be building a blood safety platform technology. The technology in terms of the process, we call this the generation one process. Basically, platelets are collected. They are transferred to a container which is a standard ELP bag container. If you're familiar with the -- I want to say CODE Gambro
Trima, the CaridianBCT Trima apheresis devices.

We connect a 35 ml solution of 500 micromolar riboflavin to that container and transfer the contents. This is riboflavin in a 0.9 percent saline solution that’s exposed to light for 6 to 10 minutes, UV light, and then it's ready to go. Right now in Europe it's validated for five days of storage in plasma.

The source product can be single donor platelets or buffy coat platelets. These are some of the specifications associated with the use of the products. We validated it at fairly high white cell contamination levels as well as fairly high red cell contaminations levels.

The treatment windows that you see here are based primarily on the growth kinetics of bacteria in these products. We looked at rapidly growing bacteria such as E. coil, how rapidly did they grow up to titers at which issues of pyrogenecity become the problem.

It's possible, for example, to inactivate products at that end of storage or during longer terms than what we have identified here in terms of this treatment window. But at that point the bacteria has
probably already grown to a level, if it's a pyrogen-containing organism, where that's going to cause a problem in transfusion aside from whether or not there is active bacteria that are growing or replicating.

For the plasma process, very similar, basically the set is changed just to contain one additional storage bag connection here. That storage bag connection is done so that you have a container that’s suitable for freezing and long-term storage. And again, these are the specifications which you can read in the handout regarding the use of the product. It's validated for both whole blood single units, as well as for apheresis-derived plasma and storage conditions as described here.

In terms of effectiveness, and again, I won't dwell on these since it wasn't the topic today, but there are a lot of articles which we have published in peer review journals. I am happy to make those available in much greater detail. Lot of this work was actually supported through an appropriate congressional appropriation managed through the Department of Defense for the development of the whole blood treatment system, which I am going to talk about little bit shortly here.
But again, a broad spectrum of effectiveness against virus, enveloped, non-enveloped, intracellular, extracellular bacteria species, parasites as well. And I’ll talk a little bit about -- more about those, as well as inactivation of white blood cells. And again all of this is published, literature and full manuscripts describing white zone activation, cytokine production, in addition graft-versus-host disease done in xenotransplant model, as well as some studies that were done with Wink Baldwin's group at Hopkins looking at alloimmunization and transplant rejection in an animal model.

We have evaluated a number of parasites, again spiked into different components. Studies with leishmania were done with Lisa Carter's group at Walter Reed Army Institute of Research. The malaria work done with red cells here was actually done with Loyd Lippers' group at Walter Reed Army Institute of Research. Again, all of these are published. I'd be happy to provide copies of the manuscripts and reprint. We've also just recently completed, it's only an abstract form, the same work with Jim Sullivan's group at Center of Disease Control in Atlanta for the platelet and plasma products with malaria.
I believe we were able to boost the detection level to claim greater than five logged inactivation with that. The Chagas studies were done at Lisa Carter's group. Babesiosis is actually work that was done with David Leiby's group at the American Red Cross here at Holland Labs. We are gearing up to now repeat those studies with the whole blood product, the whole blood processes as well. The Orientia work was done by Frank Rentas at Walter Reed Army Institute of Research with all three components. And again that is reported as well.

Just to give you an idea of the development timeline with this, we did -- started with this project back in '98, '99, prototype design and development. There was a trial which was done in South Africa. This was a dose range finding study, dosing of the UV light on the product. That is published. There was a design verification study done at two sites in the U.S. under an IDE. Jim Obichun (phonetic) was the principal investigator of that study. That is published. And we are in the process of wrapping up publication of this data which comes from a study that was done in France, at six sites actually, and finished at the end of last year.
That led to the CE marking of the product in October 2007. And since that time we have been commercializing the process in Europe. The plasma product has gone through a similar development cycle. We received a CE mark on that product in August of 2008. And it will go into limited release here shortly. And we are currently working, as I mentioned, on a red cell/whole blood project which I'll describe in a little bit more detail here.

Just to give you a bit of a flavor of some of the results from the trial with platelets that was done in France, again there were actually six sites, five sites doing apheresis collections, one site doing buffy coat. Two of the sites eliminated gamma radiation in routine. The Mirasol and the reference platelets were both stored in a 100 percent plasma. This process can be done with additive solutions, in fact we're working on validating that for release in Europe. But it doesn't require the use of additive solution. It works just as well in a 100 percent plasma. So there were no additive solutions in the study. Initial volume, murine concentration et cetera in the storage for these products was up to 5 days.
We had an independent data safety monitoring board independent from the company and the investigators who reviewed all of the data in terms of safety. This is the conclusion which was -- which appeared -- it's taken as a quote directly from the final report in the study. Just some of the general conclusions, no adverse events associated with the use of the device, no evidence of new antigen formation, photo product accumulation in subjects.

We don't remove the riboflavin or the reactants after the process. They are all naturally occurring agents that are already present in blood. So those are infused directly. It's immediately available for use after the treatment.

Elimination of gamma radiation, at least in several of these sites was possible without any indication of problems associated with that. We saw a 20 percent -- 24 percent delta in the CCIs at 1 hour, that reduced to a 14 percent delta at 24 hours. Why we call that an interesting trend. All of that delta was associated with the first two transfusions. For transfusions three onward, which on an average patients received about 45 transfusion on average, those transfusions were identical,
if not higher for the Mirasol platelets.

And again, no evidence of increased use of platelets in the Mirasol group, no evidence of increased red cell used. And the platelet yield from collection to transfusion was virtually identical which you would expect, because all of the treatment and storage is done in the original container that the product is collected in.

The full details on this will be presented at this year's AABB meeting. I would like to mention briefly some of the work we are doing with whole blood, as I mentioned under sponsorship from DOD support. The Mirasol system for whole blood uses the same illuminator as the system for platelets and plasma with minor software modifications to allow dosing of the red cell component appropriately. The storage set is equivalent to the set of plasma with that additional storage bag. So we're actually treating whole blood, you had the same 35 mils of riboflavin, treat the product. It's about a 20 to 30 minute process right now. And then the product is ready for separation into components.

The riboflavin solution content volume are
identical to that that’s used in the system for platelets and plasma. In 2008, I mentioned here when these slides I submitted was initiated, test for the recovery and survival of the RBCs from Mirasol treated whole blood. The IDE was actually approved by FDA 2007 for feasibility trial. The way the trial is setup, we're treating the whole blood, separating it into platelets, plasma and red cells.

We're storing the red cells for 42 days in AS-3. At the end of that an alloquat is radiolabeled, re-infused into the autologous donor, and we measure recovery and survival. Platelets and red cells, the PRP and the plasma component are monitored in vitro for 5 days. We are also -- these products are not leukoreduced, so we're using white cell inactivation as a measure of the efficacy of the procedure which is measured separately as part of the protocol.

Because it's a DOD protocol, it has to go through human use approval. It did that. Since it goes through an IRB approval locally, at Hoxworth Blood Center, which is where the initial study is being done. We expect the secondary approvals to be obtained prior to the final
HSRRB approval from DOD. We actually obtained those, and actually yesterday we enrolled our first subject in this trial. So we're hopeful to be able to report the results from this clearly within the next several months. Thank you.

(Applause)

MR. MIED: Thank you, Raymond. I'd like to ask the speakers to join me for the panel discussion.

I think we're doing pretty well time wise. I'd like to start off with a question similar to number 1 up on the screen. Ritch, this would be for you.

We heard David talk about testing of donors in blood drives. Barbara mentioned some testing that's going on in New York and in Rhode Island. What I like to get clear on is, in the Red Cross system or maybe in the non-Red Cross system, how much donor screening for antibody is actually going on, on a routine basis?

MR. CABLE: None. It's all a research study, and we're testing about 5,000 donors a year in Connecticut --

MR. MIED: 5000.

MR. CABLE: -- and Massachusetts. That's it.
MR. MIED:  But you're using those results to defer donors?

MR. CABLE:  Yes, yes, as a part of these research study and IRB approvals and consensus and so on they are told if they are positive they will be deferred and they have done.

MR. MIED:  Right.

MR. CABLE:  We are now wondering whether we can modify that, but the re-entry study doesn't support that. So we aren't planning any donor re-entry today.

MR. MIED:  Right. Yeah, I think we also heard that it's difficult to define when someone has really cleared the infection. We can define with certainty whether or not they are cured. It leads me to ask can we really -- any donor who previously has had an IFA titer be safely re-entered? What do you think the prospects are for future re-entry schemes?

MR. CABLE:  I think that the FDA has been very conservative, and so I wouldn't expect that until there is a better test than IFA to support it, and data. You know, I -- as I said, we did this, because we felt some pressure from the research subjects themselves that they didn't
after -- even though they consented to it, that now they are having biased remorse, and wish to be blood donors again.

And we were encouraged to look at the data. We did. This is our conclusion. We are doing longer term testing now, so that they are going to be asked to come back annually. And when we get that data in 2 or 3 years, we'll look at it again. By then there might be other, you know, other answers available.

MR. MIED: Yes. Celsa.

