FOOD AND DRUG ADMINISTRATION
CENTER FOR BIOLOGICS EVALUATION AND RESEARCH

BLOOD PRODUCTS ADVISORY COMMITTEE

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CASET Associates, Ltd.
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Table of Contents

P R O C E E D I N G S

Agenda Item: Opening Remarks

DR. HOLLINGER: We have a pretty complete day today and tomorrow. As you know, today we're going to start the session this morning with some committee updates of three workshops or committee meetings that have occurred in the latter part of last year and the first part of this year. Then we have informational presentations to occur after that on XMRV. This will be followed this afternoon by some presentations on *Babesia*, in which the committee will be asked to vote on some questions that they have before them.

Tomorrow will be a session on hemoglobin and hematocrit, donor hemoglobin and hematocrits, inter-donation time intervals and so on, in which again the committee will be asked some questions and will be asked to vote on some questions.

What I would like to do, to just start out, since there are some new committee members here, is for the committee to -- I'll start with myself, and we'll go around the group here -- I would like you to give your name, your title, and where you're from, so that we and others will know who is on the committee.

(Introductions)

Just to tell the committee, there are some

buttons on here, as you can see, for voting later on, for yes, no, and abstain. These will be done simultaneously and recorded, so that we can see how everyone is voting on the various questions.

I'll ask Brian Emery if he would read the conflict-of-interest statement for the committee.

LCDR. EMERY: The Food and Drug Administration is convening the July 26-27, 2010 meeting of the Blood Products Advisory Committee under the authority of the Federal Advisory Committee Act of 1972. With the exception of the industry representative, all participants of the committee are special government employees or regular federal employees from other agencies and are subject to the federal conflict-of-interest laws and regulations. The following information on the status of this advisory committee's compliance with federal ethics and conflict-of-interest laws, including but not limited to 18 USC 208 and 712 of the Federal Food, Drug, and Cosmetic Act, is being provided to participants at this meeting and to the public.

FDA has determined that all members of the advisory committee are in compliance with federal ethics and conflict-of-interest laws. Under 18 USC 208, Congress has authorized FDA to grant waivers to special government employees and regular government employees who have financial conflicts when it is determined that the agency's

need for a particular individual's service outweighs his or her potential financial conflict of interest. Under 712 of the Food, Drug, and Cosmetic Act, Congress has authorized FDA to grant waivers to special government employees and regular government employees with potential financial conflicts when necessary to afford the committee their essential expertise.

Related to the discussions at this meeting, members and consultants of this committee have been screened for potential financial conflict of interest of their own, as well as those imputed to them, including those of their spouses or minor children and, for the purposes of 18 USC 208, their employers. These interests may include investments, consulting, expert witness testimony, contract and grants, CRADAs, teaching, speaking, writing, patents and royalties, and primary employment.

The committee will discuss for topic 1 the risk of *Babesia* infection by blood transfusion and the status of laboratory tests. This is a particular matter of general applicability.

For topic 2, the committee will discuss blood donor hemoglobin/hematocrit qualification standards, donor iron status, and inter-donation interval. This is a particular matter of general applicability.

In addition, the committee will hear updates on

several topics and an informational presentation. These updates and the presentation are not for discussion by the committee, and therefore committee members were not screened for financial interests relating to these updates and the informational presentation.

Based on the agenda and all financial interests reported by members and consultants, no conflict-of-interest waivers were issued under 18 USC 208(b)(3) or 712 of the Food, Drug, and Cosmetic Act.

Dr. Donald Baker is serving as the industry representative for topic 1, acting on behalf of all related industry. Dr. Baker is employed by Baxter BioScience. Dr. Celso Bianco is serving as the industry representative for topic 2, acting on behalf of all related industry. Dr. Bianco is employed by America's Blood Centers in Washington, D.C. Industry representatives are not special government employees and do not vote.

This conflict-of-interest statement will be available for review at the registration table. We would like to remind members, consultants, and participants that if the discussions involve any other products or firms not already on the agenda for which an FDA participant has a personal or imputed financial interest, the participants need to exclude themselves from such involvement, and their exclusion will be noted for the record.

FDA encourages all participants to advise the committee of any financial relationships that you may have with the sponsor, its product, and, if known, its direct competitors.

Thank you.

DR. HOLLINGER: Thank you, Brian.

We're going to start, then, with the committee updates. So that we can keep on time, I would like to ask the presenters -- for those who have 15- or 20-minute talks -- if they would leave about two or three minutes for any questions that the committee or others might have, and for those that have 25, 30, or more minutes of presentation, if they would leave about five minutes of time for any questions, so we don't get behind as we go through the talks.

The first talk on a committee update will be an update of the HHS Advisory Committee on Blood Safety and Availability and a summary of the June 10-11, 2010 meeting that took place. We are asking Dr. Jerry Holmberg to fill us in on the results of that meeting. Jerry?

Agenda Item: Committee Updates

Update from HHS Advisory Committee on Blood Safety and Availability

DR. HOLMBERG: Thank you, Dr. Hollinger. It's good to see you all. I don't think some of us believed

that this meeting would take place this morning, if they were like me. I'm still without power. It's good to see Dr. Linden on the committee here.

I'm going to give you a quick overview on our last meeting in July on the topics. But since there are some new members to the committee here, I would like to just give a high level of what the charge is to the Advisory Committee on Blood Safety and Availability, so that you understand the difference between BPAC and the Advisory Committee on Blood Safety and Availability.

Our committee, the ACBSA, provides advice to the Secretary and to the Assistant Secretary for Health, and really provides information and advice on multiple areas of interest:

· Definition of public health parameters around safety and availability of blood and blood products.

· Broad public health, ethical, and legal issues related to transfusion and transplantation safety.

· The implications for safety and availability of various economic factors affecting product cost and supply.

The July meeting was July 10 and 11, and the issue was that the Advisory Committee on Blood Safety and Availability met to discuss the current policy on men who have had sex with other men, or MSM. FDA's current donor qualification states that MSM at any time since 1977 are

currently deferred as blood donors, and the BPAC has discussed this on numerous occasions over the last several decades. The most recent discussion that took place on MSM was really a workshop on behavioral-based deferral in March 2006.

Our June agenda addressed:

· The scientific rationale for the current policy.

· The epidemiology of transfusion-transmitted diseases in individuals practicing high-risk behavior compared to the general population.

· Risk assessment in the United States and other countries.

· Computerized inventory management in blood-collection establishments.

· Reliability of donor questionnaires to address high-risk sexual behavior.

· Hemovigilance and data sets.

· Societal considerations for MSM deferral policy.

The ACBSA engaged in deliberations and discussed the following:

· The important factors to consider in making a policy change.

· Scientific information, including risk

assessment.

· Whether any additional studies are needed before implementing a policy change or following a policy change.

· Monitoring tools and surveillance activities that could be implemented, and whether those should be implemented before implementing any policy change.

· Whether additional safety measures, if any, are needed to assure blood safety under a revised deferral policy.

The first question that the committee was asked was, should the current indefinite deferral for men who have had sex with another man even on one occasion since 1977 be changed at the present time? The committee was split on the vote there, 6 yes, 9 no. But the committee went on to make some very clear recommendations. I have to say that these recommendations were unanimously supported by the entire voting committee.

The HHS ACBSAQ is sensitive to the blood system and broader societal issues related to the current deferral policy for men who have had sex with another man even one time since 1977. Whereas we believe that the current donor deferral policies are suboptimal in permitting some potentially high-risk donations while preventing some potentially low-risk donations, we find that currently

available scientific data are inadequate to support change to a specific alternate policy. Therefore, until further evaluation, the committee recommends that the current indefinite deferral for men who have had sex with another even one time since 1977 not be changed at the present time.

To develop and validate candidate alternate policies, we recommend research in the following areas:

· Validate modifications to the donor questionnaire that would be better differentiate low- versus high-risk MSM and heterosexuals, including studies to investigate transfusion-transmitted infectious disease and sexually transmitted disease markers in potential donor subsets.

· Establish ongoing national hemovigilance programs for TTID markers in blood donors linked to analysis of demographics, behavior, and other risk factors.

· Obtain a baseline on prevalence and incidence of TTIDs.

· Characterize risk in different donor subgroups.

· Use above characterizations for continuous quality improvement of the donor deferral process.

Here are some more recommendations:

· To determine the feasibility of ht donor pretesting to limit risk while characterizing donors who

might be recruited under modified eligibility criteria.

· Investigate the impact of revised donor criteria on the global availability of plasma products.

· Evaluation of data from other countries that have changed their high-risk donor evaluation programs, including MSM.

· Periodic assessment of transfusion safety, including consideration of multiple and cumulative blood product exposures to recipients.

Additionally, the committee went on to say that the following actions should be brought forward:

· Implement the January 2008 recommendation to adopt pathogen-reduction technology for all transfusible blood components.

· Create a more robust donor education program focusing on high-risk behaviors that is more inclusive of all donor groups, emphasizing the link between safe donations and recipient health.

· The Department should take action to investigate and reduce the risk of quarantine release errors in blood-collection establishments.

· Recognizing the relationship between acceptance of risk of transfusion and protection from harms, further consideration should be given to mechanisms for compensation for blood injuries, consistent with the

recommendations of the Institute of Medicine and the U.S. Congress.

It would be nice if I could present to you sort of a timetable of different actions that are in the works for some of these recommendations. I do want to remind the committee that the recommendations that were brought forward by the Advisory Committee for Blood Safety and Availability are nonbinding for the Department, but the Department has very seriously looked at all of these recommendations, and we continue to put a process forward to address these issues so that we can address the deferral policy in the near future.

Are there any questions?

DR. HOLLINGER: Anybody have any questions?

DR. TRUNKEY: How many potential donors are being eliminated by the current policy?

DR. HOLMBERG: That's very difficult to put a finger on. There have been estimates on if there was a one-year deferral and a five-deferral. I believe that the five-year deferral would bring in about 75,000 -- or is that 150,000?

Jay, would you mind addressing that?

DR. EPSTEIN: FDA performed, in association with external collaborators, quantitative risk assessment, and within that estimate, it was indicated that if there were a

five-year deferral, there might be 14,700 additional donors in the first year. If there were a one-year deferral, there might be 75,000 additional donors in the first year. It's difficult to predict out-years.

This also does not take into account what some might call a watershed effect. We know that there have been solidarity movements, especially on college campuses, where blood drives have been canceled. We don't actually know the net effect of discouraging sympathetic youth.

DR. HOLLINGER: Thank you.

The second update is a summary of a workshop that was held December 14 and 15 of last year on "Emerging Arboviruses: Evaluating the Threat to Transfusion and Transplantation Safety." Deborah Taylor will give us that update.

Agenda Item: Summary of "Emerging Arboviruses: Evaluating the Threat to Transfusion and Transplantation Safety" Workshop

DR. TAYLOR: I'm summarizing the workshop on emerging arboviruses which was held December 14 and 15 of 2009.

We would just like to acknowledge some of the cosponsors. We had financial support from many branches of the Public Health Service and America's Blood Centers.

I just want to provide a little bit of background

and rationale for holding the workshop. Arboviruses, which get their name from arthropod-borne virus, are found around the world, most commonly spread by blood-sucking insects. Their circulation depends on the presence of transmitting vector and vertebrate host amplifiers.

Arthropod-borne diseases are becoming increasingly widespread. Of global concern are dengue virus, Japanese encephalitis virus, chikunguna virus, tick-borne encephalitis virus, and West Nile virus.

For most arboviruses, humans are not the amplifying host, and human infections are rare. Arboviral infections occur mostly during warmer and wetter months. In mild climates cases occur year-round.

Hunan infections have an asymptomatic viremic phase, posing a threat to transfusion and transplantation. Arbovirus-transmitting vectors are present and expanding their distribution in the U.S.

There are large gaps in knowledge about arboviruses. The potential for another arbovirus like West Nile virus to reach and establish epidemic status in the U.S. is of concern and required preparedness, risk assessment, and strategic action plans.

The scope of the workshop was determined by the Scientific Steering Committee. With the help of the Steering Committee, we refined the scope and the agenda.

The scope was to facilitate dissemination of scientific knowledge among government, academia, blood establishments, and other industry, such as test-kit manufacturers. It included a discussion of biology, epidemiology, and pathogenesis of vector-borne viruses of public health relevance. We discussed the potential risk for transmission by transfusion of blood and strategies for prevention of vector-borne virus transmission. Another part of the scope was to promote discussion on strategies to address public health needs, should there be a potential emergence of these pathogens, and what the impact on transfusion and transplantation safety would be.

These are the highlights of the workshop that were gleaned from the workshop. The relevant risks for arbovirus emergence are travel, trade, demographics, poor sanitation, urbanization -- meaning populations moving from rural to urban areas and deforestation -- and climate change. The transmitting vectors for several arboviruses are in the U.S. and expanding their range. Hosts in the sylvatic cycle may be important, but not always necessary, because urban cycles can support epidemics.

Dengue and chikungunya viruses have been reported mostly among travelers in the U.S. Dengue is an immediate problem, since epidemics are occurring in the U.S. -- namely, Puerto Rico and Key West. Japanese encephalitis

virus has been reported among travelers, and its lifecycle is similar to West Nile virus. But a licensed vaccine is available.

We heard during the workshop that dengue and chikungunya viruses are of most concern. Autochthonous transmission of dengue virus has occurred in Puerto Rico, Hawaii, Texas, and Florida. Both viruses can be transmitted by blood transfusion, and the transmitting vector is abundant in parts of the U.S.

With respect to detection and prevention, the pros and cons of availability of a safe blood supply during an outbreak were discussed. Many dengue patients need transfusions and are at risk of receiving blood containing a different serotype, which may worsen the clinical picture. Recent outbreaks in Puerto Rico had multiple co-circulating serotypes.

For blood screening, it was determined that there is a need for a dengue virus nucleic acid test. The American Red Cross is planning to screen blood in Puerto Rico using the Bio-Rad NS1 antigen assay, which is currently under IND.

Test-kit manufacturers, however, indicated that they have prototype assays for dengue virus 1 through 4 and chikungunya virus. However, it was firmly stated that there are no plans for development of these assays in the

near future, even for research purposes.

It was also discussed that pathogen-reduction and inactivation technologies, if successful, may help ensure the safety of blood in the absence of tests for known and unknown viruses. It was recommended that resources be dedicated to realize this goal.

Vaccines were discussed. They concentrated mostly on dengue virus vaccines. It was thought that, although a dengue virus vaccine may affect the outcome of infection, it may not prevent infection. Natural immunity to dengue virus is lifelong. However, neutralizing titers tend to wane. It was also discussed that an effective dengue virus vaccine must be tetravalent. Several are now in clinical trials.

Vector control was argued to be the most appropriate and efficacious measure for prevention of arbovirus infections. Vector control will help to ensure public health and will impact the safety of the blood supply.

CDC staff stated that there are effective mechanisms for arbovirus surveillance and reporting through ArboNet. Zika virus, chikungunya virus, and Japanese encephalitis virus have been found as a result of the surveillance. However, CDC mentioned that ArboNet has been recently affected by the reduction of funding to state

health departments and the CDC.

The tolerance threshold for risk in the blood supply was also discussed. There is a low tolerance threshold for risk associated with blood.

Dengue virus is the most common arbovirus throughout the world. It affects children preferentially, and the outcome in children is worse than in adults.

So the take-home messages, among these other highlights were that successful measures adopted to mitigate West Nile virus transmission by blood should be used as a model for future needs and that the cooperation and communication among stakeholders was critical for West Nile virus and will be critical for success in dealing with other potential epidemics.

We would just like to acknowledge the Steering Committee and the speakers and discussants at the workshop.

Thank you. Any questions?

DR. HOLLINGER: Dr. Linden?

DR. LINDEN: We have been observing in New York that over half of cases thought to be likely West Nile, in fact, turn out to be Powassan virus encephalitis. Has that been considered, how that fits in with West Nile?

DR. TAYLOR: I'm sorry, I didn't hear the full question.

DR. LINDEN: We have found that there is more

Powassan virus encephalitis rather than West Nile in our state. Clinicians are sometimes surprised to hear that. I was wondering if that has been considered in these discussions.

DR. TAYLOR: The reasons for encephalitis were not discussed, no.

DR. HOLLINGER: Also, Dr. Taylor, just a couple of questions. Did I hear that there is a licensed vaccine for Japanese encephalitis virus in the United States?

DR. TAYLOR: That's my understanding.

DR. HOLLINGER: Okay. Secondly, you mentioned that CDC, through surveillance, had detected, if I saw this correctly, Zika, chikungunya, and Japanese encephalitis virus in the United States.

DR. TAYLOR: No, that was not in the United States, I don't believe.

DR. HOLLINGER: I had not thought that they had detected these.

DR. TAYLOR: There may have been instances of dengue virus and chikungunya virus detected in the United States. They are now reportable through ArboNet. But those are probably mostly through travelers.

Yes, Hira?

DR. NAKHASI: Just a clarification. I think the zika was found in Samoa and some of the territories of the

United States. But the point here is that in the ArboNet -- they have a good surveillance program -- they could detect chikungunya coming into this country from travelers to Italy, for example, and things like that. But the point was made that the funding for that ArboNet is going down, so it may impact their surveillance capability.

MS. BAKER: You mentioned in your report -- and thank you for the report -- that among the test-kit manufacturers there were no plans to develop the assays for dengue virus and for the chikungunya virus. Was that clarified?

DR. TAYLOR: CBER has reached out to the manufacturers to understand what's been going on, why they aren't interested. It looks to be a financial issue, where, if there isn't going to be testing throughout the United States, they are not willing to invest in producing kits, even though they have them already developed -- somewhat developed.

But that has been a big problem. We think that's a very big problem.

DR. HOLLINGER: And is the FDA working on these kits?

DR. TAYLOR: We have been discussing with the test-kit manufacturers and the industry stakeholders how to facilitate the process for the manufacturers so that we can

move forward.

DR. RENTAS: Regarding the IND ARC that you mentioned in Puerto Rico, is there going to be a confirmation test associated with that? Do you have any idea how long those donors are going to be deferred for?

DR. TAYLOR: Bio-Rad has submitted an IND and has made the IND public through a press release. But the details of the IND still remain proprietary information at this point.

DR. NAKHASI: Maybe Sue can -- do you want to comment?

DR. STRAMER: Yes, thank you. Susan Stramer, American Red Cross.

We started dengue screening for Red Cross collections on the island of Puerto Rico on March 8. We have now screened over 22,000 donations and have 12 positives, for a rate of about 1 in 1,800 donations screened. This is considered the non-epidemic season on the island. The epidemic season is usually considered August through the end of December, during the rainy season. But in 2010, the rainy season has started early, basically, the first half of the year. They have a raging dengue epidemic now, as was mentioned.

Regarding confirmation, we work with the CDC branch, the dengue branch, in San Juan, Puerto Rico, and

they do confirmation by serotype-specific RT-PCR, IgM, IgG assays, and quantitative PCR assays as well. We have a donor reentry program -- that was your question, how long donors are deferred -- for a minimum of 120 days. They can't be reentered unless they test negative on follow-up of all tests.

DR. HOLLINGER: Sue, before you leave, it's my understanding that of the 10 NS1-antigen positives, none of them were found to be infectious. Am I correct in that or incorrect?