MR. BIANQUA: Yes, Celsa Bianqua, America's Blood Centers. We heard in the previous session how difficult it is to determine sensitivity, specificity of those tests. And I think the problem that Ritch has is not exactly with the positives to be re-enter. It is with the negatives. Could you call them Babesia safe donors because they tested negative? And you can't. We don't know that. And those are what I would like to hear from Ritch and from the group. It's what kind of studies are required to really re-assure that we know what is length of the persistence of antibody, even in animal models. What is the length of persistence of DNA, and how we are
going to make sure that one of them is feared, because if not, I prefer to use pathogen in activation.

(Laughter)

MR. CABLE: Well, our study wasn't designed to, nor are we claiming blood safety. So I think -- well, I don't have to answer your question, because they are filing for an IND, an IDE or whatever. And I guess the FDA will ask him those questions, and I am interested in their answer. I have no opinion on that. It's above my pay grade.

MR. MIED: Yes. Matt.

MR. KINARD: Matt Kinard, CDC. I wondered since question one here, it says, the deferral question is have you ever had the babesiosis? Has that question ever been validated? In other words do we know how well that question works as far as correctly identifying people who have the babesiosis, either, you know, using laboratory testing or any other method?

MR. CABLE: I know how it came about. Mark Boboski reported the first case. And this showed up in an AABB newsletter. That's how it started. So no, it wasn't validated, not that I'm aware. I would think that if a
person said they had babesiosis you would assess their
general credibility and competence, medical competence,
and then you would have to take them at their word. Why
would someone make that up?

    MR. KINARD: No, no. What I was asking was, is
-- you know, there are a lot of factors where that
question could be misunderstood. It could be
mispronounced. The person might not understand what they
had, what disease they had, and doesn't even, you know,
know what it is. They know they had a tick thing, but
that may be all they know, or all they were told. So I
was just wondering, we sort of hold this up as this is our
babesiosis safety measure is this question. I just
wondered if it's ever been evaluated.

    MS. CARR-GREER: Paul, could I address? This is
Allene with AABB. This question was on a part of the
questionnaire when it did go through a validation study.
But what we didn't have in the groups -- this was -- the
validations were done by NCHS. We, to our knowledge there
was no one in the study group that actually had babesiosis
had had. But so it would depend, I guess a lot on if a
donor was aware that they had the babesiosis.
MR. MIED: Thanks Allene. Presumably we have had that yes answers.

MR. SPENCER: In the -- Brian Spencer, Red Cross. In the New England region where we have 400,000 collections a year, the numbers of those self reporting history of a babesiosis are very small. I don't know exactly, but it's one or two dozen or fewer per year.

MR. CABLE: Red Cross can certainly give you aggregate data to those, yes, but it's very, very, very small as you'd expect.

SPEAKER: All right.

SPEAKER: Such -- in terms or validating infectivity of a seroreverter, I am just -- this is a naive question to the real experts. Are there any large animal models that you could infect with B.microti to which you could put a whole unit, because that's one way of getting at it. And then the concept that, because it might take several hundred hamsters, you can't do it, it’s disapproved, because in various vCJD experiments, things of that nature precisely have been done, even with interest of rubella inoculation. So we didn’t rule out the possibility of an in vivo infectivity determination.
Obviously it would be easier in the very small number of large animals, but it's not impossible at a large number of small animals.

MR. MIED: Maybe Sam has a --

MR. TELFORD: Yeah, certainly the large animals will be primate, non-human primate. But you wouldn't want to go there. That's very expensive. Not a cut down -- hamsters are pretty unwieldy. I’ve taken these in SCID mice. But those are pretty expensive.

SPEAKER: If I could just follow up. You know, in the early days of HIV we used chimps. For a very long time for HPV we also used chimps. For other animal models where it's been feasible, smaller non-human primates Marmosets et cetera had been useful.

So again, I think we need to take a more critical look. If it's an important question, whether we can re-enter seroverted donor, then I think that an animal model in vivo infectivity experiment becomes very important. And you know exactly what resources it would be require, and how we mobilize them. Of course you have to sort out. But I don't think we should start out by ruling it out if in fact they are potential animal model.
MR. KUMAR: So that's the question. I mean then what Dr. Epstein is asking, has anybody even tried Aotus monkeys, or (inaudible) monkeys, or squirrel monkeys? Doesn't seem like.

MR. TELFORD: The studies that were done previously were on Rhesus.

MR. KUMAR: Those are (off mike).

MR. TELFORD: Yes. But I would imagine any monkey will do.

(Laughter)

MR. MIED: (Off mike.)

MR. NAKHASI: Yes, I think --I just want to go -- I have a couple of questions. One is going back to this validation question of the babesiosis. Ritch, in your donor testing, obviously you are asking them the question, have you had babesiosis? And if they said, yes, then you will test them. If they said, no, do you test them still? How do you validate them?

MR. CABLE: No, no. These are blood donors who routinely donate, and they wouldn't have been taken had they answered yes to the question. So these people all said no. They had no babesiosis. We select tubes in a
kind of a random fashion, sequester the units, because they are quarantined until testing is completed, do the testing and notify positives of their status and deferral and recruit them into the natural history study.

MR. NAKHASI: I see.

MR. CABLE: We don't--

MR. NAKHASI: Reselect

MR. CABLE: We don’t -- I mean, they have had to have answered no to the babesiosis to get it -- to be a blood donor. And they have to be a blood donor to be selected for -- randomly for this study. We put the informed consent documents on every blood drive. But as I said, we test about 5,000 out of 150. Well -- yeah, something like 5,000 of 150,000 in an effort to find research objectives, not for any other reasons.

MR. NAKHASI: I have another question for the other two gentlemen, especially for Dr. Corash. In your--those samples when you--treatment with these you know, agents, did you show any--you said there were 100,000 platelets which were used. And was there any adverse events, or any adverse resections to that reported? What was the safety profile, in other words?
MR. CORASH: Yeah, so safety has been evaluated in a randomized control clinical trials, but the experience that I referred to there was in the post marketing experience. We have conducted an active hemovigilance program, in which the people who participate fill out a form for each transfusion, whether or not an adverse event has been reported. And we have had 30,000 transfusions that have been documented in that program.

We've not seen any unexpected adverse events that related to platelet transfusion. You do get reactions to platelet transfusions. The reaction rates that we have seen have been very low, in the range of 1 to 2 percent of transfusions that resulted in -- in all cases they've been minor reactions to these transfusions. So we think on that basis it has a very good safety profile in addition to what we done in clinical trials.

MR. MIED: Sanjai, you had a question?

MR. KUMAR: I have a question for Dr. Cable or maybe anyone in the audience who knows. How many donors are deferred each year for babesiosis risk?

MR. CABLE: Very small.

MR. KUMAR: Any idea of numbers?
MR. CABLE: I would think under 10th of a percent, probably 100th of a percent. Let’s say couple of dozen over 400,000, right.

SPEAKER: Very small.

MR. CABLE: That's in Massachusetts. Kansas ought to be lower, you know.

SPEAKER: (Off mike)

MR. CABLE: Yeah, we can get that figure for the national Red Cross system pretty quickly for you. But it's not -- you know, it’s just extremely low, really, really low.

MR. KUMAR: May I just ask one question (inaudible) Dr. Goodrich. Out of 30,000 transfusions how many positive units you have interdicted for any pathogenic bacteria?

MR. GOODRICH: I'm sorry. Out of 30,000 transfusions?

MR. KUMAR: How many units you have interdicted actually?

MR. GOODRICH: Oh, as of today I don’t have the exact count. We just introduced the product in the end of October. I would say that it's probably approaching just
several thousand.

MR. MIED: Dr. Goodrich, you didn't specifically say, but those riboflavin work the same way by intercalation and cross linking?

MR. GOODRICH: It doesn't work by an intercalation process. It works by an electron transfer process. But it does associate with DNA, RNA by virtue the hydrophobic portion or the planar portion of the molecule. And what it does in the body is it acts as an electron transfer agent. What we use it for is to act with as an electron transfer agent when it's excited by light of a specific wavelength.

And in that form, what it wants to do is act as a recipient from guanine (phonetic) basis, primarily, which is a good electron donor, and that leads to a reaction in the DNA or RNA that causes a change essentially in the nucleic acid, and prevents replication process -- processes from occurring.

MR. MIED: Yes, Lou.

MR. KATZ: Yeah, so it would seem like pathogen reduction might allow us to avoid a very difficult to imagine test for babesiosis for the operational reasons
that Ritch outlined. What do you guys think about the ability not to do some test we do already?

MR. CORASH: So -- you know, the practical experience that we’ve had is in Europe where it has replaced bacterial detection in countries that were requiring bacterial detection for platelet components. It's replaced CMV serology for patients who require CMV negative platelets or CMV safe platelets on top of leukoreduction. And it's replaced gamma irradiation for prevention of graft-versus-host disease in patients at risk for graft-versus-host disease.

So there is now an experience that is developing in the island of La Reunion during the epidemic of Chikungunya virus which has abated somewhat now. They were asymptomatic donors. That's now clearly known. And they only had available for part of that time a research test which had limitations in terms of sensitivity.

All of the recipients for these products in La Reunion have been monitored for transfusion-transmitted infection. And we have not seen any cases that involves, about to date about 2,000 transfusions in about 500 recipients.
MR. MIED: Steve?

MR. GOODRICH: I'm sorry. Sorry, I was just going to say I think our experience has been much more limited just because of the timing difference here with the product being available but very similar. I think bacterial screening obviously, gamma irradiations, CMV, these are the items that people consider in Europe at various locations to consider or actually drop.

I think it also changes the dynamic a little bit. And this goes back to something I heard Bernie Horowitz (phonetic) say, I think it was in this room, or a similar auditorium, years ago, talking about solvent detergent plasma. As a new agent emergence into blood supplier, our first question become if we have these techniques in place are we able to deal with them?

We can then figure out all the other issues about serology, about the nature of the agents, about whether perhaps even they are being transmitted by blood or not, which was mentioned earlier as one of the criteria, it has to be transmitted by blood to really be interest as a transfusion-transmitted disease. I think that having the technologies in place allow people to
enter a different dynamic relative to how they deal with these emerging agents. That's one of the potentials for them.

MR. MIED: Steve.

MR. KLEINMAN: Yeah, Steve Kleinman, AABB. Question for Larry and Ray. Do we know what happens to the red cells, I mean the particular red cells that are infected with a parasite like the Babesia or plasmodium when pathogen inactivation is used? I mean, does it lyse these -- I realize the DNA is inactivated, but do those red cells lyse? What happens to the quality of the unit?

MR. CORASH: Well, in -- the easiest way to look at that is in the serolin experiments. The red cells remain intact. So we seed Babesia infected red cells and Plasmodia infected red cells and we can tell that they don't lyse. In the unit of whole red blood cells the number of infected cells there seated into a whole unit of red cells would be very difficult to tell what's happening to them, because you see there are very low levels of hemolysis. So I don't think you could pick it up --

MR. KLEINMAN: Right, but you do have the experimental data to show that they don't lyse --
MR. CORASH: We have the data from the plasma and the -- from the plasma and the platelet experiments that you can find those red cells.

MR. GOODRICH: I think David actually did the experiments with platelets and plasma with our process. Maybe he could tell you directly what happens to those. I could just answer from the standpoint of work that we've done white cells and looking at inactivation of white cells.

What happens to the white cells is, again, as in this setting where these are not leukoreduced units. And we've looked at the phenotype, cytokine expression proliferation responses. One of the interesting things in whole blood, it appears that the cells stay about the same amount, to about 8 days, relative to untreated product. And after that you start seeing their proliferative response has dropped down in storage.

With the treated products, we see phenotypically they look the same. But they don't make cytokines during that initial 8-day period, or beyond. They don't fall apart. They don't -- they're not able to act as antigen-presenting cells. And they don't react in a mixed
lymphocyte reaction. So it is as if they are being killed from the inside out, in the sense that if you look at them phenotypically, they would be about the same. I would expect the same would occur with the parasites that are present inside the cells, but I can't say for certain.

MR. MIED: Hira.

MR. NAKHASI: Given what we heard from both you, and Dr. Corash, and Dr. Gaven (phonetic) that the safety, the intactness of the cells is there. How sure are we that these parasites? For example, for the case of in Babesia now here is completely inactivated. And how you guys have done an experiment where you take this blood samples and inject into animal models like Dr. Epstein was suggesting, and see, especially the SCID mice, you know, why they move so close to (inaudible) probability of seeing a small amount of parasite, because all it needs is one parasite to get it back in disease there. So have you guys done those kind of experiments?

MR. CORASH: Well, the experiments that I described are in infectivity experiments. So the treated samples are then inoculated into sensitive animals. And the animals are then followed for a period of time,
samples are taken, and then those samples are re-injected into sensitive animals, or you use in vitro culture assays.

We for Hepatitis B and C, we did a whole chimp experiments where you take an entire treated unit, you put into a chimpanzee, and then you follow that animal for 6 months. That scale of experiments has not been done for Babesia.

MR. GOODRICH: We've done similar experiments with Babesia, that's done in a murine model. Orientia was also done in an animal model. And of course, the white cells phase I mentioned, the xenotransplant model was done looking at microchimerism, graft-versus-host disease, (inaudible) graft-versus-host disease under those conditions if there were cells that were surviving, and in contrast with untreated products where you do see those effects.

MR. MIED: Peter you had a question?

MR. KRAUSE: Just my own curiosity. You do immediate safety studies with these products. And I assume you do longer term follow-up studies. Do you do that? And how long out do you look at folks when they
receive these products?

MR. CORASH: So for the -- we have some centers that have been using these products for 2, and now 4 years, where you have patients who've had longitudinal exposure. Long-term safety studies in patients, particularly because platelets are going into patients, a large percentage of that population have neoplastic diseases, and are receiving very complex chemotherapeutic agents, are extraordinarily difficult to do. And a registry has not yet been formally established.

So the long term safety is really based on appropriate animal models where you can repeatedly expose mice or other animals for long periods of time, and do validated carcinogenicity assays or look at many multiples of clinical human exposure through a substantial life portion of an animal. I think that as the technology is used in more and more patients or for longer period of time, the patient registry could be established. But I think, it's a difficult thing to do due to the nature of the primary diseases, and the fact that oncology patients are actually getting very complex mixtures of toxic and potentially carcinogenic medications on top of their
platelet transfusions.

MR. GOODRICH: I would concur. I think that those types of studies are very difficult to do. You know, I guess one thing as we’ve learned just initially getting into this, and looking at following the quality of the data that you get back in some of these cases varies dramatically.

And where you are not in, the reasons obviously why randomized perspective clinical trials are done, you have much higher power to be able to detect certain types of events. And in these kinds of studies that power drops off dramatically, because they are not randomized or not controlled in specific ways.

So you get a lot of data points, but they may not be as powered, let's say as if you -- yeah have perhaps a smaller study at a much higher power to -- specifically looking at those types of events. So perhaps there is a balance in there somewhere where you need a little bit of both.

MR. MIED: Sanjai, you had a question?

MR. KUMAR: Well, I just was coming back to the question that was asked, what happened to the red cell
infected Babesia plasmodium, whether they survive or not? So perhaps you may not see lyses, immediate lyses, but I would imagine that they are trial structural changes, so elaborate in those erythrocyte membranes, that they might be much more amiable to splenic clearance once they are injected in vivo. And that's an easy thing to test.

You can have high parasitemia cultures, you treat them with whatever you treat them with, then radiolabel them and look at the rate of radio -- splenic clearance in non-parasitized dead cells and parasitized dead and compare. My hunch is that probably they will not survive in vivo but we will get cleared by spleen.

MR. CORASH: I agree that they would most likely be cleared at an accelerated rate if they were parasitized and had a dead parasite in them. You would expect the particular endothelial system to act -- to clear those cells. Now, theoretically, in a platelet component or a plasma component and even in a red cell unit that should be a very small fraction of the total cell that's in that unit. I mean -- you know, we can gamma irradiate red cells and leukocytes and those cells are dead and cleared as well of course.
MR. KUMAR: I agree with you there. I mean, perhaps your paracetimias (phonetic) will not be that great. So I am talking about red cell units only here. So you can take that kind of losses. If 0.1 percent red cells are gone, probably that's okay, and then the pathogen is gone along with. I just wanted to make the point that probably those red cells will not have long-term survival.

MR. CORASH: I would agree with that. I think they shouldn't.

MR. MIED: Before we go to break I want to ask Ritch, I want to pursue the seven state strategy for a moment, or maybe be put it to bed. If we had a test, a test that worked well, high sensitivity, very high specificity, and had reasonably good throughput and we define, say the top seven endemic states from data from the public health labs or wherever. And if we were to test residents of those states, which I think you said would be about 16 percent of donors, as well as travelers, maybe within an overnight stay to those states, that’s another 10 percent of donors. So we're talking about 26 percent of donors. If we did that testing, do you think we would effectively
interdict most of Babesia contaminated units?

MR. CABLE: Yes.

(Laughter)

MR. MIED: All right. Why don't we go to break and be back at 4:00 o'clock for the last session?

(Recess)

MR. NAKHASI: All right, so I guess, sorry -- in the interest of time, I guess we should start -- get started. The purpose of this last session is really free-for-all discussion and a sort of brainstorming what we learned from this morning -- this whole day's session with Babesia with regard to all these aspects which we discussed and then what should -- at least, should be -- in scientific terms, what should be our future directions to go. So with that I would like to introduce my panelists here, Dr. Sanjai Kumar, Dr. David Leiby, Dr. Barbara Herwaldt, and then Dr. Robert Wise, Dr. Peter Krause, Dr. Sam Telford, and Dr. Ritch Cable.

So I think we -- you heard all of them in the respective presentations. So we have, I guess, set of questions here for -- just to get started here, and -- but then anybody can ask any questions. At least from my
understanding, this discussion this morning, we discussed quite a bit about the blood transfusion, epidemiology, what kind of tests are out there, are there tests -- is the -- are these tests good tests, can we, you know, for screening donors.

Also we heard about technologies which can inactivate these pathogens and how good those technologies are. We need to really validate those things. And also -- so therefore -- but we didn't hear, at least, my mind, we didn't hear -- there is another component which is very important, I guess, you know, Matt (phonetic) will attest to that, is there any transmission cases reported through organs, tissues and other cells which -- not the blood and so I would like to ask experts on the panel here, as well as out in the audience, people who have experiences, have they seen any things, or should we be worried about those things? Who wants to take it?