DR. STRAMER: There are 12, and we are still working on confirmatory. There is a range. It's difficult because NS1 doesn't perfectly correlate with RNA, and we have never done prospective donor screening before. So we are seeing a lot of unexpected findings. Some are confirming with very high viral loads -- that is, RNA viral loads of up to 10^{10}. Some are confirming with the presence of antibody. Some donors just have persistent NS1 antigen that appears to be specific, but may not be necessarily related to RNA. If that's related to resolution of long-term infection or some type of carrier state, that's unresolved as of yet. The research is just beginning.

DR. HOLLINGER: Thank you, Dr. Taylor.

We'll move to the next update. The next update is again on a workshop that was held May 11 and 12, 2010 on

"Emerging Infectious Diseases: Evaluation to Implementation for Transfusion and Transplantation Safety." We have a duo, I guess, talking, Paul Mied and Melissa Greenwald.

Agenda Item: Summary of "Emerging Infectious Diseases: Evaluation to Implementation for Transfusion and Transplantation Safety" Workshop

DR. MIED: Thank you, Dr. Hollinger.

The FDA workshop on "Emerging Infectious Diseases: Evaluation to Implementation for Transfusion and Transplantation Safety" was held on May 11 and 12. I'll summarize for you day 1: "Evaluating Emerging Infectious Diseases, or EIDs, for Transfusion Safety."

The goal of the EID workshop was to explore strategies for EID threat detection, intervention, and the prioritization of effort in dealing with EIDs.

We asked some key questions right at the start:

· How do we and should we characterize the risk to blood safety from an EID?

· What are the criteria to prioritize EIDs that pose a threat to blood safety? We acknowledged that prioritization has always been a complicated process.

· How should regulators, blood organizations, manufacturers, and other stakeholders develop a response to the threat from EIDs?

When we speak of EIDs, we are referring to new infections, reemerging infections, drug-resistant infections, whose incidence in humans has increased within the past 20 years or whose incidence threatens to increase in the near future.

In the workshop our keynote speaker addressed the many diverse factors that contribute to the emergence and spread of infectious diseases, such as physical environmental, genetic and biological, ecological, social, political, and economic. Among these are:

· Human demographics, behavior, and sanitation.

· Closer human contact with wildlife and its habitat.

· The failure of control measures.

· International travel and commerce, which results in population movements and transport of agents, reservoirs, and vectors.

· Microbial adaptation and change.

· Human susceptibility to infection.

· Even climate and weather.

We emphasized the importance of public health surveillance in being prepared. Surveillance is the ongoing systematic collection, analysis, and interpretation of outcome-specific data which needs to be closely integrated with the timely dissemination of those data to

those responsible for taking public health action.

We heard that about 70 percent of our 68 or so EIDs have been zoonotic, or able to transmit from animals to humans, with wildlife being an increasingly important source as reservoirs or vectors for disease. Our keynote speaker said that the key will be to unite human and veterinary medicine to anticipate potential threats to blood safety, to be vigilant in early detection, improving our predictive capability, coordination, and communication. We have learned from some recent outbreaks that for this we need strong national and international partnerships, including with the human and animal public health sectors.

We looked at various current effective methods of horizon scanning, the systematic examination of potential threats, opportunities, and likely developments, and the ability to detect novel and unexpected issues, persistent problems, or trends.

We learned about the characteristics of the current repositories of specimens that have been established through the years and the specific purpose for which each was established, especially the large-scale TTVS, RADAR, and TRIPS linked donor-recipient repositories. We heard about the contributions of each repository in being so valuable for the evaluation of transfusion transmission of known agents, and that they may also be

very useful for that purpose for new and future EID agents. We learned how to access those repositories for such studies.

The critical information about a particular EID comes from answering such questions as:

· Is the agent blood-borne?

· Is there an asymptomatic blood-borne phase?

· Have transfusion transmissions been observed?

· Does the agent cause disease?

· What's the prevalence and incidence in donors? Is that significant?

· Are interventions available?

· What would be the impact of those interventions on resources?

We heard a description of the AABB transfusion-transmitted EID four-year project, with the goals of doing three things:

· To describe EID agents for which transfusion transmission is documented or its potential exists, and no effective intervention exists.

· Secondly, to create fact sheets for the agents.

· Thirdly, to prioritize agents as to their blood safety threat.

In our panel discussions, some of our speakers gave us their perspectives on prioritization. One said,

“Prioritization is not the main function. In itself, it’s not all that helpful. We get a feel for which ones have significant risk that requires us to take action.”

Another said, “If you can develop a multi-pathogen chip or pathogen reduction, the whole question of prioritization becomes moot. Or you can try to prioritize quantitatively and put them into an equation. But it’s more difficult in the end to factor in public perception and the societal concerns about an EID agent.”

Also, “Over the last 20 years, we acted because a test was available, but the paradigm has changed,” as we have already heard this morning, “for the test manufacturers. If we had tests available, we wouldn’t have to do things so differently.

And, “We always acted in the face of a disease. For simian foamy virus, we didn’t have a transfusion-transmitted disease. For XMRV, we don’t know if we have a TTD.”

The panel discussion raised some key questions on prioritization:

· What is acceptable risk? That’s a question we have wrestled with for years.

· When do we act with an intervention? And when an intervention is introduced, can it be removed if it is no longer needed?

· Really, how do we know when a trigger for action is reached?

To explore these questions further, we looked at two EID case studies for *Babesia* and XMRV, two agents that are different stages in the decision process. *Babesia* is an emerging transfusion-transmitted agent that is expanding geographically. Regional testing is conceivable. For XMRV, on the other hand, no transfusion transmission has been observed. There is no known causative relationship to disease. Donor prevalence is unknown. Test methods have not been standardized. The literature is controversial, with inconsistent findings for viral markers.

The precautionary principle is one tool that has been used in making decisions. You can state the precautionary principle as, action should be taken even if its value cannot be proven -- that is, even if there is only a theoretical risk of harm. If risk is possible, then we must err on the side of caution. But that action that we take must not be disproportionate.

The question to focus on is, how do we prioritize our response to an EID threat? Our panel discussion members suggested that we could develop a scoring system or a formula for prioritizing. But the American Red Cross has actually developed an EID agent priority matrix. Thanks to Roger Dodd and Sue Stramer, who developed this matrix.

This shows public concern or regulatory concern on the y-axis, from absent to very low, to low, to moderate, to high, versus scientific evidence of blood safety risk on the x-axis, from theoretical to very low, to low, to moderate, to high. This led to the suggestion that dengue virus, *Babesia*, and vCJD should be the EID agents that we should prioritize for intervention. These prioritized red agents have in common that they are known to be transfusion-transmitted, they are increasing in worldwide frequency, they cause disease in recipients, and there are no specific interventions for them.

One of the panelists said XMRV is an excellent model for dealing with potential EIDs. What we have done with it so far has been very deliberate in trying to standardize the procedures, the sample preparation, et cetera. We are asking, does it cause disease? Is it transfusion-transmitted? There, because the science is not clear, the societal issues are weighing in. XMRV is a model for the future.

Another panelist said that we may not know enough about XMRV to declare it to be the model. West Nile virus was a very good model. Petersen and Biggerstaff developed a model for how to deal with future EIDs, saying that West Nile virus is likely to be transfusion-transmitted.

So we have the stages. Infection was detected.

Disease causality was established. The picture of epidemiology was there. Nine months later, we had a donor screening test in place.

But in the end, the question is, what's the appropriate action now? For the two EID case studies, implementing blood donor testing for *Babesia* is an option that could be considered, it was said. But for MMRV, we could continue research and perhaps consider implementing an interim blood safety intervention until the mail questions about XMRV as an EID that could threaten blood safety are answered.

We learned about the existing decision-making framework that Health Canada has for managing risks such as EIDs:

· Issue identification. You identify a possible risk to blood safety.

· Risk assessment, such as donor risk of exposure, by surveillance and hemovigilance, and also benefit assessment.

· Risk management. Identify and analyze options, such as testing, select and implement a strategy, and then monitor and evaluate the results, implementing surveillance measures to assess residual risks to the system.

We learned about risk assessment, that it has components such as:

· Hazard identification, which is easy for some EIDs, but not for others.

· A dose-response assessment for the infectious agent.

· An exposure assessment, in which parameters are represented as distributions, not as point estimates.

· Risk characterization, which is a synthesis of all of that information in a form that risk managers and stakeholders can understand and use.

· Risk management. It considers risks and benefits, and compares potential mitigation "what-ifs," such as, how does the risk change by testing all or by testing some, or by changing the questionnaire? With that comes the number of cases of transfusion transmission that are prevented for each mitigation.

One of our speakers said that as the way forward, we need stronger links with other governments, regulatory authorities, and public health to manage EID risk; increased networking of researchers and global coordination of responses to EIDs in the area of blood safety; collaboration and communication with our domestic and international stakeholders to prepare for new EIDs and for new technologies to combat them; and we need a forum to describe how the decisions that were made were made.

But the question is, what's the appropriate

vehicle or process to put this forum into action?

You can see that we still had a lot of unanswered questions.

Some tools were described to address EIDs. The TessArae high-multiplicity resequencing pathogen microarrays were described, technologies for prion protein assays and blood filters that are under development, and we heard about the contrast between expectations and realities for the perceived costs for implementing pathogen-reduction technology versus the potential benefits of lower infection rates and eliminating some current tests. But this approach was questioned by our speaker. It was suggested that combining methods may offer advantages. An orthogonal process approach was suggested that combines two independent methods, NAT and pathogen-reduction technology, to cover the window period. Could testing plus inactivation or removal equal reduced deferrals?

The suggestion was made to consider where and when it makes sense to apply pathogen reduction in combination with or in lieu of testing or deferrals. The point was made that no decision can ever be without risk. One method can’t be perfect or solve all of the problems, nor does it need to be.

We were left with challenges to consider, some additional profound questions, such as: What do we do

about lack of interest and participation from the manufacturers due to the small market and margins for new tests? Where will the funding come from as threats emerge or current technology becomes antiquated?

These are questions that we are going to be addressing with more intensity in the months ahead.

So that's what happened at the workshop.

DR. HOLLINGER: Thank you, Paul.

Melissa, why don't you go ahead? Then we'll get to questions after that.

DR. GREENWALD: Good morning. I'm Melissa Greenwald, from the Office of Cellular, Tissue, and Gene Therapies. I'm here to tell you about day 2 of the workshop that we had back in May.

Since I'm from the Office of Cellular, Tissue, and Gene Therapies, unlike Paul, we didn't talk about blood. We talked about tissues and cells, as well as organs. I want to point out that human-derived cells and tissues that are intended for transplantation, infusion, and transfusion into humans are regulated at the Center for Biologics, in my office. We do not regulate human organs for transplantation. Those aren't considered cells and tissues regulatory-wise. But we have a lot of common donors and a lot of common issues in evaluating our donors. That's why we included the organ community in our

discussions.

I'll talk a little bit about who was at the meeting. By way of overview, I'll go through a few slides that I used to introduce the meeting so you can see why we had the meeting, and then just review the major discussion points.

We had lots of representatives from the Department of Health and Human Services. I would like to thank Jerry Holmberg for this slide. Jerry was there. The Health Resources and Services Administration does oversight of organ transplantation. Of course, FDA, CDC, NIH were also there.

While we are talking about the regulators, I would also like to thank Health Canada for participating in the meeting. They worked very hard at preparing for the meeting, and I really appreciate their participation. We learned a lot from it.

In order to get a better idea about the types of products I'm talking about, this is a list of the transplantation community stakeholders present. I think this will give you a clearer vision of why, when I go through our discussion, we had a very different discussion on day 2 than was held on day 1.

What we call conventional tissues are things like bones and tendons. Then we have our cell therapy products,

like hematopoietic stem cells. We have ocular tissues, reproductive tissues, as well as organs and blood products. There were people from the blood transfusion community who also stayed for our part of the meeting. We learned a lot from you.

The reason we had the meeting was pretty much the same reason why the Office of Blood had their first-day discussion. There are always new, emerging diseases, and they are potentially transplant-transmissible. We really can't screen for everything all at once. We just want to look at developing a more coordinated process to prioritize and scientifically evaluate those pathogens of concern. My main focus in organizing the meeting was really that this was the beginning of a public dialogue with our major stakeholders to develop a path forward in evaluating new and emerging diseases.

When I introduced the meeting, I explained that we are responsible at FDA for screening and testing policy for donors of human cells, tissues, and cellular- and tissue-based products. Even though we don't regulate the solid organs for transplantation, as already described, we do have shared donors, and FDA does regulate the tests that are used to test the organ donors. The application of scientific information is different between the two communities, but we really need the same basic information,

and safety of the recipients is our common goal.

I'm going to skip this slide and come back to it in a second, because I realized I have them out of the order that I prefer.

Overall, the incidence of transmission associated with allografts is thought to be low, but we really have much fewer data than you do for blood transfusion directly assessing the risk of transmission of potential pathogens by tissues and organs. We do have to make policy decisions based on the best available information in order to try to improve transplantation outcomes. When specific data are unavailable, the resultant donor screening and testing policies tend to be conservative, erring on the side of caution.

During the meeting, everyone agreed that the ultimate goal is patient safety. Because we have so many different types of products, the way you view this approach to safety can vary based upon lots of factors. The urgency of transplantation is very different for organs -- life-saving transplants -- than it is for many of the tissue products. Then you have the cellular therapy products, which are going into people who have often been myeloablated prior to receiving their cell therapies.

The transmission risk is different between all these products, and oftentimes we don't really know what

that transmission risk really is.

There are costs involved in all of this, including development and deployment of testing methods, and really developing a research agenda at its base has to have some funding sources in order to have research occur. We are working to coalesce these into a coherent program, but that is going to be quite a challenge. We will need to deploy significant resources in order to move a research agenda forward.

Ultimately, though, the cell, ocular, tissue, and organ communities are charged with evaluating the infectious disease risks of transplantation. Collaboration is going to be the key to advancing transplantation transmission research. That collaboration includes government.

Now I'm just going to quickly go through each of the three sessions. We started off with an introductory session, where FDA and HRSA and Health Canada provided some regulatory perspective. Then we went into the scientific sessions.

The first session really focused on surveillance approaches. The surveillance, obviously, is important for assessing the safety of the cell, tissue, and transplant products. However, there are a lot of limitations to surveillance information. Underreporting is common. Some

of the notable improvements that we need currently include communication of data between stakeholders, the public health authorities, regulatory authorities, and physicians. Oftentimes, things just aren't recognized, and when they are recognized, they are not always reported. Surveillance alone is unable to detect all infectious disease transmission, and current testing might also be expanded.

Some of the key points in the discussion that came out: In order to enhance surveillance efforts, there is really a need to address donor identifiers, standardize nomenclature and definitions, improve tracking, denominator data, and communications.

I could go into a lot of detail about where we are lacking in all of these, but unfortunately we don't really have time to talk about that today.

But it is important to improve the surveillance of transplant recipients -- not just outcomes in the recipients, but also surveillance from the front end, looking for emerging diseases that we should be concerned about. We always need to strike the right balance between safety and availability.

Session Two looked at the infectious disease transmission events to date, for clues for use in identify future pathogens of concern. We also had a risk assessor provide information about the types of data that are

normally needed to formally evaluate infectious disease transmission risks. Then we had a panel discussion to explore the gaps in data that are needed to assess them.

Some of the key points that came out from all those discussions: As already discussed, everyone agrees that there are infectious disease transmissions, but they are incompletely assessed right now. Risk modeling will be a useful tool that we should be using more in the future. Obviously, those risk estimates can only be as good as the scientific theory and data going into the model. So as we develop new models going forward, I think some adjustments will end up needing to be made as we gather data.

Some of the key research needs in this area:

· Seroprevalence data. We don't really have any seroprevalence data that have been gathered in any sort of collective way on any of these donor populations.

· We also don't really have denominator data available.

· There has not been a lot of research that goes into the transmissibility of these various types of products.

· We really need to complete the process that is already under way of validating a donor history questionnaire that is uniform, like is used for blood donors, and then implement the use of a standardized

questionnaire to also help with data gathering in the future.

· Improve donor testing.

· Look at sterilization and pathogen reduction.

· New approaches to transmission studies.

In Session Three we had presentations from all our various stakeholders focusing on research issues. They talked about research as being currently performed, but also focused on the challenges they face in performing research and the overall research gaps. We asked for some discussion of funding. That's always a problem. The research and surveillance needs are crosscutting.

The final key points that came out during the third session:

· It's very difficult to measure these infrequent events.

· The transmission rates are going to be variable across all the various donors and between the different types of products themselves.

· We do need to develop some protocols and collaboration in order to collect the donor and recipient data.

Tissue handling and processing affect the transmissibility, but the effects of the processing have been very difficult to quantify because the processing

methods are inconsistent, they are proprietary, they are not reviewed by FDA prior to distribution of products, and there is scant published information available for evaluation. There is a need for a commitment to open communication and cooperative data collection.

That was a very quick, very high-level overview of the discussion. Thank you all for your time.

DR. HOLLINGER: Thank you, Dr. Greenwald.

Are there questions of Dr. Mied or Dr. Greenwald about those workshops?

DR. TRUNKEY: How far along are we on prion detection?

DR. GREENWALD: I think I'll let Dr. Ashworth comment on that.

DR. ASHWORTH: There are a couple of tests that have been described in the popular press. One of them was discontinued at the end of last year. At the moment I'm afraid that blood safety relative to spongiform encephalopathy is going to depend on donor deferral policies.

DR. TRUNKEY: My concern is not necessarily just blood, but tissue. One of the most common operations we do now is to implant acellular material from either pigs or humans for treatment of hernias. There is no surveillance that I'm aware of.

DR. ASHWORTH: Fortunately in pigs, there is no natural spongiform encephalopathy that has been described. It has been a concern that for postmortem tissues there are postmortem tests that are available, but unfortunately they are all research use-only tests for human beings. There are postmortem tests for animals that are widely used in surveillance of chronic wasting disease of bovine spongiform encephalopathy and scrapie. But I'm afraid, for human postmortem tests, again, as you heard earlier this morning, we are confronting the problem of a small market, which discourages commercialization of the available tests.

DR. GREENWALD: Certainly our donor-screening policies for human tissue donation are the same, basically, as what is used in blood donors -- geographic deferral based mostly for variant CJD screening.

DR. HOLLINGER: Any other questions?

(No response)

DR. GREENWALD: Great. Thank you.

DR. HOLLINGER: The final update is on the Q fever epidemic that has been occurring in the Netherlands for the past few years and what has happened with that epidemic, particularly this year. Dr. Hira Nakhasi will give us that update.

Agenda Item: Update on Q Fever Epidemic in the Netherlands

DR. NAKHASI: Thank you, Blaine.

In continuation of the team this morning, the EIDs, and why we are concerned about the EIDs as such, we also looked at -- as Dr. Blaine Hollinger said, there is this Q fever epidemic going on in the Netherlands. What impact does it have on blood safety, and also what was our response?

Before I go to the epidemic in the Netherlands, I just want to give you a background about what the cause of Q fever is. The agent which causes Q fever is called *Coxiella burnetii*. It's an intracellular bacterium that lives in macrophages. Forty species of ticks are involved in transmission in animals. However, transmission to humans through ticks is rare.

Q fever is not a new disease. It was described as early as 1935 in Australia, and since then many cases have been reported worldwide, including in the U.S. I will tell you a little bit more about the U.S. history.