MR. TELFORD: I think given that the solid organs are perfused, the risk of blood contaminating the transplant would be minimal. I think in the case of bone marrow transplants there might actually be a hazard, except that those are going back into the same patient usually.
So that would leave the speculative exoerythrocytic or pre-
erythrocytic stage for something like B.microti, or those
that are more closely related to the tylerias, and there
you have lymphocytes which sustain the exoerythrocytic
stage, or with B.microti it could be a dermal stage at the
site of the tick bite, and those, at least the dermal
stages would not be a hazard at all, but lymphocytes, if
they carry a developmental stage could presumably be
hazardous, but then I'm hearing that white blood cells are
inactivated before -- or that they're leukoreduced.

MR. NAKHASI: Not (off mike.)

MR. TELFORD: They're not leukoreduced here?

MR. NAKHASI: Not always.

MR. TELFORD: Okay, so that's a possible -- but
that's not a -- that's not transplantation.

MR. KUMAR: But I think the concern is -- remains
that there would be lymphocyte contamination unless if you
bring very specific serotypes that did not concern with
organ donation, then the lymphocytes do always present
potential contamination problem, and depending on the
parasitemia, that risk is always there. I mean, we cannot
be 100 percent sure that, yeah, it's not possible.
MR. TELFORD: Yeah, well, you know epidemiologically there's no evidence, but it certainly there, in parasitemia might actually be a very simplistic way of looking at it because what you're seeing is what's circulating, but there may be sequestration clusters of extracellular parasites and we see this especially in the spleen.

MR. KUMAR: That's something we did not discuss in our sequestration of Babesia parasites.

MR. TELFORD: There's no evidence for adherence, but we certainly see aggregates of free parasites in the liver sinusoids as well as the spleen, but some of that might be washed out with perfusion.

MR. KRAUSE: I agree with Sam that there's certainly a theoretical risk of transmission through blood, through organ donation, and certainly the marrow would represent the greatest risk. We actually had a patient who had a marrow done, and PTR was done on both the marrow and blood and the signal was actually greater in marrow than it was in peripheral blood. The other thing I will say though is that in terms of adherence, and well, sequestration, that is blockage of vessels, I think, there it depends
again on the species of Babesia, because for a duncani this has really been shown to be the case.

That is, there is adherence, there is - there appears to be sequestration and actually pathology as a result of that. So I think duncani clearly is adhering. I find it hard to believe that Babesia would not adhere, but perhaps, I mean, there's not been good evidence for that. But bottom line is I think as everyone is saying there's a really a theoretical risk. And there hasn't been -- I'm not aware of any cases of transmission through organ donation, but I certainly think that it is a risk.

MS. HERWALDT: And just to clarify, I think all the duncani data are mouse data. Now, in terms of the organ transplantation issue, I agree with what everyone has said, and we've wrestled with this issue for other parasites as well and talked to people who are experts on transplantation. And just to reiterate what others have said, the flushing does not get rid of you know, every single red cell, so it's a theoretical risk.

And so we can't, you know, exclude the possibility and all we can say to date, which really means very little, is that the organ transplant recipients --
solid organ transplant recipients who have had recognized cases of babesiosis, it's been because of blood transfusion, but again we can't exclude it. And in terms of tissue, again it's going to depend on how the tissues are processed and then also, you know, the blood issue, and also this theoretical exoerythrocytic stage issue.

And as I mentioned in my talk and as Sam reiterated now, to date it hasn't been demonstrated. That doesn't mean it doesn't exist, and theoretically if it's demonstrated, then yeah, jump over to the tyleria genus, but that's all we can say at this point.

MR. NAKHASI: All right, before we go to the second question, I think I have reminded -- oh, sorry, I was reminded that there is a -- in your package, a survey sheet and so please fill up the survey sheet before you leave today, otherwise the gates will not open in the front door.

(Laughter)

MR. NAKHASI: Well, those who are here, they have to fill it anyway. All right, so. Okay, now going back to the business here, I -- the question everybody's mind is looming here is both on the blood establishments and the
manufacturers, everybody, what is the -- how much is the risk of transfusion, transmitting Babesia, what we heard here, and are there measures which are currently done at certain areas like Connecticut, and are they sufficient, or do we need to maintain -- do we need further something else? Ritch?

MR. CABLE: Well, I did want to point out that Rhode Island cited its data for the last 3 years, I believe, 1 in 12,000 red cells. That's in a donor population that hasn't had selective testing such as Connecticut. Over the years the two states have been roughly comparable, Connecticut a little bit ahead in the past in terms of numbers of clinical cases, right, I think, per population? That speak to the two states.

MR. KRAUSE: I would say comparable, but --

MR. CABLE: Comparable?

MR. KRAUSE: I will say that my -- there isn't, you know, super epidemiology. I would think that Massachusetts would actually surpass. I mean, Nantucket alone is just incredibly impressive in number of cases, but again, we don't really know. They're comparable, yeah.

MR. CABLE: Let's say comparable. So 1 in 12,000
actually reported cases to the Rhode Island Blood Center with a 10 to 1 reporting ratio, which is very common in public health, get it back to 1 in 1,200 which is what we've measured twice and what the estimate is.

So I think the risk of infection is on the order of 1 in a 1,000. I don't think there's any question about that. The risk for the patient; different question. I don't think we have a clue. I do wonder every so often that there might be extra hemolytic, you know, problems that because it is related to tyleria and tyleria have caused lymphoma in cattle and I'm going down another slippery slope here, but this genus is not without its nasty effects which were not mentioned today, but let me mention it now. And I go back to HTLV-1, when Dr. Sandler (phonetic) told me that when I said, well, you know, it may infect people but it doesn't cause a problem, well, you don't want any retroviruses in your blood, do you, Ritch, I mean that was his comment.

And I just have to say, I think passing on a pathogen at a 1 to a 1,000 rate despite its relative lack of penetrance is a little hard to justify and it is killing people and it's killing people in the state I live. So I'd
like to be passionate about it, and say, you bet you it's a problem and what's taking you so long, and let's not leave here today without doing something about it. That's my opinion, allow me to share it with you.

MR. NAKHASI: Yeah, I think I will just echo a little bit more. Before that I just want to take this question further, but I'll ask others to you know, continue the discussion. The -- so that's Connecticut. We heard as, you know, Paul asked you the same question. If you take the seven state, which is the -- you know, all the states you mentioned, it's 16 percent of the donor population, and the trial list is around 10 percent. Now what would you, you know, constitute whether testing, what type of testing, what should be the sensitivity, specificity, are you --

SPEAKER: If I were -- there are seven panelists, so each one of us could represent (off mike).

(Laughter)

MR. CABLE: Yeah, I think the problem is -- because we've had -- I mean, Barbara was at our session Red Cross had held a while back and you know, David mentioned discussion a while back. We didn't know as much then, so
testing was on the horizon. But the big problem is there's no test to consider, and there's no chimps to infect, because no one's spending any money on this, and they're not spending any money on it because no one's making a big deal of it. The only people that can make a big deal of it is either the blood centers who start to do testing whether you like it or not, or the FDA.

MS. HERWALDT: And we're here at NIH -- we have been --

MR. CABLE: And we're here at NIH. But if you told -- if you told the major vendors of devices that they're going to be getting 25 of us on the blood supply, I think it would get their attention, and I think they -- but they have to be sure that it was going to happen. Now I don't think this is going away, so you know, for -- what was it, for West Nile virus, you got up and said, you've got 9 months. Now, what's to keep you from getting up and saying you got 2 years? I mean, I don't understand why the FDA couldn't express some urgency that got corporate assets to develop a test and buy some chimps if you have to, whatever you have to buy to make it work, and then my suggestion is tell them the way it's going to happen is
you're going to do this, this and this, and this is how much you're going to sell, year one. You're also going to fund an IMD to do a sero prevalence study, because after all, if you're doing travelers, they're going to have to be tested, you know, testing sites and other places. So do some non-travelers, and then you know, maybe you'll get more depending on what we see and we'll track it like we did West Nile, which is to say, we'll deal with what we find. But until and unless someone gives that signal to real money people, us guys in this audience aren't going to come out and give Jay test he's going to happy with, I don't think. I mean, no way, we don't have the resources.

MR. NAKHASI: I think that's the question I keep on harping is if we need to really develop a risk versus benefit analysis here, to really say that -- you know, West Nile story is different, because it was happening -- it was spreading like a wildfire kind of a thing, whereas in Babesia, I think, you know, yes, it is seven states, it is a transmitted 1 in 1,000 that you were saying, so the question is, is it localized, is it -- how fast is it --

MR. CABLE: But Hira, you're the FDA of the whole United States, not just the other 43. You're the FDA of
Connecticut, the FDA of Rhode Island and damn it, excuse me, you have a responsibility. Now, we can help the FDA by calling our congressmen and helping them understand what's happening.

(Laughter)

MR. NAKHASI: I know you're very excited about it, but I can --

MR. CABLE: I mean, this is an attempt to take this -- don't talk to me of risk-benefit. There is a risk. It's killing people, right to the -- as we speak.

SPEAKER: That's what I'd like --

MR. CABLE: I know it's not West Nile, but it is killing people.

MS. HERWALDT: And what's the comparison in terms of any other infectious agent at this point that you're wrestling with?