The problem is that these are spore-forming, and they are very resistant to heat, drying, osmotic shock, UV, and other things. It can spread over certain distances. People can inhale it, and it causes disease. It is also thought to be a bioterrorism agent.

The microorganism exists in two antigenic forms, phase I, which is infectious, as well as phase II, attenuated and avirulent. It can be easily spread in humans through inhalation. It has been reported in the literature that as few as 10 organisms of *Coxiella burnetii* in humans can be infectious. In fact, it has been shown that even one organism in hamster can cause the disease.

Bacteria are shed in milk, urine, feces of infected animals, amniotic fluid and placenta during birthing, and contaminated wool.

On average, the asymptomatic period varies from seven to 28 days, and in some cases it has been shown up to 40 days. Sixty percent of bacteremic cases are asymptomatic. Infection can lead to acute or chronic disease.

Usually the symptoms are very innocuous, and they are often nonspecific, flu-like symptoms -- like whatever I have today is flu-like symptoms; I hope it is not Q fever -- pneumonia, hepatitis, and other things. Five percent of these develop into chronic disease.

The standard treatment is tetracycline treatment. Infection, luckily, in children is milder compared to older people. Usually farmers, veterinarians, and animal handlers are at risk for this infection because of close contact with the infected animals.

Transmission can occur through:

· As I said, inhalation.

· Ingestion of contaminated meats.

· Direct contact with infected animals or the infected material, like wool, straw, fertilizer, and laundry. I will tell you what happened in the Netherlands because of those things.

· Interdermal inoculation.

· A case of bone-marrow transplantation has been shown.

· Through the transplacental route.

· Sexual transmission. *Coxiella brunetii* DNA in semen was found after 15 months after the infection.

· Blood transfusion, one rare case in the U.S. in 1977. This was a case found in California. The person got surgery. He developed Q fever infection and was treated. When they did the background and they found out this person who had donated was a 20-year-old young guy who had come in contact -- he donated blood, and after three days of blood donation, he came down with the Q fever. Then when they did the analysis, the investigation, they found out he had come down with the Q fever. He had a seropositive for Q fever.

There are currently no FDA-licensed donor-screening tests for *Coxiella brunetii*. However, there are

in-house developed IFAs, serological tests, and in research settings, PCR is used as a list of cultures.

The Q fever epidemiology in the U.S.: As I said, it first was described in 1938 by Cox and Davis. Cases have been reported during World War II and the Gulf War among the military personnel. Iraq and Afghanistan are endemic for Q fever. There are fewer than 200 cases reported in the U.S. per year.

Q fever is considered enzootic in ruminants -- basically, these farm animals, sheep, goats, and cattle -- throughout the country.

The disease is believed to be substantially underreported because of the nonspecific presentation and subsequent failure to suspect infections. A recent national serosurvey by CDC in the U.S. suggested there was approximately 3.1 percent seroprevalence among adults aged 20 years and older.

Why are we now concerned about what is happening in the Netherlands? The story starts here. In the past, there were very few cases of Q fever in the Netherlands, approximately 17 per year. However, for the last three years, there has been an increase in the cases, and last year, in 2009, there were more than 2,000 cases and six deaths. So it was a major public health problem in the Netherlands. Some of them came with pneumonia and 20

percent of cases were admitted to the hospital.

The origin of Q fever in the Netherlands started like this. Earlier they used to do pig farming. Then, because of the swine flu, they shifted to goat farming. When they started doing goat farming in those areas, as you can see, these goat farms are very close to the human habitation. They are factory farming. That means a high density of goats, as you can see from the picture. These goats are lined up there. Then there are deep-litter animal husbandry practices. It means that the manure and everything is there. They put fresh straw on top and then keep on adding more and more, until the goats don't jump out of the thing, and then they move the manure out, which is enough for the *Coxiella brunetii* to grow there. Because of that, what happened was there was an increase in abortion waves in the goats. It looks like it's a very close indication of the infection.

Then what they did on top of that, to make the situation worse, was to put these big huge fans in those pens, so that the spores can spread all over the place. That's how the epidemic started.

In fact, in the same situation in Germany, where sheep are being bred, the farms, as well as the human habitats, are quite different. It was shown later on that these spores can travel up to 5 kilometers. Depending on

the weather and the wind, it can go all the way up to 40 kilometers.

Luckily, the epidemic was towards the southern part of the Netherlands, as you can see. Again, these slides are provided by Dr. Hans Zaaijer, from Sanquin, because he has been updating us on that thing.

So not much was going on in the case of epidemic in the northern part, and the eastern and the western part, not much.

This is the number of cases in the last three years. You can see that the number of cases has been increasing.

Why is it important for the blood safety? They did a retrospective serosurvey in 2009 that collected approximately 25,000 donations during the last year's epidemic and then tested 1,000 of them by PCR. They found six reactive, which were a weak signal, high Ct value. Only three were confirmed because of the follow-up serology. Two of the three PCR-positive donations were transfused. One recipient tested had high IgG and IgM borderline 10 months after transfusion. So it could be a probable case of transfusion transmission of *Coxiella burnetii* during that epidemic.

They also tested some samples which were serially collected during that time and found out that there was a

high seroprevalence, approximately 13 percent, and the seroconversion rate was around 2 percent. They had pre- and after sero-samples, and pre were negative, so they determined approximately 2 percent of the seroconversion.

They also did a look-back on eight donors who came down after donating -- telling the blood establishments that they came down with the Q fever, which was lab-confirmed, between 3 and 13 weeks. They tested all those eight donors. One was PCR-positive. But the recipient, who was terminally ill, could not be tested. Six recipients were PCR-negative. Two of them turned out to be IgG-positive, but then one was thought to have a Q fever before and another was living in the endemic area.

So what is happening now? In 2010, they instituted mandatory animal vaccination, culling tens of thousands of pregnant goats who were in the infected areas, testing of milk tanks. Then there is a lifetime deferral with a history of Q fever compared to a two-year deferral in the rest of Europe. In 2010, they started screening donations with the PCR, which is a homebrew, actually, not really approved.

As of July 2010, with the measures which they have taken, the epidemic so far doesn’t seem to be -- there is not a major outbreak. No waves of abortion occurred in goat farms. So far, zero out of 3,000 donations tested was

positive for *Cb*. Therefore, according to the Dutch Health Council, they believe that Q fever is not a threat to the safety of blood in the Netherlands. They have also lifted the restriction on breeding and transporting milk goats and milk sheep.

Why were we concerned? This started in the beginning of this year, when we came to know that there was this epidemic going on for the last three years. This raised a U.S. public health concern, because travelers to the Netherlands, if they come back and donate blood -- we can have an epidemic in this country.

What did we do in the U.S. health response? We started meeting monthly about the PHS agencies to monitor the epidemic in the Netherlands. FDA and DHHS participated in a meeting organized by the European Center for Diseases Prevention and Control, to find out what measures they have done and what are the things they are planning to control the epidemic in the Netherlands.

Based on the Q fever risk models developed in the Netherlands and France by the European CDC, FDA and CDC determined there is a low risk to blood safety from U.S. travelers to the Netherlands -- approximately six to 15 imported cases per year.

At that point, it was decided that we don’t need any U.S. serosurveys at this time. We were thinking at

that time, if circumstances warranted, we could consider issuing a guidance for donor deferral for travel to the Netherlands. At that time, we were thinking approximately two months. That was the current consideration at that time, because the asymptomatic period can extend up to 40 days.

The U.S. public health response: Also the CDC issued a Health Alert Network notification, which is called HAN, for the potential for Q fever infection among travelers returning from Iraq and the Netherlands, in May of 2010. We were also concerned that the infection may be coming into the country from soldiers or civilians returning from Iraq or Afghanistan. But, luckily, both the AABB and DOD continued to enforce the *Leishmania* deferral policy, which is still in force, which defers the donors by one year. Therefore, at least that way, the blood safety is maintained.

Based on what is happening, the current epidemic in the Netherlands, which seems to be subsided at the moment, it is likely that the risk to the U.S. blood safety is low. However, FDA will continue to monitor the situation.

Thank you very much for your attention.

DR. HOLLINGER: Questions?

(No response)

Hira, a couple of things. Is the disease transmitted by goat cheese?

DR. NAKHASI: Contaminated cheese. I think anything which is contaminated, yes, it can be.

DR. HOLLINGER: And is it imported in this country?

DR. NAKHASI: I have no idea whether it's imported or not.

DR. HOLLINGER: Also I understand that there was some spread to cattle, because they were fed goat milk. Is that --

DR. NAKHASI: I have no clue about that. Maybe Jerry can address that.

In that meeting did they mention anything about that, Jerry?

DR. HOLMBERG: No, that wasn't discussed.

DR. HOLLINGER: Okay, thank you.

Any other questions from the committee?

(No response)

Thanks, Hira.

Does anybody from the committee have a question of anybody, from the updates this morning?

(No response)

If not, I think we will take a break until 9:45. Let's be back here to start at 9:45 for the session on

XMRV.

(Brief recess)

DR. HOLLINGER: I think we'll move on. This session is an informational presentation on xenotropic murine leukemia virus-related virus, or XMRV. I want to inform the committee that this is an informational session. The FDA is not asking for our advice. They are not asking for any recommendations from the committee. Our questions to the speakers should be directed for clarifications or for other information as a result of that, but not for any decisional comments at this time.

First, Dr. Indira Hewlett will give us an initial introduction and background. I'm going to again ask the speakers to try to provide a little bit of time after the end of their speeches -- two to five minutes, depending on the length of their talks -- so that the committee can have some discussion, if they will.

Dr. Hewlett, will you start us out, please?

Agenda Item: Xenotropic Murine Leukemia Virus-Related Virus (XMRV)

DR. HEWLETT: Thank you, Dr. Hollinger. Good morning, everyone.

As Dr. Hollinger said, this morning's sessions is on XMRV. It's an informational presentation, primarily to provide the BPAC with updates on collaborative efforts of

the public health agencies, academia, and blood establishments to evaluate whether XMRV poses a safety concern for the blood supply.

XMRV is a newly identified human retrovirus and the first gammaretrovirus to be detected in humans. It is unrelated to HIV, which causes AIDS, or HTLV. The virus was first identified in 2006 using a viral detection microarray of highly conserved sequences of all known viruses. Since its discovery, several studies have reported conflicting findings regarding the association of XMRV with disease. It should be noted that association, where found, would not in itself necessarily imply disease causation.

In the subsequent slides, I briefly summarize the contradictory results of a number of published studies that have reported either the presence or the absence of an association of XMRV with disease, including chronic fatigue syndrome and other conditions. There is also an as-yet unpublished study on XMRV from researchers at the FDA and the NIH looking at blood samples from CSF patients and healthy controls. The authors are presently addressing issues that were raised in the review of this manuscript. Therefore, discussion of that study is premature at this meeting, pending publication of the results. But we expect to update the committee on XMRV again at a future meeting,

where this can be discussed.

In the next set of slides, I will discuss the positive findings in regard to disease association, followed by the negative ones.

Using the gene array, XMRV sequences were detected in seven of 11 highly selected prostate cancer patients, homozygous for RNase L, which is an important molecule in the innate antiviral response. Additional studies of a broader spectrum of 334 prostate cancer cases showed that up to 6 percent were positive for DNA by PCR and 23 percent for protein expression by immunohistochemistry. Taken together, these findings suggested an association of XMRV with prostate cancer.

In 2009, a U.S. study showed that XMRV could be detected in 67 percent of patients with another disease condition, chronic fatigue syndrome, and 3.7 percent of healthy controls, using PCR and serology. Viral gene sequences in this patient population and healthy controls were virtually identical to sequences from prostate cancer patients. They also showed that virus from activated peripheral blood cells and supernatant could be transmitted to susceptible prostate cancer cells in culture, suggesting that the virus was infectious.

Recently, this year, a German group reported that XMRV DNA could be detected in respiratory secretions. In

this study, 329 respiratory samples were tested, and positive findings were observed in 2.3 percent of 75 travelers from Asia with respiratory infections, 3.2 percent of patients with chronic obstructive pulmonary diseases, 9.9 percent of immunosuppressed patients with several respiratory tract infections, and 3.2 percent of healthy controls.

Now I'll talk about some of the negative findings in regard to disease association of XMRV.

Recent studies of prostate cancer patients have yielded negative findings, resulting in a controversy of XMRV association with prostate cancer. Specifically, studies of an Irish prostate cancer cohort of 139 patients and a German cohort of 589 patients showed no evidence of XMRV by a variety of techniques. In a separate German study, one of 105 samples from non-familial prostate cancer cases were found to be positive for XMRV DNA. However, one of 70 from non-prostate cancer individuals were also positive in the study.

In regard to CFS, several recent studies have reported negative findings of XMRV in the blood of CFS patients. A Dutch study of 32 patients showed negative results using PCR on blood samples. Two U.K. studies, one of 186 and the other of 142 patients, also reported negative results using PCR on the blood of CFS patients. A

study conducted by CDC in 51 CFS patients reported negative results using PCR and serology. This work has just recently been published. A U.S. study of 996 men from the Chicago multicenter AIDS cohort, which is a collection of HIV-positive specimens from MSM, reported negative results for XMRV using PCR.

The reasons for the discordant findings in the various studies are unclear at the present time. They could be due to differences in study populations, geographic differences in prevalence, case-definition criteria and stage of illness, sensitivity and specificity of test methods, the potential genetic variation of the virus, and other unknown factors. Therefore, additional studies are needed to evaluate the role of XMRV in disease using well-standardized assays.

In regard to transfusion risk, transmission through transfusion has not yet been shown, but is theoretically possible, since XMRV has been detected in blood cells and there is evidence of cell-free virus. There are a few studies that have been done in blood donors. They include a seroprevalence study in Japanese blood donors, where, using an in-house Western blot assay, they showed a prevalence of 1.7 percent. Preliminary studies in U.S. donors showed .1 percent seroprevalence using a research serology assay. A CDC study of 121 U.S.

blood donors, however, showed no evidence of XMRV by PCR and serology. In a separate U.S. study of blood donors, none of close to 1,500 blood donors were positive for XMRV RNA and DNA.

Other evidence for potential transfusion transmission of XMRV comes from XMRV infection of rhesus macaques by intravenous inoculation, which showed disseminated infection and low but detectable transient viremia between 4 and 14 days. Seroconversion occurred between 11 and 14 days, with titers peaking around day 95. The virus could be isolated from lymphoid cells and reproductive tissue. There was also evidence for virus replication in a number of organs. These findings lend support to potential transfusion transmission of XMRV, and it does suggest that there is a need for additional studies using well-standardized assays.

However, in regard to assay standardization, currently there are no FDA-approved assays for XMRV, and assays used in the cohort studies have not been standardized. For this purpose, a Blood XMRV Scientific Working Group, led by the NHLBI, was established to standardize and validate assays to evaluate transfusion risk in future studies. This group has developed analytical and clinical reference panels to validate assays for future donor studies. These coded panels are in the

process of being evaluated by six different laboratories, including the CDC, NIH, the Whitmore Peterson Institute, Blood Systems, and two labs at the FDA. You will hear more about this in a presentation later in this session.

In regard to XMRV and blood donation, as there is currently no evidence for XMRV transmission by transfusion or association with a transfusion-transmitted disease, FDA has not established donor policies specific to XMRV. A large survey conducted in Sweden and Denmark, the SCANDAT study of close to 900,000 blood transfusion recipients without a prior cancer diagnosis at the index transfusion, showed no increase in prostate cancer in recipients. Furthermore, statistically significant decreased risk was observed in site-specific analysis for prostate cancer two to four years after transfusion.

In regard to CFS, FDA regulations require that donors should be in good health and medical directors at blood-collection centers should exercise judgment in determining whether CFS patients are in good health at the time of donation.

Indefinite donor deferral has been introduced n parts of Canada, Australia, New Zealand, and the U.K., for donors who voluntarily disclose their diagnosis of CFS. We'll hear more about this from Dr. Ganz, from Health Canada, later in this presentation.

In the U.S., the AABB has issued a bulletin recommending the use of donor education materials on CFS and indefinite deferral for donors who voluntarily disclose their CFS diagnosis.

In summary, FDA intends to periodically update the committee on progress in our understanding of XMRV and transfusion safety. As additional data are obtained on prevalence in blood donors and transfusion risk, the BPAC may be asked to advise FDA on appropriate measures for maintaining the safety of the blood supply.

The rest of the BPAC session today will include the following presentations on XMRV:

· You will hear a review of studies that led to identification of the virus and updates of relevant research.

· A Canadian perspective on XMRV.

· An update from the scientific working group on assay validation using XMRV panels.

· Updates from the various PHS agencies on their XMRV studies.

Thank you.

DR. HOLLINGER: Thank you, Dr. Hewlett, for that introduction.

I think we'll move on to the next talk. We will open it up for any questions at that time. The next time

is by Dr. Bob Silverman, who will give us some information on the identification of the new human retrovirus XMRV. Bob has had lots of experience with this agent. Thank you.

Agenda Item: Identification of the New Human Retrovirus XMRV

DR. SILVERMAN: XMRV is a newly identified human retrovirus, only the third type known, the other two being HIV and HTLV, which cause AIDS and adult T-cell leukemia. XMRV is now associated with two major human diseases, prostate cancer and chronic fatigue syndrome, or ME, and has generated tremendous interest among scientists, patients, and the general public, and also concern about the safety of the blood supply. Not surprisingly for a newly identified virus, there is controversy surrounding XMRV, which I will address.

By way of disclosure, my institute, the Cleveland Clinic, licensed my patients on XMRV to Abbott Laboratories. I'm currently a consultant for Abbott and receive research support to my lab from Abbott.

The story goes back to 2002, with the mapping of a prostate cancer gene called HPC1 to an antiviral gene, RNase L, by the group of Jeff Trent, then at the NIH. We were collaborators in that study. I'm just going to present one result. This is a pedigree from one of the prostate cancer-stricken families. The father died of

prostate cancer. There were five sons, four of which had prostate cancer, and all four were heterozygous for a stop codon mutation in the RNase L gene. That can be seen from these two peaks here, because these patients are heterozygous -- these two peaks in the sequencing chromatograms. This study also showed loss of heterozygosity, loss of the wild-type allele, in tumor tissue.

The identification of a prostate cancer gene as an antiviral gene led us to hypothesize that there might be a virus involved in the etiology of prostate cancer. So at the Cleveland Clinic we designed a clinical study involving 150 patients. These were patients of Dr. Eric Klein, a urologic surgeon and my collaborator in this work. Dr. Klein performed radical prostatectomy, removing the prostate with the tumor tissue. We obtained frozen tissue and isolated RNA, and collaborated with two labs at the University of California at San Francisco, headed by Joe DeRisi and Don Ganem. These investigators had invented a wonderful virus identification and discovery tool called the Virochip, which is a virus DNA microarray representing all known viral sequences.

Screening the Virochip, it quickly became apparent that a subset of these prostate cancer cases were infected with the type of retrovirus known as a

gammaretrovirus. Interestingly, that virus appeared almost exclusively in men that were homozygous for the mutant RNase L R462Q. These are the hybridization patterns. The codes in red are the homozygous mutant cases. There was one heterozygous case that was also positive. More recently, it has become apparent that all people are susceptible to this virus regardless of this SNP in the RNase L gene.