MR. NAKHASI: No, I'm not wrestling. I think I'm responding.

MS. HERWALDT: Oh no, I don't mean this antagonistically. Compared with malaria, compared to --

MR. CABLE: It's killing a 1,000 times as many people.
MS. HERWALDT: Oh, absolutely, absolutely.

MR. CABLE: A 100 times as many people.

MS. HERWALDT: Absolutely.

MR. CABLE: One blood transfusion in the United States.

MR. KUMAR: But is it the same sense of -- this -- we hear from other blood banking establishment and media, from you? I mean, we haven't heard the same voices among others here.

SPEAKER: Oh, I think you --

MR. CABLE: -- cases in Connecticut, so it's easy for me to get more excited.

MR. LEIBY: I think you have to turn your question around. Your first question is, are additional safety measures warranted to protect. And I think the question to the audience after spending a whole day here listening to this, is there anyone here who feels that additional safety measures aren't needed?

MS. HERWALDT: Is there anyone here who, back to the scenario of if we had -- forget right now the issue of needing money. Let's pretend that even weren't an issue. Would we all agree that if we had a test, we would start
using it?

SPEAKER: I think so.

MS. HERWALDT: No. I'm -- a simple thought --

MR. CABLE: I don't know. I mean, you have to ask the FDA what their thoughts were, but I want to see it used in the state of Connecticut, personally. I'm not speaking of the Red Cross, but I definitely want to see it used in the state of Connecticut, it's always (off mike).

SPEAKER: Which test would that be? A VCI, an antibody --

MS. HERWALDT: Now we're talking, you know, a test.

MR. NAKHASI: I think Sue had raised hand there.

MS. HAMER: Susan Hamer (phonetic), Red Cross and I'm not speaking for the Red Cross either, but I echo Ritch's comments. You know, we've been talking about Babesia for years and years and years. So what is it going to take? Is it 70 cases not a threshold? Would it be a 170 cases, 300 cases? We did take action on 23 transfusion-transmitted cases of West Nile, and then on -- and seven Chagas' cases, yes, for which we have no positive recipients yet. So I mean, we have a lot more evidence
that Babesia is a much more -- is a serious -- it's our own endemic malaria, and it's something we should be taking more action on. I don't believe we can answer -- well, maybe we can answer some of these questions, but the vast majority of questions are data-driven questions, but are reliant on the tests that are available today, none of which I believe are suitable for blood donor's training.

MS. HERWALDT: I think one of the problems that we're facing is because this has been going on for a number of decades, that people have gotten -- not these people, but --

MS. HAMER: But these people are -- we are tolerant.

MS. HERWALDT: But some of us literally spend a lot of time, all the time, and at CDC we're almost always investigating transfusion associated cases. Not that there's a new one every day that we hear about, but they take so long to investigate and we're constantly investigating various cases. And -- but in terms of what you just said, when I was speaking this morning, I was thinking of you know, making the point, you know, what's the, you know, magic number. Now, I realize that life
isn't that simple in terms of screening donors, and -- but this isn't going away, and if anything I think is going to increase and again, I think we're up against not only the issue of not having, you know, this wonderful test of the moment, but also this -- again, I'm not saying FDA has this attitude, but this sort of -- well, this has been going on a while. That's why, you know, the contrast with West Nile, I'm not sure that really is a good contrast. You know, if this just started to happen in the last year or two, I think there would be a very different reaction.

MR. KRAUSE: I want to make some further comments also. I think that there is some concern potentially because of the perceived hyper concern about Lyme disease and the thought that we don't want Babesia to have this same scenario potentially. There's some potential hesitancy in that regard. I'm not sure how much a role that plays, but I know that that may have some effect. I think the other thing is people -- when you look at the health burden of disease, it's not just the incidence of disease, but it also is what that disease does obviously, how severe, you know, what happens.

The number of patients dying from babesiosis, at
least that I know about, that are recorded far outweighs that of the Lyme, for example. So the other comment I would make is, I think the fact -- I think that if you -- one can look at the data that I presented, and this is from New York State, from Connecticut, the incidence of the disease is increasing. Whether that's biologic or simply a matter of better diagnosis, I think it is increasing.

And I know also just looking at new sites now that are -- Westchester Country, for example, babesiosis now there was not there before. So I think the disease is really emerging. It really truly is. It is increasing, I think it does have a significant -- I think there is a significant health burden. The final comment I wanted to make is, the strategy for control of this doesn't have to be perfect.

I mean, it seems to me that people are concerned that, you know, well, there's still going to be some people who -- there'll still be some transfusion cases that may occur given X, Y, or Z strategy. It seems to me if you have a strategy that gets you, you know, even a 50 percent reduction, it's something that's positive. So if there's concern about costs of certain strategies, one might choose
something that's perhaps not as effective, but yet is much more cost effective, let's say. So I think that's another -- that's something else that I think needs to be considered in all of this.

MR. TELFORD: In the light of those comments, I hope people in the audience don't get the impression that the IFA is a bad test. It actually is a very good test, and in fact it's hard these days in public health. In the old days, you realized that public health people do the best they can for the most, realizing that some are left behind. Unfortunately, nowadays you have litigation, and that has made our wishing for tests that are 100 percent sensitive, and a 100 percent specific. But even if we take a lower limit for the figures that we heard to date, 80 percent sensitive or specific, that's still not a bad test.

And there are ways -- there are ways of improving the throughput. I mean, Ritch and I were just talking. I personally in a day can screen 400 sera. No big deal. He was saying with GMP, you could do maybe a 125.

(Laughter)

MR. TELFORD: If it pays more than a faculty position --
SPEAKER: You could screen half my volume everyday.

MR. TELFORD: But there're ways of streamlining this with existing -- you know, based upon the principle of the IFA. You have microarrays, for example. You do a reverse microarray. You spot the serum on the chip, and then you do a capture with the antigen, and you've got these nice laser-detection things, you automate it all and you can screen hundreds and hundreds and hundreds of sample, you winnow down the negatives and then you focus more on the positives.

SPEAKER: If somebody has to build the test, and we have recent experience with asking somebody to build a test, and that's Chagas' disease, and essentially somebody in this room said, in a crowded room where the test-builders were, if you give us a test that needs reasonable performance criteria, we will use it. So we've got a test, and we have apparently not prevented much morbidity, at a very, very high cost.

And so there's some trepidation about that. So the problem is that the way we get a test that's suitable to my little stinking blood center which has -- we need a
throughput of 700 or 800 a day, not 400 a day. Just my little center can't make it work with IFA, if we were to do big-time screening. In Iowa, where -- if I tell you that the prevalence is even measurable, I'm probably almost lying, but it maybe. And an 80 percent specific test means that I've got this huge, huge population of people I've now told this group, we can't deal with that.

So the trick is to say if we're not going to test everybody, okay, if in fact it appears at this point it's localized to some area that constitutes some percent of the blood supply, all of a sudden you might see most of the test-builders are out of your room because --

(Laughter)

SPEAKER: -- they're going "Huh, no ROI." Not all the test-builders are out of the room. So there's got to be ROI, and they've told us on malaria, if we have to do a blood donor screening with the sensitivity and specificity that we require of a blood donor screening, and you don't do the entire blood supply, it's a no-go. So then we say, well, is there a way to do it with like a 510-K, and then you don't get a screening test, you get a test that can be used selectively. You can deal with non-
specificity, if you're selecting your population with some thought.

How to do that? Is it seven states in every 2 years? Everybody else does a 1,000 donors to see what the prevalence is and see what the prevalence changes over time? And with strategies like that, localized testing in areas of high prevalence and incidence and intermittent screening in areas that are not believed to meet a threshold, can you justify the companies building a test that we can live with?

MR. KRAUSE: I would like to just discuss for a moment the concept of using PCRs as screen as opposed to antibody. Antibody screening has one negative effect, and that is, if someone is positive then depending on one's strategy, you may have to defer that donor. And you're talking about a lot of donors then. I mean, under current guidelines, if someone has a history of babesiosis they're then -- can never give blood again. And if you follow that same policy after your IFA testing, those people would no longer be able to donate, which eliminates a lot from your pool. On the other hand, if you use PCR testing, you're identifying people who are actively infected.
The problem with the PCR testing is that if it's -- if an individual -- the problem with that is that there maybe individuals who are infected and yet you get a false negative PCR. And I would submit that that number -- I'm not sure that number is well-known, or I'm not sure that number is established, but that number is probably not going to be large and one could again, the same -- again, one could argue that. But I -- no, I think that ultimately, at least for starters, you look for something that's practical that's going to have a reasonable effect, and I think that type of screening would have a reasonable effect and then you can refine the system as you go.

MR. KUMAR: I think that there's a lot of weight in the approach he has stated here.

MR. KRAUSE: I'm sorry?

MR. KUMAR: There's a lot of weightage in what he is saying here. Actually I don't think that many symptomatic carriers will not be picked up by PCR given the given sensitivity and that's an approach -- it will be very upfront, you don't lose donor, yes and no answer, and huge, I mean, it seems like at least 10 cases are being reported everywhere. Nonetheless, so you'll find out in few years
what effect it's having.

MS. HERWALDT: I'm sorry, say that again?

SPEAKER: Sanjai -- can you get closer to the mike, Sanjai?

MR. KUMAR: What I'm saying, you'll find out in few years how much PCR is helping without costing too much.

MR. CABLE: One of the things we do know from David's and other's work is the sensitivity to the PCR is very important with respect to how many you're going to find. When I -- I don't think we're going to do real-time PCR, so are we?

(Laughter)

MR. CABLE: But you know, it's already a nested PCR in most labs, and how would that work with the PCR kind of thing that blood donor screening works on? Pooled testing? I mean --

MR. KUMAR: I think we could (inaudible) PCR sensitivity too much without delay.

MR. CABLE: Well, but --

MR. KUMAR: The only thing we need to worry about, how much sample volume we're testing. I think most PCRs can test a single parasite. It's the copy number that
does not come in question here, because copy number will be there only it's not extracellular parasite. You will detect the copy number only if present in a given sample volume, so that's what we need to worry about. And I think most PCRs -- any of the bench PCR, laboratory PCRs will be good enough for that reason.

MR. CABLE: I don't think anyone's doing PCR with pools of less than -- what Sue, in the country?

MS. HAMER: Pardon me?

MR. CABLE: What's the smallest pool that --

MS. HAMER: You know, it's what Lou (phonetic) said before. I mean, when we talk about the test, we still would need to build the test. I mean, it's some -- either talking and too abstractly. I mean, I can't -- we don't --

MR. CABLE: No, I understand, but the point on PCR that everybody should understand is blood centers aren't doing it neat. They're doing it in --

MS. HAMER: A few are, but currently the automation doesn't allow the throughput for us to test every donation, that's correct. But for something like Babesia or malaria, where parasite loads are very low and infectivity of each parasite in a unit -- we are concerned
about infectivity of low parasite loads, I mean, I don't think we could do pooling in that kind of scenario.

MR. CABLE: I don't think you could, but I'm trying to say that therefore the blood bank testing site of PCR needs to realize we probably can't do pooling and what are -- how do we --

MS. HAMER: No, we need a new paradigm for this.

MR. CABLE: Yeah, so how feasible is a PCR test on every unit, when we've never done that for any of the --

MS. HAMER: Well, I mean, it depends. Something would have to be developed and you know, feasibility would have to be investigated.

SPEAKER: We are inter-testing -- looking at the issues that Ritch raised, either we are testing everybody, or we are testing seven states. Rhode Island came to us and said we are going to do patient specific testing, which is what we do currently for CMV. It's not perfect. The test has reasonable sensitivity and specificity. We still have breakthrough cases, but we have reduced substantially the transmission of CMV. If you do that then even the current test could be applied, if there was sufficient education of physicians toward the appropriate unit of
blood, for this neonate, or immunosuppressed patient.

MR. BERARDI: Victor Berardi from IMUGEN. I'm sitting here in a sense listening that there are no tests for Babesia, which is rather surprising since --

SPEAKER: (Off mike).

MR. BERARDI: Yes, I understand that, but when we're talking about technologies, that's a process and a formality. That does not mean that the technology does not exist to do this. Certainly, from the standpoint of relatively high throughput PCR -- and let me qualify this, I have no stakes. I am from a commercial company. We do not own PCR or any particular methodology, so were that to be used, I would have personally nothing to gain from it. But I do know from our experience in dealing with patients with suspect Babesia infection, the only test that we have found that is really useful to the clinician to make a treatment decision, do I treat this person, or do I not, for Babesia, that test has been PCR.

Antibody testing in an endemic population simply results in a lot of additional questions and we spent years working on IgM tests, hoping how this would be the answer, this would provide evidence of recent infection, active
infection. Unfortunately, that has not worked out. The best method we have found is PCR and using real-time PCR with automated extraction chemistry, using the appropriate chemistry, it's perfectly possible to run through thousands of specimens in a laboratory. QC, QA, that satisfies CLEP at the state of New York, and seemingly satisfying physicians. It's perfectly possible to do antibody follow-up on a patient who's tested by PCR, the plasmas available. We have found it to be an excellent test. In terms of cost when one gets down to it if one looks at the volumes of testing that would be going on, say, at the Rhode Island Blood Center, a 100,000 specimens a year, and we've done numbers -- I'm not going to stand by this but if someone asked us to do it, we would be doing it for $3 or $4 a specimen with labor.

So when it's -- when one time -- not that that's what I'm proposing, but when I hear, oh, there are no tests, there may be no FDA-approved tests, but I think there are ways of getting at this. Are they 100 percent sensitive or specific? I don't know, but they're about as sensitive and specific as seemingly as any other test we use in the laboratory. They seem to far exceed the
performance of current Lyme -- commercial available Lyme tests in terms of either sensitivity or specificity.

MR. LEIBY: I think part of the issue, Victor, and I'm hearing from you and then also from Peter, is that you're dealing with clinical cases. So you have patients who've gone to a physician saying I'm not feeling well, without knowing the issue and they send you to get a test and they're acutely infected, high parasitaemia usually you pick them up.

We have blood donors who are asymptomatic. Probably if they have parasitaemia it's extremely low levels and we're not able to pick it up. So I would not say that PCR in a blood bank environment is the way to go.

SPEAKER: No, I'd strongly --

MR. LEIBY: I mean I think it may have a role, I'm not saying it doesn't have a role, but to say that that is the method of choice I think is a dangerous concept.

MS. HERWALDT: I agree. Again, it gets down to these low-level parasitemias and --

SPEAKER: And more prevalence.

MS. HERWALDT: -- more prevalence, and you're testing, you know, this amount of blood and this amount is
in a unit and you know, maybe there is technology that can test larger samples but then you're going to be drawing large volumes of blood from donors, so it's very, very different than the clinical setting.

MR. NAKHASI: Jay, you wanted to --

MR. EPSTEIN: Well, I was going to make that point, but I want to make perhaps a more general point, you know, why did the FDA convene the workshop. It's because we want to make progress in this area, you know, we are seeing these biologic product deviations, we are seeing fatalities; we do recognize that there is apparent geographic spread; we do recognize that there is a report of increased incidence. We are feeling the same frustration that you are feeling.

But the question still remains, what is practical and feasible to do now. And I think that what we're facing is a set of unresolved questions. Perhaps the principal one is not really, should we have universal screening, because maybe we don't have a practical, feasible, strategy to do that thing.

And so at that same time that we're trying to figure out if we have well-standardized test tools, we're
trying to figure out how we would best use them and the problem is that it's a bit circular; you have to figure out, well, what's your strategy, you know, who you're going to test, what products you're going to label, you know, how you're going to manage your inventory, who needs to be tested geographically et cetera. At the very same time that we're trying to figure out that issue we're trying to figure out what's the appropriate test technology.

But the two things have to come together in the end. And the test system that you build to screen all donors, would not be the test system that you build to maintain a small volume screened inventory.

Likewise, just because the test technology characteristics for donor screening would be different from diagnostics doesn't mean there isn't a need for approved diagnostics that would also advance the field.

So I think, you know, my perspective on this is, you have a proven transfusion-transmissible disease which is capable of causing fatality. Of course we want to intervene. Where the dilemma lies is what's the strategy and with what tools. And so we've convened this workshop to try to figure out where we really are.
MR. KRAUSE: Can I make one more comment? In regard to the PCR it is true that we use this -- it has not been used as a screening tool, for people are very symptomatic.

However, just because that hasn't been done, doesn't mean that it would not necessarily work. We -- I mean the data that you folks have put together, the data that we looked at earlier are -- you know, show that people who are asymptomatic remain PCR-positive for quite a while, probably with a very low parasitaemia. We know that individuals vary. And I may be missing something in this argument, so you guys can tell me what I'm missing, but I will say it's -- okay, you have got to unload and that's fine, but I mean, in my, you know, you hear, you're picking up --

MS. HERWALDT: Your work probably deserves --

(Laughter)

MR. KRAUSE: You're -- it's again, not a perfect test, but you can pick up very small amounts of Babesia within a blood sample.

MS. HERWALDT: Okay, you say it, then I'll --

MR. LEIBY: I'll say well, he's saying something
different, or it may be different but I think the difference is that we're actually pre-selecting them because they're IFA positive. So we have a at-risk, presumably exposed and infected population. Now, if we just did PCR on the broader Connecticut population, yeah, perhaps we might find some early acute infections but we also would get false positives and a host of other problems. I'm not sure we'd be picking up many asymptomatic donors. If we would they might have antibodies as well.

MS. HERWALDT: I agree. I think the issue of pre-selection is a big issue, and I was just going to make the same point. And then the other issue is -- David showed some examples of PCR results for some of the people in the Connecticut study. But, you know, even with real-time PCR which was done for some but not all, it wasn't necessarily consistent positivity.

So yeah, this very selected population and then especially with nested, let alone with real-time or hamster inoculation, you could have positive one time and negative the next time, and so I think it's a very different situation. That's not to say that there isn't some
molecular approach that accomplishes something, but I think it's a very different paradigm in the vivo paradigm and I think it's a very different paradigm than clinical diagnostic issues.

MR. KRAUSE: I would simply say that I think it's probably worthy of a study, it may not be worthy of implementation at this point in a wide scale manner, but at least looking at the issue might be worthwhile. I mean I believe that it would be.