The presence of a retrovirus was first confirmed with nested RT-PCR for *gag* and then sequencing three complete viral genomes from three different prostate cancer cases. It became apparent at that point that this virus was closely related to a type of gammaretrovirus found in mice known as murine leukemia virus and more closely related to the polytropic and xenotropic forms of that virus than to ecotropic MLV and less closely related to gammaretroviruses that have been found in other mammalian species, such as feline leukemia virus, gibbon ape leukemia virus ,and koala retrovirus. So gammaretroviruses have been found in many different types of mammals, but this was the first one found in humans.

We gave this virus a descriptive, the xenotropic murine leukemia virus-related virus, or XMRV for short. Xenotropic in the context of MLV means the virus emerged from mice, but cannot infect common laboratory strains of

mice.

XMRV has a classic structure for a gammaretrovirus. There are two positive RNA strand genomes inside of a protein core, which is surrounded by an envelope, and in the envelope are inserted the envelope proteins, one of which docks to the receptor for this virus, a protein called XPR1. The genome has three principal genes, the *gag*, *pol*, and envelope genes that encode the core proteins, reverse transcriptase, and envelope proteins, respectively.

From the original study, we knew that there was a complete viral genome and that there were all open reading frames, but we didn't know if the virus itself was infectious. What we did was, we pieced together partial cDNAs from a prostate cancer patient. We then took that fused full-length genome into prostate cancer cell line LNCaP, and those cells then produced infectious virus. EM pictures of that virus, which were provided compliments of Abbott Labs, are shown here.

We observed XMRV in the prostate cancer tissues, in the stromal cells, and not in the epithelial cells. We used two methods. One was looking for virus nucleic acid, which is what's shown here. These are FISH assays. The green spots are XMRV nucleic acids; the blue is DAPI-stained nuclei. What we found was that the virus was in

stromal fibroblasts and stromal hematopoietic cells. The latter result is consistent with the CFS study of XMRV in blood cells.

Another lab, headed by Ila Singh, has had different results. She has found the virus in the malignant epithelium of prostate cancer, so in the actual cancer cells.

We have been looking at how this virus infects people, what causes it to grow, what causes it not to grow. I'm just going to present a little bit of the data on the former topic.

By preincubating XMRV viral particles with human semen and then infecting prostate cancer cells, we were able to obtain over a 100-fold increase in infectivity. The reason for that is the presence in semen of a protein called SEVI, which is really prostatic acid phosphatase fragments that form amyloid fibrils and enhance infectivity by HIV and by this virus. SEVI was discovered by Jan Münch and Frank Kirchhoff at the University of Ulm.

In addition, we observed XMRV RNA in prostatic secretions of men with prostate cancer cases, using nested RT-PCR.

So there is a factor in semen that enhances greatly infectivity. The virus appears to be present in semen. These results suggest, but certainly do not prove,

sexual transmission.

Another factor that enhances infectivity is androgen. These are increasing doses of dihydrotestosterone in a cell culture experiment. We obtained a threefold increase in virus replication by measuring reverse transcriptase activity. Antiandrogens, as expected, have the opposite effect. This is Casodex treatment, which inhibits virus replication by two- or threefold.

The reason androgens regulate this virus is because in the viral enhancer in the LTR U3 region, there is a consensus glucocorticoid response element which is also almost identical to a consensus androgen response element. Our group and also the group of Stiff and Goff (phonetic) at Columbia have shown that androgen treatment of cells that are infected with XMRV stimulates this promoter and that stimulates virus replication. This could help account for the fact that we find the virus in prostate.

In October of last year, a study headed by Judy Mikovits at the Whitmore Peterson Institute in Reno showed an association of this virus with blood cells in patients with chronic fatigue syndrome. That was a close collaboration with investigators at the National Cancer Institute, in particular Frank Ruscetti. Also Dr. Mikovits

spent PBMC DNA samples to my lab, and we performed some of the PCR and sequencing in that paper.

There were five lines of evidence in support of the conclusion that XMRV associates with CFS:

· There was PCR and sequencing of novel XMRV isolates. That was the only PCR method used.

· XMRV proteins were detected in activated PMBCs and T cells and immunoblots.

· Activated PBMCs from chronic fatigue syndrome patients, but not from controls, transmitted XMRV to LNCaP cells n co-culture experiments.

· There was evidence of viremia, in that plasma from the CFS patients, but not from controls, transmitted XMRV to the LNCaP cells.

· Circulating antibody against XMRV envelope protein was detected in CFS patients, but not in control individuals.

Together, these five lines of evidence provide strong support for the presence of XMRV in these CFS cases.

The nested PCR results showed that 67 percent of patients and 3.7 percent of controls were positive for XMRV DNA. This is just one result from the paper. This is an electron micrograph of type C retrovirus particles, which appear identical to our pictures of XMRV, which were transmitted from CFS activated T cells to LNCaPs.

XMRV was discovered four years ago. There have been more prostate cancer studies published than chronic fatigue syndrome studies, but with both diseases, there have been groups that have found the virus and groups that have not found the virus.

In the case of prostate cancer studies, there have been 12 studies presented -- some of these by the same groups, incidentally -- nine of which found evidence of XMRV, at least at some level. In the case of CFS, the Lombardi study is the only positive study that has been published to date. There are four negative studies that Dr. Hewlett discussed. So it's one out of five. The Lombardi *et al.* paper was the only study that involved culturing of virus, and that might be significant.

Then there is one negative study on amyotrophic lateral sclerosis.

What could be responsible for these large differences in detection rates? I have listed here what I regard as every possible reason:

· The first is lab contamination. When working with PCR, for instance, one has to be extremely careful to avoid cross-contamination of samples. However, lab contamination cannot explain all the results -- in particular, immunologic evidence of antibody.

· Geographical distribution is likely. This is

well known with HTLV-1, which has huge differences in distribution around the world.

· Sequence variants. So far all the XMRV sequences to date are more than 99 percent identical. But if there are sequence variants, one could miss it by using the wrong oligonucleotide sequence in PCR reactions.

· Clinical criteria for patient selection. Not really an issue with prostate cancer, which has well-established laboratory methods, including Gleason scores. But it is an issue with CFS. Different sets of criteria were used in different studies.

· Lack of standardized methods is certainly an issue, as is lack of widely available positive control human samples.

In the remaining time, I'm going to discuss studies that have been done in the rhesus macaque model, designed to determine the cell and tissue type tropism of the virus, while at the same time providing research material for the development of diagnostic assays. These experiments were done at the Yerkes Primate Center, in the lab of Francois Villinger, and also was a close collaboration with Abbott Laboratories and with my lab.

There were five adult monkeys that were inoculated by the intravenous route with XMRV. Blood samples were taken at regular intervals. There were two

short-term animals that were sacrificed after 6 and 7 days, one at 144 days. At 158 days, the remaining two animals were reinfected, and they were then vaccinated with a mixture of XMRV recombinant proteins to boost antibody titers at 275 days. Then those remaining two animals were sacrificed at 291 days.

Firstly, there was a productive infection in these animals. These are the viremia data using quantitative, real-time PCR. We obtained up to 7,500 copies per ml of XMRV RNA in this male animal, which then declined to undetectable levels after two weeks. So it was transient viremia.

This is a female animal. The viremia was delayed. It peaked at 14 days and then was not detected after three weeks.

In the third animal, there was no detectable viremia.

But all three animals became infected. We performed nested PCR for the envelope gene, one of the same assays done in the CFS study, and showed that the PBMCs, the blood cells, were infected. There was provirus present in the PBMCs after 7 days. Then it could no longer be detected after three or four weeks. So blood cells are a primary site of infection.

As I mentioned, these animals were used for assay

development. These are chemiluminescence immunoassays developed at and performed at Abbott Diagnostics. These are days post-infection, looking at antibodies to two different viral proteins. This is the p15E envelope protein and this is the GAG p30 capsid antibody. The solid arrows indicate times of infection. This is the vaccination.

We got a very robust antibody response, with high titers that were boosted greatly by reinfection. But also we observed that antibody titers rapidly declined after infection. This could indicate why very low levels of antibody are found in humans.

Interestingly, also, at 114 days post-infection, we observed the presence of neutralizing antibody. We took the antiserum from the monkeys, incubated it with the virus, and then infected cells, and showed that the antiserum could prevent XMRV from infecting cells in tissue culture. I think this bodes well for the possibility of an eventual vaccine, should that be deemed necessary.

What types of cells are infected? First of all, CD4 T cells are infected, which is one of the targets of HIV, of course. These are merged signals from anti-gag monoclonal antibody and anti-CD4 antibody. It’s the sixty day post-infection jejunum.

Another site is a non-lymphoid organ, the

prostate. There was a mass of focal infection of the prostate epithelium, as detected using monoclonal antibody to FFB gag. It's certainly at least consistent with prostate cancer findings. This virus apparently targets the prostate early in infection.

After 10 months of infection, we could no longer detect the virus by using antibody, but we did detect it using virus nucleic acid in FISH experiments. The virus now is found only in the stromal cells, and not in the epithelial cells. That's consistent with our findings on prostate cancer.

The early targets are lymphoid organs, CD4+ cells, but other blood cell types as well; in the lung, macrophages; and in the genital tract, the prostate epithelium. The late targets, after 10 months -- again, lymphoid organs, lung macrophages, in the prostate the stromal cells only, and in the one female animal that we infected, the cervix and the vaginal tissue were infected. This is another suggestion of possible sexual transmission of the virus.

What's the possible role of this virus in prostate cancer and CFS?

In the case of prostate cancer, if epithelial cells are the target, there could be insertional activation of proto-oncogenes, a direct mode of carcinogenesis that's

well established in the field with other retroviruses. If the target cells are the stromal cells, those cells could produce cytokines, growth factors, and chemokines that act on epithelial cells to effect carcinogenesis, and this whole process being driven by androgen stimulation and possibly other hormonal stimulation of transcription.

In the case of CFS, if the blood cells are the primary cell type, XMRV infection could lead to immune dysfunction, followed by secondary infection and eventual neurologic effects, which are observed for several other types of retrovirus infections of animals and humans.

In conclusion:

· XMRV is a novel retrovirus. It’s associated with prostate cancer and CFS in humans in some but not all studies. All individuals are at risk, regardless of the RNase L genotype.

· XMRV establishes both acute and chronic persistent disseminated infection in primates. The prostate epithelium is an early target, the stroma late. CD4 and other blood cell types are infected. However, there were no obvious pathologic changes observed in these animals.

· XMRV growth is fueled by androgen, which is a possible oncogenic mechanism.

· XMRV might be transmitted by blood transfusion.

There is now donor deferral for CFS in three countries. We need to keep in mind that this may be an emerging infectious disease, but it's also emerging science. We need to let science do its work.

· Any causal link to human disease remains to be established.

With that, I'll just acknowledge all my coworkers and collaborators. I would be glad to take questions.

DR. HOLLINGER: Thank you.

Questions from the committee? Dr. Rentas?

DR. RENTAS: Is there a difference between the way patients are diagnosed with CFS in Canada, Australia, and New Zealand?

DR. SILVERMAN: There are different criteria. There is a so-called Canadian consensus criterion. I would really rather not try to answer that question, because it's really outside of my field. It should be directed to one of the CFS experts.

DR. HOLLINGER: Have you taken plasma from the rhesus monkeys and given it to other rhesus monkeys?

DR. SILVERMAN: No, but that experiment clearly needs to be done.

DR. HOLLINGER: Do you know if there is any increase in CFS in females from patients who have prostate cancer?

DR. SILVERMAN: No, I don't. There is only anecdotal information out there. But you are dealing with two pretty common diseases, and so anecdotal information is not going to be of much use.

DR. RAGNI: Did anybody look at the wives of the prostate cancer patients to see if they were infected?

DR. SILVERMAN: No, that has not been done.

DR. HOLLINGER: Thank you, Dr. Silverman.

The next speaker is Dr. Peter Ganz, from Health Canada, who will provide us with some information on Health Canada's perspective on XMRV.

Agenda Item: Health Canada's Perspective on XMRV

DR. GANZ: Good morning. Thank you, Dr. Hollinger and staff at OBRR, Dr. Epstein, Dr. Nakhasi, and staff, for the kind invitation to speak to you today about XMRV and Health Canada's perspective on trying to manage the potential threat of XMRV to the blood system.

Just by way of a quick introduction, you have heard already from Dr. Silverman and Dr. Hewlett that XMRV is a gammaretrovirus that can cause leukemias and sarcomas in multiple rodent, feline, and primate species. But it has not been shown to cause disease in humans. I think that's a very important message.

However, xenotropic murine leukemia viruses, which are found in the genomes of many inbred and related

wild mice, can grow in non-rodent cells in culture and have the potential to infect a wide variety of mammalian species, including humans. So there is the potential *in vitro* for infection of human cells.

XMRV is closely related to xenotropic murine leukemia viruses. Recent studies that potentially link XMRV infection in humans with prostate cancer and chronic fatigue syndrome suggest that this gammaretrovirus could cause human disease.

As Dr. Mied mentioned in his introduction, Health Canada presented at the May meeting on emerging infectious diseases. Our recipe for looking at threats to blood safety from emerging infectious diseases and some of the criteria that we use to try to develop risk-management strategies are summarized on this slide:

· First off, published literature.

· Global experience is certainly factored in.

· Surveillance data, if it exists.

· Risk-modeling data.

· Other considerations, which may be focused primarily on the particular environment in which we find ourselves -- Canada being somewhat different than the U.S, particularly, as mentioned, with regard to threats of *Babesia* and other infections, because of temperature, climate, and so on.

With regard to XMRV, we really are focused primarily, at least in our deliberations, on published literature at this point in time, since we have very little surveillance data or other data to consider.

What does the science say, at least from our perspective, with regard to XMRV and its threat as a potential transfusion-transmitted agent that may cause disease? Just to get some feel for the scope of the issue -- and I'm going to focus my discussion primarily on chronic fatigue syndrome, and not prostatic cancer, for the simple reason that individuals who present for blood donation with prostate cancer are indefinitely deferred. So I'll focus my presentation mostly on trying to manage risks for transmission through chronic fatigue syndrome.

Within Canada, the percentage of the population that is suffering from chronic fatigue syndrome is estimated to be about 340,000 Canadians. That's a subset of the overall 17 million individuals that are estimated to have CFS worldwide.

As alluded to by Dr. Silverman and Dr. Hewlett, some of the science that has been published in this area has been conflicting. I'll try to summarize some of the data, at least from the perspective of Health Canada and the emphasis that we place on these studies.

The first one is a study from Lombardi that was

published in 2009, where they were looking for XMRV DNA and identified, using nested PCR, that 67 percent of CFS patients harbored XMRV DNA in their peripheral blood mononuclear cells as compared to eight out of 218, or 3.7 percent, of healthy controls.

It was also demonstrated that XMRV could be transmitted by PMBCs from CFS patients, by plasma, and by cell-free virus from activated T cells of CFS patients. Also they showed that antibodies to the XMRV envelope protein were detected by flow cytometry in the plasma of nine or 18, or 50 percent, of CFS patients that were infected with XMRV, but not in the plasma of seven healthy donors.

The authors concluded that there was a highly significant association between XMRV and CFS from these data. They subsequently noted that they have done additional work since this publication and have detected XMRV in 95 percent of CFS patients' blood samples in the study group.

In contrast to the Lombardi study, there was a study that was published this year by Erlwein, another U.S. study, that failed to detect XMRV or MLV when the DNA extracted from whole-blood samples from 186 CFS patients in the United Kingdom that was tested by nested PCR using primers targeted to an XMRV-specific sequence and a

sequence conserved among MLVs generally. This was a study that indicated that there was no association between XMRV presence and chronic fatigue syndrome.

Another study that was touched on briefly by Indira related to a U.K. study where they looked at 170 samples from chronic fatigue syndrome patients from two cohorts and 395 controls that were analyzed for XMRV, looking either for the presence of viral nucleic acids using quantitative PCR, with a detection limit of fewer than 16 viral copies, or for the presence of serological responses using a virus neutralization assay. They found no evidence of XMRV DNA in any of the samples by PCR, although some serum samples showed XMRV neutralizing activity. But only one of those positive sera came from a CFS patient. Most of the positive sera were also able to neutralize MLV particles pseudotyped with envelope proteins from other viruses, including VSV, which indicated significant cross-reactivity in serological responses. Four positive samples were specific for XMRV.

The summary from this particular study was that there was no association between XMRV infection and CFS in the samples that they tested, either by PCR or serological methods.

Another study this year by Van Kuppeveld *et al.* that was published in the *British Medical Journal* looked at

the prevalence of XMRV in patients with CFS in the Netherlands, using a retrospective analysis of samples from an established cohort. In their PCR analysis, they found zero positives out of 32 sample CFS cases, and in controls, the group found zero positive by PCR of 43 tested samples, concluding that there was no association between XMRV and CFS, at least in this group.

Another study from this year, by Switzer *et al.*, from U.S. CDC, Robert Koch in Germany, and Blood Systems Research in San Francisco, found no association between XMRV infection and chronic fatigue syndrome following testing of plasma from 51 CFS cases and 53 controls, using ELISA and nested PCR technologies.

As Indira mentioned earlier, there is another study that was authored by staff from U.S. NIH and FDA that is purported to have independently confirmed an association of XMRV with CFS as per the publication of Lombardi. But, as Indira mentioned, they are doing additional studies and will be reporting to BPAC with an update at some future time.

With regard to the XMRV transmission mode, there really is no definitive study that has been published that speaks to XMRV transmission. There was an abstract at a Cold Spring Harbor retrovirus symposium last year from a Japanese group, where XMRV-specific nucleic acid sequences

were detected in the genomic DNA of PMBCs from two of three healthy donors following exposure to culture supernatant of PBMCs obtained from a patient who had been found positive for XMRV-specific nucleic acid by nested RT-PCR. The authors concluded that XMRV is sustained in a few fractions of blood cells and can spread through blood, even though the virus replication rate was very low. The detection of XMRV in plasma and PMBCs of infected individuals also suggests the possibility of a blood-borne transmission. Dr. Silverman summarized some of the animal studies in the previous presentation.

There are also some suggestions, perhaps, that XMRV could be transmitted through other body fluids, such as semen and breast milk, but there is no direct evidence to support those routes.

What about regulatory perspectives, at least from Health Canada’s point of view with regard to some of these published studies? Certainly we feel that there is lack of consensus regarding the association of XMRV with CFS in the published studies. As both Dr. Silverman and Dr. Hewlett alluded to, there could be many reasons for this, including variation in the diagnosis of CFS patients, methodological variability that could apply to PCR and immunochemistry, and also the use of differing and non-standardized samples and reagents.

What does that say from a regulatory perspective? I think certainly one needs to look at what the risks are to blood recipients and also the fact that a lack of consensus doesn't require adherence to the status quo, which certainly in Canada has been to accept donors with CFS that are asymptomatic, up until April of this year.

With regard to potential risk to blood recipients, I have summarized a few points which we have taken into account. The general population could potentially be exposed to XMRV via various modes, including vertical or horizontal transmission, as well as transmission through transfusion and transplantation. Currently, there is no direct evidence to support XMRV transmission through blood or cells, tissues, organs. However, its detection in plasma and in PBMCs of individuals in studies does suggest that these routes of transmission are possible and could significantly increase the risk of exposure to XMRV.