MR. KUMAR: I would like to say something that will fit into what you're saying. We never know what is the parasite burden in asymptomatic clinically immune people in Sub-Saharan Africa and then we are talking about a disease which is in United States, okay. So there's something incumbent upon the field also to -- I mean it's much difficult to find answers there, but I think if field gets together you can find the answer, their quantitatives or my quantitative PCRs, you can find out what is the parasite burden in majority of asymptomatic carriers, at the tail-end, the beginning, resolution of acute disease then we will find out the answer also whether PCR is going to be useful or not. That's all I would say. I mean from
the same measure the disease is here that needs to be responded, but I think some answers can be found here too.

MR. NAKHASI: Having fired up Ritchard for being, I think why we are not screening the Babesia now, I guess you've heard in the answer from Jay and, in fact I was going to give the same answer. But the question is now, we now know that yes, we need to test, for example, in those areas.

So we also heard that what should be the testing strategy -- maybe some -- you know, and Peter would say PCR, or somebody would say Western Blot or serology. So we need to figure out here so what kind of combinations or if there is one, what type of tests we would need and what type of characteristics of those tests would be, because I think that's important than you heard from Lew (phonetic) and others that in screening blood donors, it's not the diagnostic people, you know, so obviously Jay said for diagnostic purpose, that's also market for that, but the question is for if we go that we decide that we need to screen for people, what type of characteristics we need and you know, we should not be jeopardizing more cases which you know, availability versus the donor screening, yeah.
MR. CABLE: I'd like to say just a word about the practicality. Whereas we can't do 400, we've taken folks without a college education, we've trained them in a couple weeks; there is -- get the same results as the med tech kids in our hands, we're confident in their work, they can do 125, 150 a day with good research recordkeeping. With a little bit of help on the computer-automation side, you know, they could -- one person could maintain an inventory in Connecticut with the same strategy that, well, Anne was talking about and we -- to second Salsa's idea, we proposed this to our hospitals, blood banks, and they hated it. They just hated it. It's another chance to charge money to them, what the hell's a at-risk donor recipient anyways would you tell me Dr. Cable, and you know, I ran for cover. And the next president of the AABB was leading the charge.

(Laughter)

MR. CABLE: Now, if the industry, and then if that wouldn't start -- if the FDA made it quite clear that this was a perfectly acceptable strategy like CMV before labeling requirements were made necessary to make claims for donor screening, if they just said just use your home-brew test kit, CLEA requirements for the IFA and do your
tests, I'm sure the seven regions of the Red Cross, or six regions of Red Cross could figure out how to make this available but nothing. What if they make it available and no one would order them?

So in order for that to work, the AABB would need to put on a major-league effort to educate first, blood bankers who many times don't hear about this disease they can't pronounce and then clinicians in each region to start ordering it for -- and by the way someone then would need to say -- and these are the list groups precisely defined -- the immunosuppress, that doesn't do much for me. I mean, you know, oh, that doesn't do much for me -- in terms of if I'm a blood bank director.

SALSA: And I think that you touched again, on something that I said in the beginning. Education is probably the most important factor in all that and I wish that all the experts that's sitting around the table will get together and write a very nice review for the New England Journal of Medicine or JAMA and starting promoting knowledge about Babesia and Babesia infection that we hear very little about.

MR. EPSTEIN: I don't thing we should think of
this problem as a once-and-for-all solution. I think it would be more constructive to look at a menu of alternative interventions and for each one, ask what would it take to get there, because if we can get to any of the feasible interventions we make the situation somewhat better.

So for example what you've described a moment ago is the strategy of a small-screened inventory for selective use as a tremendous education issue. The doctors have to order the screening, units have to know when to do it, have to be motivated to do it and the blood banks have to be willing and able to do the requisite testing.

It's really a different set of barriers just to go over the other end of the spectrum. It's a different set of barriers to have a high throughput, highly specific and sensitive donor screen that would be cost-effective across the whole country despite the differences in prevalence.

So the way I'm thinking of it is that you have a variety of candidate interventions, but each of those has a different set of obstacles or issues and we should be looking at all the possibilities and asking how do we deal with the respective sets of barriers and that's in the hope
that any of them might be feasible to move forward.

SPEAKER: Yeah, I just wanted to make a point about the discussion concerning why this is different from West Nile, and some of you probably remember that there was a paper that came out before the largest epidemic hit the United States, on modeling, of how many transfusion-transmitted infections might occur, and I think that's what sort of missing here is that sort of big number.

I mean we've seen fatality reports, we've seen other FDA reports, we've seen CBC reports, there are reports from various states that make it reportable, but we haven't seen it all come together, and someone just mentioned something about a review article, but I'm thinking if something that's actually models, you know, how many we could be seeing, and a lot of it is going to be handwaving and leaps of faith, but if someone or a group can do that and put it together, and come up with a number, I think that would galvanize people to think, well, this is what we're dealing with and this is not a small number, this is a big problem. And that might -- it always takes some sort of a spark, whether it was West Nile bacterial contamination, Chagas, and so the question is what that is
going to be.

The other point I wanted to make is that we might want to look ahead -- you know, there is different Babesia species, yes, but there is also the different tick-born diseases as well. So, do we want to talk about multiplex and try to tackle some of these other things that we know as transfusion-transmissible also, anaplasmosis, ehrlichiosis. Lyme's not on the list but they'd probably feel left out if we don't include them, but nonetheless, anaplasmosis and ehrlichiosis are also risks. So just two points to think about.

MR. LEIBY: Well, it seems to me that and I was thinking about that, isn't 70 a big enough number? I mean how much bigger do we have to go?

SPEAKER: Maybe not.

MR. LEIBY: And I think the difference of West Nile is West Nile is largely media-driven. So here we have an exotic agent that appeared in the United States and everyone published it and jumped on the bandwagon and you had cases.

Now, here, Babesia, first case in U.S. about '66? It's been around for a long time, 100s of cases, you know,
no one really gives a rat's you-know-what, so. And I think that we're -- I mean it's here, we've been talking about this for ever, we publish papers. Peter publishes very elegant papers in *New England Journal* about chronicity of the infections. I mean, I think lots of it is already out there.

But what I think I'm hearing, and as I sit here and listen to the workshop and I hear what the FDA is trying to say and the questions they're asking, I think we're caught in the bind where we're trying to use approaches that haven't been used in blood banking for the last -- since the early 1980s. We have an agent, we screen everything, we test and then resolve the problem. Well, now we're confronted with an issue that well, it's not everywhere in the country, it's only maybe in seven states and only in certain portions of the state.

So now we're sitting here and we're saying what are we going to do about it. And you and I have had discussions before here, and you said well, this time we're going to have to think out of the box. Well, I haven't really heard any out-of-the-box thinking, yet. I mean, I hear you saying well, give us some ideas, tell us what to
do but I don't hear the FDA saying, well, perhaps if we had a test, and maybe the states, each individual state or Connecticut figure out what they want to do and they could tell us, and we'll see if that's reasonable. Or some other approach.

I mean, there has to be an opening up to some other ideas besides the PLA and the whole screening. I mean that's -- or otherwise nothing's ever going to happen. I mean the manufacturers are not going to make a test for seven states.

MR. KRAUSE: I think that the earlier comment about a review paper and with the -- I think the emphasis on the importance in terms of the blood supply, I just think making -- I don't know that that argument has been made passionately and I'm not sure the argument is made -- maybe you have to just keep going -- saying it again and again. Well --

MS. HERWALDT: To who? I mean we're sitting here with the FDA, right.

MR. KRAUSE: Right. Well, to FDA for sure, but maybe, you know, in an academic stemming as well.

MS. HERWALDT: And we're in the process of
writing some articles, but the people we need to -- and I
don't mean this antagonistically -- yes, we need to be
educating the public, physicians et cetera. But I agree
with David, in the sense that, yes, mathematical modeling,
it's interesting and helpful et cetera et cetera, but we
know this is a problem.

Now, that -- I'm not saying that means universal
screening and we start to model, I mean, use X test, I'm
not saying that. But the people to get buy-in on -- at
least considering what the approaches are, are sitting
right here in this room. Yes, we can write a wonderful
review article and we can have this wonderful statistician
do mathematical modeling, but the bottom line, we already
know. But the issue is what to do about it.

MR. NAKHASI: Yeah, Barbara, so I think that's a
good point. I think this is the first time we are having
this kind of a discussion, and I think that's the purpose,
is to really hear and to see what can be done next, I think
that's a good beginning. I think with that I think Jay,
you wanted to say something?

MR. EPSTEIN: Well, no, really just the same
point that the obstacle is to demonstrate a feasible
strategy now because we want to intervene; we've wanted to intervene for decades. That's not really where the problem has been. It's not a lack of willingness, it's not a lack of interest, it's not a lack of awareness; it's what is the strategy proposal, because you have a different set of the validation issues for a different approach. And I guess what you're saying is you want FDA first to mandate a given approach.

Now, if we build it they would come, but we don't necessarily operate in that mode. You know, what we're looking for, looking at it the other way around is so what's a realistic proposal and what is the technology and social infrastructure that makes that feasible.

MS. HERWALDT: Well, I guess it would be helpful, and again I mean this in a very positive way, to hear from you like, you know, when's meeting number 2, or, is this meeting going to extend till tomorrow, or again, and I don't mean this to be flip, because you'd said -- and we all agree, you know, we've known about this for a long time and so where do we go from here?