The risks that are posed to recipients of blood for XMRV infection will also depend on its ability to cause diseases in humans, and that's important. The magnitude of this risk will depend on whether only a subset of recipients -- perhaps immunocompromised individuals -- are susceptible to XMRV infection/disease or not. We don't really have much information with regard to that aspect.

While the data presented to date suggests a link between XMRV infection with CFS, there are still many unanswered questions regarding its causative role in these and potentially other diseases. In synchrony with several of the other speakers, further studies are required, we think, to prove that XMRV is the underlying cause of human diseases in infected individuals. A recipe has been mapped out for carrying on additional studies that are needed.

However, at least in Canada, one of our overarching, guiding principles with regard to blood safety is the precautionary principle, which means that authorities must act even if there is only a theoretical risk of harm, and if risk is possible, then we should err on the side of caution. Ever since our Royal Commission on the Blood System that was chaired by Justice Krever in the 1980s, one of our guiding principles has been the precautionary principle, and we have exercised it with regard to blood safety in a couple of areas to date. One of the first dealt with the risk of transmission of variant CJD, where we added donor screening added to defer those with travel or residency history in high risk areas. Measures implemented took into account effects on supply, balanced against maximal risk reduction. At the time that we took these measures, it was precautionary, and

subsequently there have been published data to show that indeed variant CJD can be transmitted through blood transfusion.

In another area dealing with managing a potential risk for retroviral transmission through blood, we implemented donor screening for individuals at risk of exposure to SFV. As well, our donor demographic screens that were done prior to implementing that indicated there would be a minimal effect on blood supply with a deferral or adding a question about association or high-risk activities with non-human primates. Although we don't have any data at this point to support it, we feel that the risk of introduction of a retrovirus with the possibility of developing pathogenicity over time with spread in human hosts via transfusion would have been expected to have been reduced by deferring those donors.

What are some of the regulatory options for managing imminent potential XMRV risks?

· Status quo, with further research, which certainly is a possibility.

· Education and self-deferral, which can be considered precautionary. Certainly that's a possibility.

· Deferring donors presumed at risk for carrying XMRV. Certainly that is precautionary and is possible.

· Testing of donors for XMRV and considering

timeframe and other factors, including economic potentially. That's certainly not possible at least within the timeframe that we are looking at.

· Pathogen reduction is certainly something that we could look at, which is possible for some blood components. Clearly that would be an intervention that a regulatory authority could look at.

How do we operationalize some of these options? I'll spend a couple of slides speaking to Health Canada's current risk-management strategies that take into account some of these factors that I have just listed.

The measures that we have implemented in Canada that could potentially mitigate risk of XMRV transmission are self-deferral through education efforts -- I'll speak a little bit more about that -- donor screening and other measures that are currently in place that might, based on what has been published, mitigate risk.

The first one under the banner of self-deferral and education: Health Canada sponsored a symposium on chronic fatigue syndrome under the banner of a consensus conference in 2003. Following the review of the current science at that time, considering chronic fatigue syndrome, there was a series of recommendations that emanated from this consensus conference, including a recommendation with regard to blood transfusion or blood safety. The

recommendation was, from this particular conference, for health professionals that treat CFS patients and individuals with chronic fatigue syndrome was, as a precautionary principle, CFS patients should not donate blood, as it may exacerbate symptoms. I think that's important from the point of view of donor health. And it's possible -- and this was back in 2003 -- that some patients are carrying infectious agents in their blood. Currently in Canada a very active Chronic Fatigue Syndrome Foundation and patient group has advocated since 2003 for their members and chronic fatigue syndrome patients to not donate blood.

A second avenue of risk mitigation that we have pursued is donor screening. Currently in Canada we have in place a health status questionnaire prior to donation that asks the question, "Are you feeling well today?" and, "In the last three days, have you taken any medication or drugs?" Certainly for a number of individuals that are suffering from chronic fatigue or other diseases that result in their having a health issue, there is deferral of those donors as a consequence of at least asking the question of whether they feel well enough to donate.

DR. HOLLINGER: Dr. Ganz, could you summarize for us, please?

DR. GANZ: Sure.

As a precautionary measure, the individuals who donate in Canada at present that have a diagnosis for chronic fatigue are indefinitely deferred. That covers about 80 percent or 90 percent of Canada. We are still in discussions with Hema-Quebec about moving to an indefinite deferral. They currently defer donors that are symptomatic.

Lastly, just by way of summary, another measure that we have in place that we feel has an impact is, in 1998 we implemented universal leukoreduction so that all of the blood in Canada is leukoreduced. Given some of the data that shows that XMRV may be associated with peripheral blood mononuclear cells, we think that that particular measure may offer some protective effect.

In summary, we do have as a precautionary measure an indefinite deferral for individuals with chronic fatigue syndrome. We encourage further studies to address gaps in information in order to appropriately assess the risks of transfusion-transmitted XMRV. I have listed a couple of areas that I think are important to carry out further research that is needed in this area.

Thank you very much.

DR. HOLLINGER: Thank you, Dr. Ganz.

Any questions from the committee?

(No response)

There are how many blood transfusion services in Canada?

DR. GANZ: There are two, Hema-Quebec, which serves the province of Quebec, and Canadian Blood Services, which serves the rest of Canada.

DR. HOLLINGER: And Hema-Quebec has done what?

DR. GANZ: They have a deferral for individuals that are symptomatic for chronic fatigue syndrome.

DR. HOLLINGER: Thank you.

The next speaker is going to be Dr. Michael Hendry, who is going to give us an update on the CDC XMRV activities.

Agenda Item: An Update on CDC XMRV Activities

DR. HENDRY: Good morning, and thank you.

I want to go through and talk about some of the recent activities at CDC as far as XMRV. I want to show that this really is a crosscutting effort within CDC that involves two different centers and three different divisions within CDC. I'm chief of the Laboratory Branch within the Division of HIV/AIDS Prevention. We are considered the subject-matter experts for human, simian, and other animal retroviruses within CDC. But then certainly within CDC, regarding chronic fatigue and its epidemiology, the Chronic Viral Diseases Branch has also been highly involved in this. In the area of blood and

tissue safety, the Office of Blood, Organ, and Other Tissue Safety -- I know Willie is here and on the committee. So these are the parts of CDC that deal with the various aspects of XMRV.

I'm going to skip this because all this data is in your appendix and has been covered by the other speakers.

I will talk about what we have done at CDC, which was to develop Western blot assays for antibody detection using polytropic MuLV-infected cells. We have successfully used the same assay format to identify human infection with simian foamy virus. I'll also talk about the highly sensitive and specific nested PCR assays for both *gag* and *pol* that were developed. Again, these were nested assays that could detect down to 1 µg DNA input, and the integrity is confirmed by beta-actin PCR. We also developed sensitive mouse sequence-specific qPCR to rule out contamination with mouse DNA.

This is just to give an example of our Western blot assays. The upper panels are murine leukemia virus-infected cell extracts on Western blot that are probed with anti-Friend MuLV whole virus goat antisera from ATCC or anti-Rauscher murine leukemia virus envelope-specific antisera -- again, this is goat antiserum from XMRV -- and anti-XMRV whole virus rabbit antisera that we obtained from

Ila Singh at the University of Utah. Not shown is a rat monoclonal antibody that we obtained from Sandy Ruscetti.

This is just to show the sensitivity and specificity of the PCR assays. These are both nested PCRs. The GAG primers that we used were the same as what were in the original Urisman paper in PloS ONE in prostate cancer. We also designed our own primers targeting the integrase of XMRV POL, and you can see we can detect down to 10 copies. When we looked at blood donors, of the 41, none of those were giving us a positive signal. Again, our mouse-specific assay for contamination shows us sensitivities in the range of 10^5 copies. Again, we did not detect any mouse sequences in 117 blood donors.

This is a study that was presented at CROI and is actually undergoing internal review for publication, where we have looked for XMRV sequences in prostate cancer. We do find it, although at a very low frequency. Of 162 samples -- and these were from the Fox Chase Cancer Center prostate repository -- we detected one out of 162 with our GAG primer and three with our polymerase primers. We ruled out mouse contamination. These actually turned out to be more polytropic-like when we sequenced them. But again, when we screened the same patients by plasma by our sensitive Western blot assay, we found none positive.

Moving on to the CFS study, we looked at

archived, anonymous plasma and matching PMBCs and DNA from 51 patients with CFS and 56 matched healthy controls. This was defined using the 1994 International Research Case definition. This included both population-based sampling and physician-referred samples. Again, in both cases, whether it's population-based or physician-referred, it also included the clinical evaluation of those patients. Some of these also had sudden onset.

As I mentioned before, this was all done in blinded fashion. We used a combination of molecular and serologic assays, both at CDC and in our collaborating laboratories. We used Western blot, nested PCR that our colleagues at the Robert Koch Institute also used in XMRV using recombinant proteins in an EIA, as well as in an IFA confirmatory format. Then Graham Simmons and Mike Busch at Blood Systems also tested these, again in blinded fashion, using a *gag* PCR.

These are the results. The upper panel shows the CFS patients that were tested blindly. None of them were positive at CDC, using either our *pol* or our *gag* primers, nor by Western blot. One of 51 was positive by the Koch Institute, by their EIA, but was not confirmed by their IFA, nor was that one positive confirmed by our Western blot, and zero out of the 50 by BSRI.

In the healthy controls, those were uniformly

negative, again except for a weak positive that was picked up by the Koch Institute by their EIA that was not confirmed by IFA, nor was it confirmed by our Western blot.

Some of the additional samples that we have subsequently looked at for antibodies: None of HTLV-1- or 2-infected individuals, zero of seven HIV-1 positives, zero of six HIV-2 dual positives, zero of 121 U.S. blood donors, zero of 20 U.S. IVDUs. We also received plasmas from the Whitmore Peterson Institute that were identified by them as being positive. Again, in our hands, all 20 of those samples were negative by Western blot.

This just shows a sample of our Western blot with CFS patients. On the left are some of the positive control antisera. The upper panel is the infected HeLa cells; on the bottom are the uninfected. Again, although you get a certain amount of background -- and, again, this is just a sample -- we do not detect any XMRV-specific bands on our Western blot.

This is the data from the Koch Institute. You can see the two samples, one of which was from a healthy individual, one of which was from a CFS patient, which were positive, although weakly reactive. The far right shows their positive control.

This shows again examples of the CFS patients using our polymerase nested PCR assay done at CDC. To the

right are the positive controls, showing both the first-round and the second-round results that are uniformly negative.

These are the results from Graham Simmons at Blood Research Institute, again showing the second round, as well as the housekeeping genes that are uniformly negative.

These two studies have also already been mentioned, the negative results from the MACS study, as well as the negative results by Gen-Probe and the American Red Cross, as well as also negative results from blood donors.

In conclusion, we have developed highly sensitive assays for detection of human infection with XMRV and other MuLVs. We didn't find any evidence of infection with XMRV in our study population of CFS patients and controls, done in blinded fashion. PCR and serologic were performed independently in three different laboratories blinded to the clinical status. The testing included generic PCR and two serology assays, reducing the possibility of false negative results caused by divergent viruses. Many people have alluded to this: Differences in patient population, complexities of defining of CFS, lab methods, strain differences may explain the contrasting results. However, our results do not support an association of XMRV with the

majority of CFS patients, and certainly more research is going to be needed to look at the prevalence in the general population, look at modes of transmission, and standardized testing across labs.

Thanks.

I'll take any questions.

DR. HOLLINGER: Questions from the committee?

(No response)

Dr. Hendry, did you try to reproduce, using the same primers and methodology that was used in the Lombardi study? Did you happen to use those --

DR. HENDRY: The *gag* primers that we used in our PCR are the same as what was used in the Lombardi study.

DR. HOLLINGER: Thank you.

If there are no other questions, we'll move on. The next talk will be by Dr. Indira Hewlett, who will give us an update on the FDA/OBRR XMRV activities.

Agenda Item: An Update on FDA/OBRR XMRV Activities

DR. HEWLETT: I'm going to give you a very brief update on the XMRV laboratory activities in the Office of Blood Research and Review at the FDA. We are in the Division of Emerging and Transfusion-Transmitted Diseases in the Office of Blood.

We actually initiated our XMRV research

activities subsequent to the publication of the 2009 paper in *Science*, where it was reported that 3.7 percent of healthy controls were positive for this virus. So we felt there was a need to initiate some research to address questions relating to the blood supply and blood safety. So we have a number of different projects that we have initiated.

The first is to establish and develop highly sensitive and specific PCR assays for XMRV -- we are also working on immunoassay at this point -- and to evaluate assays using well-characterized panels. We wanted to use these assays to test samples from blood donors and HIV-positive individuals from the U.S. and Africa. The reason for our interest in Africa is because it's a geographically distinct region that has not yet been studied. We also have an ongoing HIV diversity or molecular evolution study, particularly in Cameroon. We are also working with some investigators in Uganda on HIV diagnostics. So we had a collection of these samples, and we thought it would be useful to look at them.

We are also interested in developing FDA reference panels for future lot release of assays, should it become necessary to institute and implement testing for XMRV. We are also initiating studies on transfusion transmission of XMRV using an appropriate rhesus macaque

model. I think we heard a little bit about this from Dr. Silverman's talk. Of course, we are interested in the issue of XMRV tropism, infectivity, and pathogenesis.

The assays that we initially set up in our lab -- and this is an ongoing effort. We continue to develop more sensitive and specific assays. This is the *gag* PCR primer set from the Urisman paper that you have heard about already from the other speakers. We have also implemented envelope PCR testing, but we find that the *gag* works much better. Using the plasmid DNA, we can go down to .1 femtogram of the plasmid DNA that is detected by this primer set.

This is the real-time PCR assay using plasmid DNA. We can go down to about 6.7 copies. Therefore, to summarize these results, one round of PCR and qPCR could achieve a detection limit around 10 copies of XMRV plasmid DNA per reaction, while nested PCR could detect one copy of XMRV under our current assay conditions.

We have also established methods for extraction of nucleic acid from plasma and DNA using the traditional QIAGEN viral RNA and DNA kits for extraction of DNA from whole blood.

These are results from XMRV testing of plasma from HIV-positive and negative blood donors from Cameroon. This is just a representative data set. You can see that

we are able to detect HIV-1 in these samples, but we are not able to detect any *gag* sequences. This band you see in lane 6 is actually not an XMRV band, as confirmed by sequencing.

This slide summarizes the testing we have done so far in our laboratory using the Urisman primer set and these extraction methods. With blood donors, we have tested 105 specimens, plasma specimens, from blood donors from Cameroon, 19 PBMCs. We have also tested 50 PBMC culture supernatants where we could isolate HIV. We thought it might be useful to look at them to see if XMRV would be present in these culture supernatants. We have also tested 94 plasma specimens from HIV-positive patients in Uganda. As you can see, we were unable to detect XMRV sequences in these specimens.

In summary, we have established RT-PCR, qPCR assays for XMRV detection. Ongoing assay improvements for whole blood and plasma are under way. Using our current assay, XMRV was not detected in a total of 268 samples of plasma or PBMC from blood donors in Cameroon and HIV-positive patients in Uganda.

Additional blood donor PBMC and plasma, including U.S. blood donor specimens, are currently being tested for XMRV DNA and RNA using more improved assays. We are also planning to test well-pedigreed CFS patient samples in the

future.

In a separate effort, we are developing FDA lot release panels. These lot release panels are used to establish standards for licensure of assays and postmarket surveillance of licensed assays. We envision potentially three ways that one could test for XMRV. One is testing of RNA using plasma, testing of DNA in whole blood, and, of course, serology testing. To be prepared for all three potential strategies, we have initiated lot release panel development for all three measures.

For RNA testing, we have cultured a supernatant from 22Rv1 cells and DU145 clone 7 cells, which were a gift from Bob Silverman. We have estimated the copy numbers using PCR assays targeting the *gag* region. The virus stocks have been heat-inactivated. No infectious virus was detected after heat inactivation, but there was also no significant effect on copy numbers. We are in the process of putting this panel together for copy-number-value assignment, based on consensus of testing in different laboratories, particularly the six laboratories that are involved in the blood XMRV working group.

This slide just shows the virus titration. Our titers are very high. This is about 10^{10} copies in the supernatant. For DNA testing, of course, we would need whole blood and cells, so we have cultured 22Rv1 and DU145

cells and prepared aliquots of these cells. They will be sent to different labs for copy-number evaluation and assignment, and eventually the cells will be spiked into whole blood and consensus values of cell copy numbers will be obtained prior to formulation.

In terms of serology, we currently do not have any materials to proceed with serology panel; development, so efforts are under way to obtain XMRV-positive control specimens, including human and/or animal sera for FDA panel development. If there are any investigators who have such materials, we would be very interested in talking with you about acquiring these materials for our panel development work.

We are setting up in-house serology assays based on purified whole viral lysate. We plan to develop an EIA and Western blot, similar to what you saw with the CDC project. The assays will be standardized using SFFV antisera and known XMRV-positive human and animal sera. We will also be using these serology assays to characterize materials for future FDA lot release panels.

Finally, we are interested, of course, in the issue of infectivity and tropism. We will be initiating an animal study looking at transfusion transmission, to do exactly the experiment that was discussed earlier -- that is, to take plasma and blood from the pre- and post-

seroconversion phases of infection and transfer them to other animals to see if it can be transfusion-transmitted. We are, of course, interested in studying viremia, viral kinetics, host responses, seroconversion, and antibody profiles.

We also have initiated work to look at XMRV infectivity for cells of lymphoid and epithelial cell origin, studying host factors and signaling pathways to identify biomarkers of infection.

I would like to close by acknowledging the people in the laboratory that have been involved in this work, from CBER/FDA and, of course, support from the Office of blood, and all our collaborators, who have either provided us with reagents or samples for our research, and, of course, the Blood XMRV Scientific Working Group, for helping us evaluate our assays.

Thank you. I'll take any questions.

DR. HOLLINGER: Thank you. Questions? Yes, Margaret?

DR. RAGNI: Have you looked at any multiply transfused recipients? HIV may not necessarily be a transfusion issue in many of the groups that were looked at.

What about in any of the cases where a positive sample was identified -- I understand, maybe not from your

group, but did anybody look serially to see how these things change over time, if they did have a positive result?

DR. HEWLETT: I believe in the one publication where positive results were found, I don't believe there are any serial samples. But perhaps Bob or somebody who was part of that study might want to address that.

I just want to mention that we have not looked at multiple transfused recipients. I think that's a good population to look at. We just have not had those particular samples to study.

Maybe Judy --

DR. MIKOVITS: I'm Judy Mikovits. I can address that. We have multiple serial samples from patients taken over 20 years, and we have isolated virus from 1984 and the same patient in 2008. In all of the samples that were given to the CDC, we isolated and sequenced whole virus from every one of those 20, suggesting that that assay isn't clinically validated.

DR. RAGNI: That was for each year or multiple samples? Or were there just two, like before and after?

DR. MIKOVITS: We have more than 600 actual isolates from CFS patients.

DR. HOLLINGER: Indira, when you looked at PBMCs, were these activated PBMCs?

DR. HEWLETT: No. They were unactivated.

DR. HOLLINGER: Any other questions? Dr. Baker?

DR. BAKER: Dr. Hewlett, you used a phrase which caught my attention, “well-pedigreed CFS patients.” Could you describe what constitutes well-pedigreed? In general, I would be curious, given the striking differences that we have seen in these various studies, if you had any thoughts around why that should be. Is there a subset of CFS patients that are more likely to be infected?

DR. HEWLETT: Actually, we are talking to some investigators who have been involved with some of the CFS cohorts out on the West Coast, independent from the Incline Village cohort. Of course, the term “well-pedigreed” may be a bit of a misnomer with CFS, because there is a rather wide definition. But our understanding is that, based on the revised criteria of the CFIDS Foundation , some of these samples that we hope to get may, in fact, fall into this new criterion of definition. In that sense, perhaps they are more rigorously defined as CFS patients.

But I agree that the “well-pedigreed” is in some ways a little bit subjective definition. That’s the best answer I can offer at this time, not being a CFS treater myself.

DR. HOLLINGER: If there are no other questions, we’ll move on. The next speaker will be Dr. Graham

Simmons, from Blood Systems Research Institute, who will give us an update on Blood XMRV Working Group studies.

Agenda Item: An Update on Blood XMRV Working Group Studies

DR. SIMMONS: I'm going to represent the NHLBI-organized XMRV scientific research working group. The mission of this working group is to design and coordinate research studies to evaluate whether XMRV poses a threat to blood safety.

The working group includes representatives of key federal agencies, as well as field leaders in the topics of transfusion medicine, retrovirology, and CFS.

We have four initial aims:

· Firstly, to evaluate XMRV nucleic acid and antibody assays developed by the laboratories represented in the research working group.

· Secondly, to establish the prevalence of XMRV in blood donors.

· Thirdly, using existing repositories, to begin to determine if XMRV is transfusion-transmitted.

· Finally, to determine if transfusions are associated with a disease outcome.

This is example of the roster. It's chaired by Simone Glynn of NHLBI and co-chaired by Jerry Holmberg. As I said, there are six collaborating laboratories in the

working group that have designed and are using assays, including myself at BSRI and the CDC group that we have heard from, as well as Indira's group at FDA, the other group at FDA, the Lo lab, which has been able to detect XMRV in chronic fatigue patients, and John Coffin's lab at NCI, and Judy's lab at WPI. At least from our end of things, the funding is coming from NHLBI.

We divided the work into four phases. The second phase will be some pilot clinical studies to really see if the samples we have in our repositories will be useful for looking at transfusion transmission of XMRV. The third phase will be an expanded clinical sensitivity/specificity panel of pedigreed clinical samples to really look at the clinical sensitivity of the various assays. Fourth will be the actual blood donor clinical panel to begin to estimate XMRV nucleic acid prevalence in blood donors, as well as also initiating some seroprevalence studies.

The first phase is really to design some analytical panels, to really compare the different assays in the working group, comparing limits of detection and accuracy of viral load studies, et cetera, and also to standardize performance of future assays that may become available.

Again, because we were going to apply these assays to existing repositories, which exist mainly as

either plasma or frozen whole blood, we really designed these analytical assays similarly. We either spiked in XMRV-positive cells, the 22Rv1 cells, which were shown by Dusty Miller's group (phonetic) to contain at least 10 copies of XMRV -- spiked into whole blood. Similarly, plasma from these cells, which a number of labs in the working group estimate the viral load to be about 5 x 10^9 RNA copies per ml -- were equivalently spiked into plasma.

This just describes the two panels. For the whole blood, we used just a single whole blood unit from a single donor. For the plasma, we had two plasma component units. These were pedigreed as negative by nucleic acid, by ourselves, CDC, and Judy. She also pedigreed them as far as culture-negative, and CDC and Frank Ruscetti at NCI pedigreed them for being serologically negative.

We then spiked in the cells that are supernatant into the two panels, did serial dilutions, and demonstrated here, starting 9,900 cells for the whole blood, a quarter of a million copies per ml of the plasma. Each dilution was represented in triplicate. We also had six negatives. There are two blinded panels of 36 samples each to the participating laboratories.

There are a lot of details on here that I won't bother going through. These are the participating labs' whole-blood assays, the six labs, using various different

extraction methods. Most of these assays were the assays that these labs have used in previous studies, including the published ones, apart from the WPI's assay, which is a quantitative PCR rather than a nested PCR. I think for the other labs -- the CDC, ours, and the two FDA labs -- it's more or less the assays they were using in the previous studies.

Similarly for the plasma panel. In this case, there were five participating labs. Most of the labs use ultracentrifugation in order to concentrate the virus in the plasma before extraction. The NCI also used an internal standard of spiking in mouse sarcoma virus.

These are the results of the whole-blood panel tested by the six laboratories -- again, replicates of three. Red is three out of three replicates are positive, orange is two out of three, yellow is one out of three, and then green is one out of six of the negative controls being positive. As you can see, all the labs demonstrated pretty good detection limits, everything detecting at least 13.6 cells per ml, which correlates to about 130 proviral copies per ml. Four out of the six labs were somewhat better. Unfortunately, the dilution series wasn't sufficient to really do statistical analysis to work out the actual sensitivities, but it looks like the CDC lab performed particularly well, the other three slightly less well, but

still being able to detect to the end of the panel. The FDA Lo lab had one false positive, which turned out to be upon subsequent sequencing a nonspecific band of human genomic collagen. The WPI lab also had one out of six positive.

Similarly for the plasma panels. There is general agreement with the sensitivity. We have to take these copies with a pinch of salt, because obviously it's kind of a circular process. The same assays are used to determine what the input copies per ml were. It may be not be entirely accurate. But basically all the five labs were able to detect RNA 80 copies per ml. The FDA Lo lab, which developed this assay specifically for this test -- it wasn't previously used in the plasma assay -- was somewhat less sensitive in terms of getting all the replicates positive.

To conclude the Phase I studies, all the Western blot assays detected at least 136 proviral copies per ml, and four out of six of the assays were even more sensitive. As I said, the study was too small to conduct statistical comparisons. The whole-blood panel lacked sufficient dilutions to each endpoint. Four out of five of the plasma RNA assays also performed similarly, with limits of detection of at least 80 RNA copies per ml.

The one major limitation we have to point on this

panel is that, due to availability, we were using the 22Rv1 cells, which are clinically infected with the 5062 prototypical XMRV isolate made by Dr. Silverman's lab from prostate cancer. It's possible that this may not adequately represent the diversity of XMRV chemical isolates in blood in patients. So further work on analytical panel development definitely needs to be performed.

Going to the Phase II pilot studies, which, as I said, were developed in order for us to determine if the existing repositories we have -- donor/recipients and other repositories -- which are mainly composed of frozen blood and/or plasma or serum -- if these would be adequate for looking at transfusion transmission of XMRV. We wanted to compare whole blood versus PBMCs versus plasma.

Similarly, just in terms of processing the samples, both for our preliminary clinical panel and also for these repositories, there is usually a delay of between one and four days before the samples are processed. We also wanted to just make sure that this didn't affect the sensitivity for XMRV detection. We designed a pilot study in order to look at the time to processing of the samples.

Judy at WPI collected four samples from chronic fatigue syndrome patients. These had all previously been identified as XMRV-positive in the Lombardi *et al.* paper by

PCR, serology, and viral isolation. She separated the samples and either processed them immediately or after two or four days. Then each sample was processed into PBMCs, whole blood, and plasma. Then it was distributed to the CDC labs, as well as Joe Coffin's lab at NCI, and Judy is also processing these samples. We also retained one copy at BSRI for future testing.

This is the first example where the CDC lab also will be testing by NAT assay some of the validated clinical samples from the WPI for XMRV virus. That will also be an interesting side result from this pilot study.

I believe most of the labs actually have results back from the study, but we haven't actually gone through the results yet. Hopefully in the next week or two, we should have some preliminary results from these studies.

The Phase III study is really an extension of the last study. We are going to include more clinical samples from the WPI. Judy will collect 25 samples. All will be from patients reported in the Lombardi *et al.* study to be positive by multiple techniques. Then we are also validating a larger number of negative samples as this is initiated. Some of the PCR detection assays, as we have shown for one of the false positives in the analytical panel, may amplify nonspecific human-derived genomic sequences. So we wanted to make sure there was more

diversity on our negative panel by including at least 10 negative donors, which would then be multiple replicates of each to get up to 30 negative donor samples in each panel. As I said before, these have been pedigreed negative by various means by the participating laboratories.

Finally, the Phase IV study: At BSI we have a collection site in the Reno-Tahoe area. We decided to collect a number of donations from this site, as there is clearly a high incidence of chronic fatigue syndrome in the Northern California and Nevada regions. We have collected nearly 400 samples that were given by apheresis. So we have a large number of replicates of both whole blood and plasma for this donor panel. These aliquots have been anonymized. We are now putting together a panel which will consist of approximately 300 blood donor samples from the Tahoe-Reno area, including 25 confirmed XMRV-positive samples from Judy and 30 pedigreed-negative samples. Then these panels will be blinded and again distributed to the participating laboratories. At least four laboratories will assay the whole blood and four will assay the plasma samples. We hope to correlate the results between the whole blood and the plasma testing within the laboratories and between all the different participating laboratories, and get a preliminary XMRV prevalence in blood donors. Obviously, this is still a fairly small sample set at 300.

Future studies hopefully will expand this to even more numbers.

This is the ultimate goal, to tap into some of these repositories which are already existing, such as the REDS RADAR study, TRIPS study, which have both donor and recipient samples, many of both plasma and frozen whole blood, and start to look at transfusion transmission of XMRV in these repositories.

Thank you.

DR. HOLLINGER: Questions? Dr. Ragni.

DR. RAGNI: In your samples, how do you determine the sample size that you are going to use? I'm just curious. Do you do sample size calculation for each of those? I just don't know how you determine that.

DR. SIMMONS: Mainly it was determined on how many samples the participating laboratories were willing to accept. Obviously, we would like to do more than 300, but at this time no one has any high-throughput methods of processing the samples. So 300 was decided to be the maximum that people could accept.

DR. HOLLINGER: Thank you.

The last talk for today is going to be by Dr. Stuart Le Grice, from the National Cancer Institute, who will talk to us on an update on NCI XMRV assay development.

Agenda Item: An Update on NCI XMRV Assay Development

DR. LE GRICE: Since I have been asked to provide an update on the NCI's efforts in assay development, I won't be giving the results of any one study, but just showing you how we have put our efforts into developing a number of complementary assays over the last nine months or so.

This is just to give you the scientific perspective of XMRV over the last three years. We have gone from one paper in 2006, dealing with prostate cancer, to 32 papers in 2010. The right-hand side of this slide in my last presentation, actually, had a fruit bowl. That was how we were describing the current status of XMRV assay development about six months -- that there was no standardized assay, there was no good serological assay, no good nucleic acid-detection assay. This is why we decided at the NCI to use our in-house resources to develop nucleic acid, serological, and virus culture assays.

Just to give you an idea of what we have been doing over the last year, this started in 2009, July 2009, when we called a meeting at the NCI of both extramural and intramural scientists to discuss the public health impact of XMRV infection. We then established a planning committee within the NCI, in October of 2009. I

implemented two mandates. One was to get a variety of complementary assays ready that we felt confident with. The second issue was to make reagents available to the scientific community, so that they could use our reagents, reagents that we felt were standardized, for their own purposes. I want to give you an update on how we worked on both fronts.

You can see that over the last six or nine months, we have managed to develop single-copy assays for XMRV DNA, XMRV RNA. We developed serological assays. Clearly, the speed of any of these assays is controlled by its slowest component. I'll get to that with some of our serological assays. We have now over the last months moved through DNA and RNA nucleic acid testing to serological assays.

I also want to deal briefly with this issue, a very sensitive XMRV indicator cell line that we developed that allows us to detect the virus within three days of culture.

Finally, down here, this is how we are trying to serve the extramural community. Just recently, 64 XMRV expression clones have been distributed to the AIDS reagent repository, and these are available free on request by any intramural or extramural researcher. That will obviously be complemented with protein reagents, but this is Phase I.

So how can we do this within the intramural program? I thought it was important to bring to your attention that the ability to develop these complementary techniques actually builds on an NCI HIV effort which has been ongoing for almost 25 years. That means that the infrastructure was in place, and this required minimal reprogramming of personnel and resources from our HIV program to our XMRV program. What do I mean by that?

The Protein Expression Laboratory at NCI-Frederick is charged with producing recombinants proteins for any project that is requested. It was a very simple matter of reprogramming so that this lab would prepare all eight XMRV antigens. I'll talk about those later.

The Viral Technology Laboratory has been involved in serological diagnosis of multiple viruses. It was a relatively simple step to take proteins developed here and move them over to the Viral Technology Laboratory for serodiagnosis.

The AIDS and Cancer Virus Program was originally the AIDS Vaccine Program. It was charged many years ago with large-scale production of HIV. Again, it was a relatively simple matter of moving from large-scale HIV production to large-scale XMRV production, as a source of what we might call natural viral antigens.

The HIV Drug Resistance Program has been in

existence now for 11 years. One of their primary goals was to develop highly sensitive HIV assays. Again, those assays have been modified to develop what we call X-SCA, which is a single-copy XMRV DNA and RNA assay.

Finally, the Laboratory of Experimental Immunology and the HIV DRP have worked together to develop virus culture assays.

So that is the infrastructure that we had in place that simply required a little bit of modification to allow us to very quickly move from HIV to XMRV.

I want to start with the work of the Host-Virus Interaction Unit of the HIV Drug Resistance Program. Several years ago, they developed a single-copy assay for HIV. They were charged with developing an equivalent assay for XMRV. I think you can see here some of the amplification curves. They amplified between 10^6 copies of virus down to one copy. Three ultrasensitive assays -- the HIV single-copy assay, developed at the NCI, is regarded as the gold-standard assay. We now believe we have an equivalent assay for XMRV.

A point I want to make as well is that the Host-Virus Interaction Unit is not only responsible for developing the assays, but implementing them. These assays have been transferred to laboratories in Sweden, Australia, Vietnam, South Africa. Developing the assay is one thing,

but transferring it to a laboratory where it can be reproduced is clearly important when we are talking about a single-copy assay. Contamination is a huge problem. So the ability to transfer these reagents is very important.

This was just referred to in the last talk, that the NCI has been part of a recent analysis. The outcome was that in this analysis of the 72 blinded samples we were given, we could detect XMRV to single-copy sensitivity. It was detected in plasma and whole blood with 100 percent accuracy, and there were no false positives or negatives.

In future, the Virology Core is going to use is going to use the X-SCA assay to detect XMRV nucleic acid in a panel of samples tested across multiple platforms.

Serodiagnosis: This was initiated with the Protein Expression Laboratory. What you are seeing here is the purification of all XMRV antigens. This project got the green light on October 23. By December 6, all antigens had been purified to homogenate. As I said, the speed of this assay relies on the speed of its slowest component. The most difficult was the surface glycoprotein. This took a little while to express in eukaryotic cells, but is now available in multi-milligram quantities.

We started by preparing all XMRV antigens. We didn’t know which one we would put into our serological assay. We didn’t know which combination would be best. We

felt it was better to have all antigens and test them individually or in combination.

It's also possible at NCI-Frederick to culture virus in large -- the ideal viral antigen is actually virus itself. What you are seeing here is a culture of XMRV. This is done by the AIDS and Cancer Virus Program, large-scale virus production. We are seeing here high-performance liquid chromatography fractionation of XMRV proteins expressed in these cells. The proteins that people are most interested in are p30, the capsid protein, and gp70, the surface glycoprotein. Taking these cells now and simply inactivating them and breaking them up and separating them by gel electrophoresis, you can see in here all of the XMRV proteins. Then probing those with antibodies to murine leukemia virus capsid -- you see the XMRV capsid -- or with MLV gp70, we see the surface glycoprotein of XMRV.

I should say, at the same time, a panel of monoclonal antibodies is now being developed to increase the sensitivity of our serological analysis.

These are the questions we would like to answer:

· Obviously, the general prevalence of XMRV.

· What do we define as reactive and non-reactive?

· The levels of antibodies in XMRV-positive subjects.

We developed a so-called training set at NCI-Frederick. This is 116 samples from the research donor program of NCI-Frederick -- we have donor plasma -- and also subjects from the Lombardi *et al.* study.

We assess the utility, then, of eight individual XMRV antigens.

This is the result of our serological analysis with this small panel. This is a chemiluminescence assay. We see reactivity to the capsid protein of XMRV, the transmembrane protein, the surface glycoprotein. I'm not sure how well you can see this. You will see, for example, the triangle here comes up well when we assay with capsid. It does not come up well when we assay with the transmembrane protein or when we assay with the surface glycoprotein. So there are obviously different levels of antibodies in serum. This poses a slight problem for us.

However, to summarize our initial serological work, we see good reactivity with the capsid protein -- this is an internal protein of the virus -- the transmembrane protein, and the surface glycoprotein. We see reduced reactivity to p12, the matrix, and the nucleocapsid protein. These are some of the structural proteins of the virus.

What's under way at the moment is the inclusion of antigens reactive to antibodies into a scoring

algorithm. What do we mean by that? With the reagents we have, how do you define “positive”? Is it reactive to one antigen, two antigens, multiple antigens? We have to define a criterion for positivity using these assays. Again, what we will be doing now is confirming these results using Western blot and PCR. Again, the Western blot will make use of whole virus, as, in our opinion, the ideal source of viral antigen is XMRV itself. These are purified in tens-of-liter amounts. But it’s in the Cancer Virus Program, which is -- it’s a frightening amount of virus, but it’s routine work for this group at the NCI.

I would like to finish with the last test, which is just coming online, and that is the ability to culture virus. There have been a lot of issues in the literature with the ability to detect protein, detect antibodies, not detect DNA. What does that mean? It seemed to us that the best method of detection was virus itself -- in other words, develop a virus culture assay. This is going back to the original work of the Lombardi *et al.* paper, who actually managed to culture virus in the blood of their human patients.

At the NCI-Frederick, we developed an indicator cell line which we have designated the DERSE cell line. The important thing is that, until this time, growth and detection of XMRV itself took approximately three weeks.

The DERSE cell line reduces the three weeks to three days. I have been told recently that we have managed to improve even on that. So we hope soon to have a test available that within one to two days, can detect infectious virus -- not viral DNA, RNA, or protein, but infectious virus itself.

Again, this has to be modified a little bit -- detected in EDTA- and heparin-containing samples. In the fall of this year -- this only is a legal issue -- we plan to deposit this DERSE cell line with the NIH AIDS reagent repository. So again, another set of reagents will be available.

How does it work? Here's a little cartoon here. The indicator cell line contains the gene that encodes normally fluorescent protein. In the indicator cell line, this protein is interrupted, and therefore it's no longer fluorescent. Once we infect with XMRV, once a virus infects and replicates in this cell line, the progeny cells now remove, for today's talk, the little gray part in the middle, it reconstitutes the green part, and these cells fluoresce. It's a very simple test. It's actually quite an old test in terms of retrovirology. It's what we call a reverse intron. This is a cartoon.

This is it in real life. At three days after infection, we have these bright green cells. As I said

before, we have a system in the pipeline now to reduce this even further, to one or two days to detect infectious virus.

So where are we going at the NCI? This is another study that has just been established using what we call the NCI cancer panel. This is a collaboration with the Urologic Oncology Branch at NCI-Bethesda. Its goal is to take all of the assays that we have developed -- our primary interest at the NCI is prostate cancer. We are collecting prospectively 100 samples. We are collecting serum. We are collecting plasma. We are collecting tissue. This will be analyzed in-house for XMRV DNA, XMRV RNA. Serologically, using an ELISA, we will look for XMRV antibodies. We will also complement this by a Western blot analysis. A virus culture assay will be done using the DERSE cell line. Immunohistochemistry will be done, both at the NCI and Johns Hopkins University.