It sounds like -- from what you said, that you agree something needs to be done, and the details of
course, you know, where and how and da-da-da are what need to be decided. So in what context can that be discussed in more detail?

MR. KUMAR: Perhaps this occasion could be used to form some sort of expert panel from here, and you go from there, and you come up with some recommendations, some solid strategies, and then we can look at that and we will respond to that and we can respond -- rather, turn around pretty rapidly.

I mean, you have all the people here, not necessarily people on this table need to be on the panel, but you can form an expert partner, come up with solid recommendations. I think it should come from you; it will have more meaning for you also.

SPEAKER: There's a questioner there, yes, sir.

MR. ADAMS: Trevor Adams, Ortho-Clinical Diagnostics. I want to question today for Leiby, because I think we've got a classic chicken-and-egg situation here. David, if you had a test for Babesia that was as good as a test for Chagas, it is licensed, would that solve the problem? Let's not worry about which state we're testing it. If you have such a test available, would that solve
the current problem as you see it from you expert opinion?

MR. LEIBY: Well, first of all the tests would have to be licensed by the FDA, so I mean that's the first obstacle.

MR. ADAMS: Right, but that's the chicken and the egg.

MR. LEIBY: That's where we really are and that's kind of the -- I mean --

MR. ADAMS: But at least it gets the technology part off the table as far as the solution, which I believe it, because I believe that although PCR's got a role, I don't think it's going to be a screening role. I think the antibody test is the one to go with, so if we had a test that had the sensitivity and specificity of a Chagas-like test for Babesia would that solve the problem, because then it throws the question back to the FDA what do they want to do about it, how are we going to get a test that is same thing for Chagas?

I mean you said yourselves that we are testing and all the people said over in this side of the room that we're testing for Chagas and it's probably less serious and I think listening to your talk that's what you said. Yet
we haven't done anything for Babesia. How can we break this deadlock between the experts and the FDA?

I think if there was -- if there's consensus on antibody screening testing, that is sensitivity and specificity would be the technical solution, then it throws the question back to the folks, the decision-makers of the FDA, how do we approve some and get the manufacturers to come in and build such a test.

MR. LEIBY: I clearly don't speak for the Red Cross, but I think in many instances I've said that would be a solution if there was a test. But then we get into complex issues about whether or not the test has improved, whether or not it's mandated, will the Red Cross -- if it's not mandated, then the Red Cross doesn't really collect money for it, then we're losing money and our organization will not put the money out for the test, so it gets very complex.

And that's why sometimes we turn it back on the FDA rightfully or wrongly, we say, hello, if you guys say we have to have a test then we can go to our organization and say we were told we have to have the test and we can have it. And then we go round and round, we all have these
same discussions.

MR. EPSTEIN: Yeah, well, I think that it's really a distortion to position FDA as the obstacle here. The obstacle here has been the failure to bring forward a feasible technology. Now, the -- what you're saying, David, is, if we create a mandate, you know, if there's a suitable screening test we'll require it.

In a sense that's what happened with Chagas. We had a meeting, I forget, in 1989, we asked the committee if they were suitable screening test or should we screen the blood supply the answer was "yes." They were, what, five cases in North America at the time.

And then we had a series of events, you know, we had some companies had brought forward their diagnostic test and said I want to be a blood screen. And we brought those to the committee and the committee concurring with FDA, said, well, no, they don't have suitable sensitivity and specificity, operational characteristics are poor, these are not suitable tests.

And then the committee said, but, so FDA, you know, what's your idea of a suitable test. So then we had another public meeting brought to the committee and we
said, okay, here are the criteria for a test and so then you know, eventually, companies came forward and a suitable test was licensed, but there was no immediate movement by the blood organizations. Some blood organizations then went ahead and adopted the test. Others did not.

And so, you know, we're still in this limbo situation where because we discovered something we hadn't expected, which is negative lookbacks, and so now we're in quandary. So we have some of the system testing, some of the system not testing, and you had all these issues about geographical risk.

I mean, after all the proxy system that they're testing are the ones that they think have higher risk, proxy systems that are not testing think they have lower risk. So, what I'm trying to suggest here is that all the players have to pull together. It isn't just a question of FDA coming forward with a mandate. We tend not to mandate technologies that aren't there, you know, we don't mandate things in theory.

And I think another point that needs to be understood about a workshop, we're not going to make policy here. That's just not happening folks, because it not
legal. So we're here to listen, we're here to gather information, we're here to get ideas, and what I'm trying to express is that we're not resistant to considering alternative ways forward.

What's needed is to frame them, you know, what does that look like, and what does it take to get to that strategy from here. And you know, various options exist, I mean there's geographic-limited testing. Okay, it's got a failing because people travel and blood travels. But after all it might capture the bulk of the problem, right?

I mean, there's pretty high incentive in Connecticut, New York, New Jersey, that makes sense. Is it a full solution? No, it's a partial solution. Should we resist it? No, I don't think we ought to resist it, but we would still want it to be under GNP, right? If you're going to label blood as negative, and if you're going to have some negative predictive value of a screen, that ought to be valid.

So, I'm just saying here, that I think simply saying, well, FDA is the obstacle, because we haven't made a policy, overlooks the fact that what we're dealing with is -- it's taken a long time. But in evolution and our
understanding of what we're dealing with and what the options to intervene are, and I'm not sure there would be a consensus today to screen all the blood in United States. But maybe that's what it takes. I mean if that's what it would take we can bring that question to an advisory committee, we can make policy. We can do it today.

MR. CABLE: I think you've pointed out an important thing, which is the industry has often been desultory on this point as well, and I've been critical of that too. So perhaps the way forward is some kind of effort between the TTD committee of AABB, the FDA and the CDC with associated experts to form out a working group to just kind of work, you know, define four options forward and work them, bring the manufacturers into the process and keep it moving.

The concern I've got is, you know, we all go home and have our margarita which we're going to do and forget about it. And I -- this is about my last chance on this one, because you know, I'm moving on to -- but I do think it's a big problem, and I no longer have to worry about the Connecticut blood supply unless my wife and I need blood. But that may happen, and I'd be concerned about getting
blood in Connecticut today that -- in which an intervention hadn't taken place.

And so I'm speaking as a customer, a consumer at this point. In my passion -- pardon me for being -- overstating the thing, which I often do, but I would like to see the industry and the FDA and the scientists, CDC, and others, figure out a way to work forward, because they've certainly done it for some diseases that are not as important, I believe.

MR. NAKHASI: So, I think, you know, that -- thank you Ritch -- I think the important part of this discussion was that to really bring this out here. I think this -- I can -- I'm glad that people are fired up, that we need to do something about it and I think, David, to respond to your question that we need to have out of thinking box -- out-of-box thinking -- and then this is the opportunity for us to really propose different solutions.

It cannot be the same as I said, in the past, that you know, every pathogen will need different solutions here. We need to come as a group, which many people suggested, a sub-group, where we discuss these issues, what are the various strategies we could, you know, to screen
all the seven states, or to screen only at-risk people, things like that, and if that is the kind of strategy we have, we can then bring it to the -- you know, have a again discussion about it, or as Jay said, we can bring it to the advisory committee and ask that question. And I think that's the way they do it, and I think we will -- that's the process of moving here. So we all are collectively responsible now to think about it, how to move from point A to point B with definite proposals, the criteria and then we can move forward.

MS. HERWALDT: Well, here is an out-of-the-box and this is truly not meant to be flip, and it truly is not the solution, but it's just to get people thinking. An out-of-the-box idea is back to Kansas, Ritch Cable, and again, this is just to perk people's brains.

In theory, these high-risk people, whether they're defined by where they live or defined by, you know, the recipients, in theory, if they got blood from Nebraska, or whatever, are there -- and again, this is not the long-term solution to have, you know, no blood donors in Connecticut, all the blood donors come from Kansas and Nebraska, that's not what I'm saying, but I'm just throwing
this is out in case anything sparks anything in some blood bankers' mind that there is some state that there is just tons of blood all the time and no one uses it and it could be used for these high-risk recipients -- you give me -- it's not the solution, but I just want to throw out.

SPEAKER: But it is a bee's nest.

MS. HERWALDT: Oh, yeah, yeah, no, no, and I'm not saying, it's the solution. I'm just -- because --

SPEAKER: I personally think it's fantastic.

MR. NAKHASI: Okay, so it's a hour, 5:31 now. I think we have to leave the room, but I think before we leave, I think I just want to thank everybody for -- all the speakers who came, and I think it was a very good discussion. I think before we leave, the action item for us I think is to have a sub-group as many proposed, come up with some concrete proposals, and I think also ideas like, you know, to give this something to the manufacturers that if we want a test, what type of test.

As Matt (phonetic) suggested once, that what kind of a test we want. Maybe it could be a multiplexing, you know, because those are the various options we have to think about, because we cannot be just thinking only one
because there has to be some incentive for the manufacturers also to have a buy-in in there.

So I think we should have a task force or whatever you call it where we could have the discussions and come up with some solutions and then we can present it to the advisory committee down the road.

So with that, I think I want to thank everybody on the panel, the audience, and the questioners. So thank you very much. Good night.

(Applause)

(Whereupon, at 5:34 p.m., the PROCEEDINGS were adjourned.)

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