I think most of us understand that this has been a very controversial area. Swapping reagents, swapping samples is obviously important. We feel it’s important to start with six assays in-house. Very simply, if we have a problem, I think it’s important that we sit amongst ourselves and try to understand where our assays have the problem, before we send those out to anybody or before we disagree with anyone else’s assay.

So, as I said, these are six complementary assays that will be under way, we hope, by fall of this year, to look at 100 prostate cancer samples.

With that, I would like to just thank the people at the HIV Drug Resistance Program and SAIC-Frederick. This is the virus culture assay, the nucleic acid detection. This is large-scale purification of viral antigens, either as recombinant or from virus. Rachel Bagni has been involved in developing the serological assays. This is the group that is involved in large-scale virus culture, and down here, Kathy Jones and Frank Ruscetti have been involved in the virus culture assay.

Thank you. I'll take any questions.

DR. HOLLINGER: Questions?

(No response)

I hope you can get this licensed by the FDA, these techniques.

DR. LE GRICE: I think first we will compare it with the FDA's assay. This is not the XMRV assay. Our goal is to develop a series of assays that we feel confident in and test those head to head with other assays. I think that is really important at the moment.

DR. HOLLINGER: A very nice approach. Thank you, Dr. Le Grice. We appreciate that.

If there are no other questions from the

committee, I think we're going to open this up to an open public hearing. There is something I need to read here. We have at least one person who has asked to speak from the floor. Then we will see if there are any other questions.

Both the Food and Drug Administration, FDA, and the public believe in a transparent process for information gathering and decision making. To ensure such transparency at the open public hearing session of the advisory committee meeting, FDA believes that it is important to understand the context of an individual's presentation. For this reason, FDA encourages you, the open public hearing speaker, at the beginning of your written or oral statement, to advise the committee of any financial relationship that you may have with any company or any group that is likely to be impacted by the topic of the meeting. For example, the financial information may include the company's or a group's payment of your travel, lodging, or other expenses in connection with your attendance at the meeting.

Likewise, FDA encourages you, at the beginning of your statement, to advise the committee if you do not have any such financial relationships.

If you choose not to address this issue of financial relationships at the beginning of your statement, it will not preclude you from speaking.

With that as a background, Mr. Nicholas Caddy (phonetic) has asked to address the committee. He will be given five minutes for his presentation. Mr. Caddy?

Agenda Item: Open Public Hearing

MR. CADDY: Good morning. I would like to thank you for giving me the opportunity to speak.

I would like to talk about the importance of blood safety, and specifically the deferral of MSM from donating blood.

My name is Nick, and I contracted HIV from a blood product in 1987, when I was 3 years old. For 15 years, I had a single-digit CD4 count, which means I was an end-stage AIDS patient for most of my life. My virus proved impossible to treat, even after the advent of HAART. As my doctors joked, the good news is, you can't get any worse.

I'm finally healthy, and only now do I understand just how sick I was. It was all I had ever known.

Many people have presented this deferment as an issue of civil rights. But Oliver Wendell Holmes once said, "The right to swing my fist ends where another man's nose begins." We have to consider the right of every person who requires blood products to have the safest possible treatments.

Regrettably, MSM experience rampant

discrimination in this country. However, this is not an instance of that discrimination. Giving blood is not a right. We can all agree that I certainly have no such right. Having experienced HIV-based discrimination throughout my life, I empathize with anyone who feels stigmatized or rejected by this policy.

I am dismayed to see that this issue has been politicized, when it should be ruled by science. There is scant scientific data to support overturning this policy, and the data that exists is troubling. According to the CDD, MSM account for nearly half of all HIV-positive people in this country and more than half of new infections, despite comprising 4 percent of the population. One study found that half of HIV-positive MSM did not know they had HIV. Now we are told by the Red Cross, the blood banks, those in the business of selling blood -- and I assure you, it is a business like any other -- that lifting the deferment is safe enough -- the same industry that 30 years ago balked at the cost of testing blood for pathogens, the same industry that told my parents and many like them that the blood supply was safe enough.

Today we are told that there is sufficient testing available to make the lifetime deferment unnecessary. Yet this does not account for human error. In the last seven years, the Red Cross has been fined

repeatedly by the FDA for failing to follow safety procedures in the collection and distribution of blood.

I agree that there are inconsistencies in the current policy regarding so-called high-risk groups. Perhaps there is a subset of the MSM population that would be good candidates for donating blood, and when there is scientific data that shows that lifting the deferment would not increase the risk of viral transmission, and only when, can we lift it in good conscience.

At the ACBSA meeting last month, the very people arguing that we should lift the ban said doing so would increase risk by only a small amount -- only. In statistics we use abstract ratios, like 1 in 10,000 or 1 in 1 million. It's far too easy to forget this: That one is a human being. That one is somebody's brother or mother or spouse or friend. Two decades ago, that one was me.

I'm here today to ask that you do everything you can to ensure that what happened to me never happens to another 3-year-old.

Thank you.

DR. HOLLINGER: Thank you, Mr. Caddy.

Any comments from the committee or anyone else?

(No response)

Are there any other individuals in the public section that wish to address the committee or the FDA at

this juncture? Yes, please. State your name, affiliation, financial relationships, et cetera.

MR. LANSON: Thank you. My name is Joe Lanson (phonetic). My financial interest is my own, as I am slowly spending my savings, unable to work full-time, as a CFS patient.

I ask very simply for, very soon, the release of the Alter and Lo data and other papers that may be pending.

Thank you very much.

DR. HOLLINGER: Thank you.

Is there anyone else?

(No response)

We have had some very interesting discussions this morning, some very interesting updates, as well as the informational presentations on XMRV, which have gone through the pathogenesis of the disease, the virus itself, some of the risk assessments and analyses that are going on, some of the new testing that is being introduced. Obviously, there is much work still to be done on these issues.

We are early, but we are not going to change when we start the second session, since there will be some people who will come in for that particular time period. So the next session will start at 1:30. You can go shopping, help the economy. We will start at 1:30.

I want to thank all the presenters today for their presentations.

(Whereupon, at 11:50 a.m., the meeting was recessed for lunch.)

AFTERNOON SESSION

DR. HOLLINGER: Good afternoon, everyone. Welcome to the second session today. This session is going to be of risk of *Babesia* infection by blood transfusion and the status of the laboratory tests.

There are actually three questions here, but there will be one question that the committee will be asked to respond to with a vote. I'm just going to tell it to you right now so you can be prepared when you are listening to this. The question is going to be, do the FDA risk analysis and the available CMS and CDC data sets together support the concept of regional testing of blood donors for *Babesia* infections?

That's the question that this committee will be asked to vote on. Then there are a couple of other questions for comments and so on that we will deal with.

Before we start, though, Dr. Epstein has a few words he would like to say.

DR. EPSTEIN: Thank you very much, Dr. Hollinger.

I have a bittersweet assignment, which is to bid farewell to one of our committee members who is rotating off the committee. This is Dr. Willarda Edwards' last meeting. Actually, there are several other committee members rotating off after this meeting, but Dr. Edwards won't be with us, for whatever reason, tomorrow, so I would

like to take this opportunity to thank you publicly, invite you up and present you with a plaque to thank you for your service. FDA is well aware that it is quite an effort that the committee members put in.

Once again, thank you.

(Applause)

DR. HOLLINGER: Thank you, Willarda, for your participation in this committee also.

I thought Jay was going to fire me.

Let's begin the session today. The first talk is going to be an introduction and the status of test technologies for *Babesia*. Dr. Sanjai Kumar is going to start this off.

Agenda Item: Topic I: Risk of *Babesia* Infection by Blood Transfusion and the Status of Laboratory Tests

Introduction and the Status of Test Technologies for *Babesia*

DR. KUMAR: Good afternoon. Thank you, Dr. Hollinger.

As you might have noticed, the title of my talk has slightly changed. I'm going to start this session on *Babesia* and lay the groundwork for this.

Here is the issue before the committee: Based on the risk analysis, do the available data support development of a regionally selective donor screening

strategy to reduce the risk of transfusion-transmitted babesiosis? That's the first issue.

The second one is, please comment on the suitability of donor screening either by a nucleic acid-based test, an antibody test, or both, given the current technology limitations.

Having that out of the way, I guess a bit of background is in order.

Babesiosis, a tick-borne zoonosis, is caused by infection with intraerythrocytic protozoans of genus *Babesia* that belong to phylum Apicomplexea. There are some very famous partners to Babesia in this phylum Apicomplexea, the most famous ones being malaria and *Toxoplasma*. Those are the parasites of great global public health and economic value. But I think *Babesia* is getting there, too, really, catching up with them.

In the U.S. the most prevalent species is *Babesia microti*. The other species have also been documented in the U.S. Dr. Barbara Herwaldt is going to tell you all about those. But I would just like to make a few points. There are important distinctions with the *microti* and these other species as well. For example, in terms of the tick vector they use for transmission, they are phylogenetically distinct from *microti*. Also the disease they cause in the host in terms of the virulence profile and the pathogenesis

is quite distinct, too.

The reason I am talking about this is, as the ecology and the infection rate caused by these other non-*microti* species grow, they will have important relevance in terms of donor --

A bit of lifecycle here. Apicomplexensis involves an invertebrate host, which is a tick here, in the case of *microti*. We see *microti* is a deer tick -- here is the natural host, white-footed mouse, which supports the -- infection here, while the sexual cycle happens in the tick here, which, after sexual zygote formation in the gut, produce esporidites(?). Those esporidites are inoculated during the blood meal, either in a mouse here -- this is the white-footed mouse -- this incidental host to human, that again go on to produce this pyriform blood -- parasites. Then here, this question of transfusion-transmitted babesiosis -- that's why we are here today. The deer, although they don't get infected, are really important in the whole picture, because they provide blood meal, sustain these ticks, and also provide transportation much faster than these ticks can do on their own.

Some more background here. Endemic transmission for the last many years has been demonstrated in northwestern states, the Mid-Atlantic, and upper Midwestern states, including the states listed here. That's where

natural transmission has been documented. There are several other states which are not recognized prevalence areas, but they have also reported babesiosis cases due to infection. Those are acquired during travel to endemic areas.

The clinical range of the disease is from silent, asymptomatic infection in many individuals to mild flu-like, to life-threatening severe disease. The severe disease mostly happens in neonates, immunocompromised aplenic persons, and elderly. Those are at the highest risk.

We have the dubious distinction of having the highest number in global terms of any other country -- highest number of clinical cases and transfusion-induced infections. Also currently babesiosis is not a reportable disease in all the states, and also is not a notifiable disease. But I think Dr. Barbara Herwaldt may have some update for you on that.

This is the geographical distribution of babesiosis. The way we did was -- it’s not a reportable disease in all the states. It’s not a notifiable disease. So there is no national surveillance system. So the hard data to look at the clinical cases nationwide is hard to come by. We had to look at the picture for the entire country to do follow-up. Colleagues at the Office of

Epidemiology needed hard data for each state and nationwide data. Dr. Mark Walderhaug and his colleagues wanted the data. They relied on the CMS data set. These are babesiosis beneficiary claims filed in the states. He was able to collect data for the last three years. He is going to tell you a lot more about the data-mining methods that he used.

Here are the CMS data for 2008: total, 1,453 cases. But the thing to look at here, the important thing, is that the states which showed the maximum number of claims here, with a few exceptions -- for example, California here -- in most of these states these are known, established babesiosis cases. So that gives us some sort of confidence in terms of the data that we got from CMS data set that it is a true reflection of babesiosis in this country.

Here are the northeastern states, Mid-Atlantic states here, and Wisconsin here, California here, Florida. Cases were seen, actually.

Looking at the transfusion transmission of babesiosis, the first case was reported in 1979, perhaps published a year later. Since then, more than 100 cases have been reported. That came with 11 deaths as well. The majority of cases have been caused by *Babesia microti*, but a few non-*microti* cases have also been reported. I think

Dr. Herwaldt is going to discuss that a lot more in detail.

It's a particular concern in neonates also. I'm just showing here two instances. That is not to say these are the only two instances that occurred. In one case, in 2007, at a Rhode Island hospital, three neonates got infected from a single donor. A similar thing happened in a Virginia hospital. Three neonates were infected from a single donor, just because the blood volume that is transfused in neonates is so small. This illustrates how wide this problem is and how much these silent infections -- how much problem they cause.

While underreported, *Babesia microti* is still one of the biggest challenges to blood safety and most frequently reported transfusion-transmitted infectious agents.

Looking at the death cases -- you may have seen this data before -- the last prior known was case in 1998, then nothing for the next six years, but in the last five years, we had 10 cases here. That is again to say how this problem is going. Especially in these transfusion recipients, which probably have other underlying causes, *Babesia* is causing more severe consequences.

The *Babesia* transmission is regional, while transfusion transmission of babesiosis risk is systemic. The most transfusion transmission is acquired in the same

region where blood is collected and used. However, TTB has occurred in non-prevalence areas from donors who had traveled to a recognized endemic area. When we looked at these fatality cases, among nine fatality cases, what we found was that the four implicated donors and five recipients did not live in an endemic area. This is data from American Red Cross Hema-Vigilance. Seventeen antibody-positive implicated donors were implicated in causing transfusion-transmitted babesiosis. Eleven were from endemic areas, while four were from non-endemic areas. So what really clearly shows together here is that the infections in these donors who were implicated in causing transfusion-transmitted babesiosis, including the ones which cause fractal(?) infections in this -- were acquired in non-endemic areas. That makes it very difficult to define where the risk of *Babesia* may come from.

Donors who normally reside in endemic areas may donate elsewhere. Also there is very solid data to show that also. The blood products are often shipped between widely separated regions across the U.S. Shipment of blood is very well known to this blood establishment community.

This is from Dr. Barbara Herwaldt. She is going to talk about this much in detail. But just to give you a snapshot, based on her data, between 2004 and 2008, when this data was collected and used, there were 63 cases of

transfusion-transmitted babesiosis. Again, most cases happened in the states where the disease is endemic, northeastern states, Mid-Atlantic, and the upper Midwest states. But the infections occurred in some other states as well -- California, Texas, and Florida. In some cases, obviously, the infections were acquired, as is definitely the case here in Indiana and Ohio. It's known that infection was acquired elsewhere. But it just shows how systemic the problem is also.

Clinical presentation: The signs and symptoms of transfusion-transmitted babesiosis are no different from natural infection, more or less the same. As I said earlier, it may remain asymptomatic or present as a flu-like infection or fulminant febrile illness, usually within one to nine weeks post-transfusion and maybe up to three months. I have seen one report goes up to six months also. The fatality rates may be a high as 20 percent, and possibly more. There are the underlying causes, such as -- or immunocompromised.

This flu-like illness is accompanied by hemolytic anemia. That's a characteristic feature of the disease. Altered mental status, in some fractions, coma, renal failure, pulmonary compromise, ARDS and DIC are all seen in severe cases.

This is what the established data looks like.

This is mostly extracted from the *Babesia* workshop we had in 2008. Around 16 percent of the population is known to live in endemic areas. These are basically seven states. Those are Dr. Alex Cable (phonetic). He's among us here today. He has identified that these are the main *Babesia* transmission states here.

But the problem is expanding, and expanding fast, into other areas also.

This is the data which you will hear from Dr. David Leiby. The endemic area of *Babesia* infection varies greatly and is highly localized. So within the endemic state, the disease can be very localized. He is going to present his donor testing experience in Connecticut, so I'm not going to talk about that.

The areas of *Babesia* transmission are expanding, as I said earlier. One point I did not put here is the reclaiming of the agricultural lands now, so more reforestation. Also the crux of the issue is here, increased recognition and diagnosis of babesiosis. That may seem like the transmission is expanding now. So areas which were not known to be *Babesia*-endemic areas -- infections are showing up here. Probably one reason could be better recognition and could be more infected ticks also expanding.

This is the suburbanization, the way we live

now -- increasingly closer human habitat to ticks now, people living closer to wooded areas. Deer populations are growing and exploding. There is no doubt about that. Since these ticks depend on these deer for their food, they are growing as well.

Tick-borne transmission is seasonal, while transfusion-transmitted babesiosis is all year. The main transmission season is known to be between May through September. The peak clinical season is known to be from July through September. But eight of nine fatal transfusion-transmitted babesiosis were transfused between August and December. The data that I did not put here, looking at the data released by Rhode Island Health Department -- some 43 percent of the clinical cases were reported. That's the data within certain years. Forty-three of the clinical cases were reported in July, while only one-fourth of transfusion-transmitted babesiosis cases were reported in July. So the transfusion-transmitted babesiosis does not exactly coincide with the clinical cases. I think that's because of the asymptomatic carriers.

Others have shown it, and Dr. David Leiby is going to show the data that seropositive and parasitemic donors have been found all year round, although he has shown that the antibodies peak between the months of July

to September. But then again, antibody positivity and parasitemia have been seen year round. So the seasonal testing scenario may not be very effective.

What are the cell components that cause transfusion-transmitted babesiosis? Red cells, obviously -- parasite -- have been shown to cause -- and in some cases, whole blood platelets have been caused -- infected red cells -- transmitted babesiosis. In one case blood stored at 4 degrees for 35 days has been known to cause transfusion-induced infection. Cryopreserved red cells can cause infection. The leukoreduction -- obviously, because the parasite is not in leukocytes. Normally the radiation dose used for blood is not sufficient to kill parasites.

What are the current strategies to prevent transfusion-transmitted babesiosis, which are not much and also, obviously, are insufficient? They are using a standardized FDA-recognized donor history questionnaire. Donors are asked if they have ever had babesiosis. Donors with a clinical history of babesiosis are deferred indefinitely. However, most babesiosis is asymptomatic and persists for months to years, rendering questioning largely ineffective. Tick bite history is both poorly sensitive and non-specific. Donor deferral for residence in or travel to endemic regions of the U.S. is impractical, due

to the impact on the blood supply, since you just heard that 16 percent of the population lives in endemic areas and then people travel all the time within the country.

There is on FDA-approved laboratory test available to screen blood donors for *Babesia* infections, and this compounds the problem.

What we have been doing in this regard -- the FDA held a workshop in 2008. Many of you here today did attend that workshop. We have the scientific agenda here. We discussed the biology, pathogenesis, transmission, epidemiology, and so forth. Risk of *Babesia* infection through blood transfusion and blood components was discussed, the status of the current laboratory tests to detect *Babesia* infections in blood donors, what are the possible approaches to minimize the risk of transfusion-induced infections. Then we had panel discussions at the end of each of the scientific session.

Babesia-endemic regions were recognized in several parts of the U.S. The current and more detailed epidemiological maps of *Babesia* endemicity are needed to identify donors, and therefore recipients who are more at risk.

The bottom line here is that these maps need to be current, actually. It’s a moving target, and constant work will be needed to keep abreast of the current

epidemiological status.

There was sufficient data provided, and many speakers emphasized that donors who are asymptomatic carriers with low-grad parasite burden present the greatest risk to recipients. Again, you will hear about this data more by Dr. Leiby. PCR in some studies has been shown to be positive in approximately 50 percent of seropositive donors. Additionally, studies are needed to determine the rate and duration of infection and parasite burden in asymptomatic carriers.

There was general agreement that further studies are needed to determine the utility of available laboratory testing, identifying asymptomatic donors. Then also novel methods are needed that can distinguish between currently infected versus those with past infections. I think that's something that is most needed at this time.

Novel testing strategies are needed to better identify, defer, and potentially reenter *Babesia* at-risk donors. Selective testing strategies may be needed in some areas, screen negative components for use in neonates and other immunocompromised recipients. The Rhode Island Blood Center has just started doing exactly that. They are testing selected donor populations to provide blood for neonates and sickle cell populations. So that's already happening.

Something that came out most substantial from this workshop: Based on recommendations from a panel of experts, a task force was established under the purview of AABB. It's led by AABB. We have members from ABC, ARC, CDC, and FDA. The focus is promoting advancements in donor testing technology. Again, I hear that there will be a presentation in the open public hearing about the work that is done and what has come out from the work being done by this task force.

So what is the nature of the challenge for donor testing for babesiosis? Asymptomatic carriers are the primary source. Infection can persist, low-grade parasitemia, for months or possibly two years or more. We don't know what is the minimal infectious dose that can cause transfusion-transmitted babesiosis. Neither do we know the level of parasitemia that is present. What is the minimum parasitemia that is needed to maintain infection? That is not known. Together, these two problems make it difficult to where to set the sensitivity limit even for direct detection.

There is no recommendation to treat persons with asymptomatic *Babesia* infections. These are silent infections, asymptomatic infections. People could be infected and, in a sense, could be serving as a live human reservoir of infections, actually.

Both clinical cases and TTB cases are underreported. That underreporting is a cause of many problems.

What is currently available? Whatever is available, basically, for malaria parasite is available for *Babesia* also. Direct microscopy is available, but obviously is not sensitive enough for use in donor-screening settings. Blood can be inoculated and amplified in sensitive animal models, but, for obvious reasons, is not good enough for donor-screening purposes. Personally, I believe it's probably not sensitive enough either.

Both a nucleic acid-based test, NAC, and antibodies have been used, based on laboratory tests. But they both have limitations in the way they are currently used in donor screening. There is lack of sufficient genomic/proteomic cross-reactivity.

There is evidence that the tests developed for *Babesia microti*, both IFA-based and antibody-based, and possibly PCR tests, are not sensitive enough to detect non-*Babesia microti* species. I think that's a very important thing to keep in mind. I don't think we have sufficient genomic and proteomic information right now. But some headway must be made in this direction. For example, when developing a NAT-based test, maybe it's better to use primers which work across these different species.

Looking at NAT now, several versions of PCR tests are available. Sensitivity of PCR has been demonstrated as equal to or superior to blood-film microscopy.

I was really puzzled. I looked in the entire literature, talked to people. Nobody has even truly demonstrated the analytical sensitivity of the NAT that has been used, actually, in *Babesia*.

The parasitemia may be very low during the early phase of acute infection. That's the window period. The parasitemia may be again very low during the asymptomatic infections. But, truly, we do not know what is the parasite burden in asymptomatic carriers.

Because of that, in the absence of clinical studies, it's really difficult to truly say that what will be the value of a highly sensitive nucleic acid-based test in interdicting these infected blood donors.

As a result, a few infected red cells may be potentially present in a unit of blood. To be effect, a NAT test must be highly sensitive to begin with, and the limit of detection of NAT for *Plasmodium falciparum* -- so where should be the limit? The limit should be what is biologically feasible and what has been gone. If we look at -- another parasite, *Plasmodium falciparum* -- people are looking at it by using the ribosomal RNA gene, which has five to seven copies for *Plasmodium falciparum*. I don't

know how many copies of ribosomal RNA gene are there in *Babesia microti*. But using that test, it can detect 20 parasites per ml of blood. So that just gives us some sort of clue of what should be scientifically feasible.

Possibly there could be other molecules which are higher copy number, and they may be a good target, actually.

Other things could be done in terms of sample concentrations, since this is an intraerythrocytic parasite. Larger blood volumes could be used to improve sensitivity. Concentration of infected red cells, if that's possible, before testing might improve sensitivity. Then again, as I said, it may be a good idea to become more creative and look for some other novel multi-copy gene targets as NAT.

Antibody testing: IFA, ELISA, and Western blot have been used for diagnosis and epidemiology. IFA is sensitive for acute babesiosis cases. But the biggest problem is -- not need to be even said -- that it's not adapted for high-throughput donor screening. Similarly, Western blot is not adapted for high-throughput screening.

If we look at the literature, the sensitivity -- the antibody positivity goes in the range of .3 percent -- in Connecticut, up to 17 percent. That shows these assays have low specificity. There are a lot of asymptomatic

infections that we don't know about. IgG titers may persist for months or years, possibly, and therefore they cannot be used for distinguishing current from past infections. Then we may be losing some donors because of that issue there.

We certainly need -- if antibody tests have to be of value for donor screening, we certainly need a novel test, but to be effective, this should be highly sensitive, able to detect early window period and asymptomatic infections. It must be very specific, so it should be of low false positivity. It should be adapted for high-throughput donor screening platform.

I'm going to just present very briefly what Dr. Mark Walderhaug is present. So what is the strategy to mitigate TTB risk? Identification of *Babesia* risk area in individual states based on clinical cases of babesiosis, transfusion-transmitted babesiosis cases, and based on FDA risk-assessment model that Dr. Walderhaug will talk about, and an evaluation of current methods for the value as donor screening test. We are proposing a two-phase strategy for donor testing for *Babesia*. Either this is a nucleic acid-based test or antibody test. They must be highly sensitive and specific.

Here is the BPAC agenda here. Next you will hear from Dr. Barbara Herwaldt. She will talk about *Babesia*

epidemiology, about including some testing experience from CDC, and then transfusion-transmitted infection cases she will discuss in detail.

Dr. David Leiby will talk about the American Red Cross experience with testing blood donors for *Babesia*

Dr. Mark Walderhaug will present FDA risk model to analyze risk of *Babesia* infection in U.S. blood donors.

Then I will come back again and present this earlier proposal.

I just want to thank the people who helped with this presentation and with the FDA position: Dr. Nakhasi, Dr. Mied, Dr. Biswas, Dr. Asher, Dr. Epstein -- they are all here -- Dr. Mark Walderhaug, Dr. Forshee, and Dr. Anderson, our colleagues in the Office of Biostatistics and Epidemiology, and Dr. Barbara Herwaldt, from CDC, who so generously gave her unpublished data and transfusion-transmitted cases.

I would just stop here, leave some time for questions, and just leave the questions here. You have seen this, but just to get you more primed with the questions.

DR. HOLLINGER: Any questions for Dr. Kumar? Dr. Bower?

DR. BOWER: You mentioned that there were four implicated donors from non-endemic areas. Do you know if

they had traveled to endemic areas?

DR. KUMAR: They did travel to endemic areas, yes. I didn't say that. They reported travels to endemic areas, yes.

DR. BOWER: Thank you.

DR. HOLLINGER: Any other questions?

(No response)

I think we'll go on, but Dr. Kazmierczak is here. Since you are new to the committee, your name and where you are from.

DR. KAZMIERCZAK: I'm Jim Kazmierczak. I'm the state public health veterinarian for the Wisconsin Division of Public Health. I think I'm here because for years I have been the tick-borne disease coordinator for the state.

DR. HOLLINGER: Thank you. Appreciate it.

We'll move on then to the next speaker. That will be Dr. Barbara Herwaldt, from the CDC, who will give us a talk on the "Epidemiology of Babesiosis, Including Transfusion-Association Infection."

Agenda Item: Epidemiology of Babesiosis, Including Transfusion-Associated Infection

DR. HERWALDT: Thank you very much for honoring me with this invitation to speak to you today. I have been wrestling with a cold, so my voice may sound a bit hoarse.

I am going to be emphasizing basics about

babesiosis -- in a sense, an overview. I'm going to touch on many topics, and I cannot do justice to any of them. I'm not going to be talking about risk or rates or regulations. I think that's very important to emphasize.

I have to include the requisite disclaimer.

Also I want to point out that a lot of what I say may seem self-evident, but it has often been a persistent source of confusion. So as I speak, you may be thinking, "Why is she saying this? Isn't it obvious, or we wouldn't be here today?" It's because I want to underscore again what may seem obvious, but often is missed. Again, I want to focus on fundamental principles and perspective.

Throughout my talk, I'm going to have points and then counterpoints. I want to give a perspective about the bulk and the range. I am by no means implying that everything I talk about is highest priority or will need to be addressed in Phase I. I want to give a sense of the scope of the problem, some of which can't be addressed in the near future and some may need to wait for the longer term.

Starting with point and counterpoint: All of the points on this slide will seem self-evident. Yes, *Babesia* species are intraerythrocytic microbes, but they are not *Plasmodium* parasites. There are similarities and there are differences. I don't have time to talk about either one,

the similarities or the differences. But whenever we extrapolate or generalize, I think it's very important to do it consciously and carefully.

Yes, *Babesia* species are tick-borne in nature, but they are not bacteria. And, yes, they are transmissible by transfusion, but they are not viruses.

I am oversimplifying here. Viruses are the same. But, in essence, what I call and what others call the viral paradigm doesn't necessarily apply.

Te next slide makes some of the same points, but in a slightly different way. Don't assume that ring forms are malaria. Consider babesiosis. You're thinking, why am I making that point here? This whole talk is about babesiosis; this whole session is about babesiosis. Even in the best hospitals in the heart of babesiosis land, babesiosis often is the second consideration. Malaria continues to be the default -- and again, I'm oversimplifying -- but the default to the detriment of patients.

Don't assume the route of transmission is tick-borne. Consider blood transfusion. Obviously, that's why we are here. We will be talking about why, regardless of region or season, even though clearly the risk is not equal.

Don't assume the species is *B. microti*. Consider

other etiologic agents.

Throughout my talk, I'll be focusing on big-picture temporal and spatial dimensions, including with the historical perspective.

Over 120 years ago, back in 1888, Viktor Babes determined that febrile hemoglobinuria in what were called "Rumanian cattle" was caused by an intraerythrocytic microbe. The *Babesia* genus was named after him.

Staying with cattle, but switching over to the United States, a few years later, Smith and Kilbourne determined that Texas cattle fever was caused by a tick-borne parasite, *Babesia bigemina*. This is no longer a problem in cattle in the U.S.

This was the first demonstration ever of an arthropod vector for a pathogen.

Switching back to Europe, over 50 years later, in 1956 was the first documented zoonotic or human case in what we would call the former Yugoslavia. Switching back to the United States, the first documented zoonotic case here was in California. The species wasn't determined, and it's not possible to determine it in retrospect.

In 1969 was the first documented case on Nantucket Island in Massachusetts. It was caused by *Babesia microti*. Ten years later -- so over 30 years ago, 1979 -- was the first described transfusion case.

By the way, every year I give in this slide and the rest of my talk is year of occurrence.

This case was also in Massachusetts. It actually was associated with platelets, though most of the documented cases that have followed have been associated with red cells.

Worldwide, the geographic distribution of reported human cases of babesiosis -- I'm talking about tick-borne -- the bulk have been in the United States, several thousand, some in Europe, several dozen. Recall that the first documented zoonotic case was in Europe, but most of those that have followed that have been documented and reported -- that often means published, though not always -- most of them have been in the United States. So babesiosis is endemic here; it's not an imported disease. There have been a few scattered in various other regions of the world. Of course, in places like Africa, you can imagine that there may be cases of babesiosis that are misdiagnosed as malaria.

Everyone likes maps, and I am going to show a few, but not always with the usual sort of intent. Again, I'm focusing on big-picture issues, including transcontinental and intercontinental issues.

Remember that I said the first documented U.S case was in California in 1966, in an asplenic person.

There was another case in 1979 in California, an asplenic person again, the species not determined. But then California sort of seemed to fall off the map temporarily. Don't forget about California. It's really still there. The movement, figuratively, was from the Southwest to the Northeast.

Here we have Cape Cod, Nantucket. This is all thanks to NASA. I'll be introducing you to some of these other islands momentarily. Some of you know them very, very well, like Martha's Vineyard and Block Island and the end of Long Island here. This is what people often think of when they think of babesiosis. Although I'm going to talk about the fact that the epidemiology and other aspects have evolved, it's not as if these areas are no longer hotbeds. They still remain key areas of concern. And the species I'm talking about now is *Babesia microti*.

This is a real public health notice from the 1970s, the equivalent of our *Federal Register* now. It's like, "Take note," which is what we're doing here today. Ignore the numbers over the islands. It had to do with concern about tick-borne *B. microti* infection on offshore islands and also in some of these states.

One of the recurring themes of my talk and Sanjai's and, I'm sure, David's will be the fact that *B. microti* is not limited to these offshore islands. Remember

that historical perspective slide I showed you. It's like, "Oh, cattle, that's not our problem." "Oh, Europe, that's not our problem." You could think, "Oh, these offshore islands, that's not our problem."

It's still in these places, to varying degrees, but it's not just in offshore islands. It's not just somewhere out there. We'll talk later about the fact that it's not uniformly distributed in these states.

I want to show another map, thanks to NASA, that gives us some perspective, literally and figuratively, about the states and their locale. This is in the context of a hurricane approaching the East Coast. Here again we have Cape Cod and Nantucket, Martha's Vineyard, Block Island, Long Island, and Shelter Island. You can barely make out Fire Island. We have New Jersey coming down here. I think it's very important to keep in mind throughout the talk that when we talk about states -- for a state as big as New York, we are not saying that it's every place in New York. It may be spreading up the Hudson Valley, for example. That is not to say that it's equally present in the magical seven states or that those are the only states in which it's present.

Which is the segue to this slide, which you have seen before. But now I'm making a different point, which you have already heard, which is that tick-borne

transmission of *B. microti* is predominantly -- not solely, but predominantly -- seasonal and regional, particularly, but not exclusively, in evolving foci in the Northeast and upper Midwest. I have listed these states alphabetically; otherwise, no other significance in terms of the list. I really almost hate to show you this slide, but it provides an opportunity for me to reemphasize that when we think of these sort of magical seven states, it's not at all to imply that all of New York should be considered endemic for *B. microti* or that all of Wisconsin or all of Minnesota, or that all of these states had *B. microti* emerge as a zoonotic issue at the same time. Babesiosis is reportable, under surveillance, in these and multiple other states. It will become nationally notifiable in January of 2011. Later, if time permits, I can discuss the distinction, which often is difficult to understand, between reportable and notifiable.

When we think of *B. microti* in U.S. foci, we have the parasite -- and, by the way, it's probably not homogeneous. There may be strain differences. There may be a species complex, particularly when we consider babesiosis in other areas of the world, where there are *B. microti*-like organisms. We have a tick vector and various reservoir hosts, particularly deer mice. As Sanjai has already pointed out, where are the humans? Humans aren't

in this picture. They are incidental hosts. But, unfortunately -- which is why we are here today -- there can be secondary transmission through blood transfusion and other routes.

This point cannot be emphasized enough. The risk for zoonotic transmission is dynamic in time and place. As Sam Telford, an entomologist, has pointed out before -- and he wasn't restricting this remark to temperature -- some years are hot and some years are cool, and predicting the risk for humans on the basis of ecologic data would be very, very difficult.

Also, just as a point to stick in, as you know, don't expect to see ticks. I don't know how many of you know how small the nymphal stage of the *Ixodes scapularis* tick is. This, of course, is before it's engorged. Tiny, tiny, tiny. And, of course, don't expect to see tetrads. You are not going to expect to see them on a donor's blood smear, but even on a patient blood smear they can be few and far between.

I just want to touch on a point that we are going to talk about. Not necessarily me so much, but we will talk about the window period. There is also something called the grace period. For *B. microti* -- it's actually different for *Borrelia burgdorferi* -- let's say this nymph is infected. It has to stay attached to you probably for

48 to 50-some or 60 hours for the blood meal and then for something called sporogony to occur and to develop infective sporozoites. An adult tick has to stay attached probably five or six days for that to occur.

As Sanjai pointed out, he wanted me to discuss other -- meaning non-*B. microti* zoonotic -- agents. I'm just going to briefly introduce them to you on this slide and then talk a bit more about them in the ensuing slides. I'm going to go quickly. The point is not for you to focus on all the individual details, but to have a big-picture perspective.

Since the 1990s, sporadic U.S. cases caused by other -- meaning non-*B. microti* -- agents have been described, such as -- and this is not an all-inclusive list -- *B. duncani*, the WA1-type parasite, and related organisms in several western states, *B. divergens*-like agents, such as MO1, in various regions. These agents -- by the way, I'm calling them agents because not all of them have reached the species status yet. They may in the future. Their geographic ranges, tick vectors, and reservoir hosts largely remain unknown. Of course, it varies somewhat from organism to organism.

A key point is that infection with these parasites is not detected by the currently available molecular or serologic tests for *B. microti*.

Briefly, Taxonomy 101: I'm not going to go through phylum and order. Here we are in the *Babesia* genus. It has lots of *Babesia* species -- lots of them. Reportedly, 100 or so *Babesia* species, quote/unquote, that infect mammals have been described. On the one hand, this could be an underestimate. There are lots of animals that have *Babesia* species, and the more you look, the more you find. On the other hand, some of these could be synonymous. Traditionally, taxonomy has been based primarily on morphology and host specificity. Some of these, if they were looked at with some of the newer tools, might be found to be synonymous.

But what we are talking about with some of these other agents -- well, for *Babesia* in general -- it's a zoonosis. Some agents cross the line -- excuse me, they are not normally zoonotic. They are normally in the reservoir hosts and the ticks, and then humans can become incidental hosts. That's when we call it zoonotic. How often that happens -- this gets very complex. It gets back to some of the issues I alluded to before.

Examples of some of the novel agents -- I'm first going to discuss these briefly. Why am I calling them novel agents and putting that in quotation marks? I've already told you why I'm calling them agents. Not all of them have reached species designation. Novel -- we don't

know if they are new, in the strict sense of the word "new." They may have been around for hundreds and hundreds of years. But they had not previously been described. Of course, there is no bank of DNA from all these other animal species for us to know if these could be a species previously described.

Starting with the WAs -- WA for Washington -- WA1, 2, 3 is the order in which they were recognized. These were true isolates from people obtained by animal inoculation. The patient and the year -- WA1, from the index patient in 1991. So it's easy to remember -- WA1, 1991. The very next case that was documented was transfusion-associated, 1994. This isolate was from the recipient; this was from the donor.

It underscores multiple points, one of which is that you can have a blood donor who is feeling fine and infected with this organism. Although I think Sanjai may have alluded to the virulence issue, we don't know enough about this organism to be able to say if there are big differences in people. There are differences in hamsters and jirds in terms of virulence.

The initial WAs and CAs -- I have already introduced you to WA1 index case patient. Isolated? Yes, an isolate from an animal. Anything in pink is transfusion-associated. WA2 and WA3, 1994. Yes, isolated.

I'm going to stop before I get to the CAs to make a key point, which is that, since this organism was isolated from an animal host, it was possible to develop an IFA test. We do have an IFA test for this organism. But again, since there have been relatively few cases that have been documented, it's not as if we have a good sense of what the threshold should be for reactivity or positivity. There does seem to be more background noise with this IFA test.

For the CAs, CA1, 2, 3, 4 -- again, convenient, 1991, 1992, 1993, 1994 -- DNA only. We -- and whenever I say "we," it's the collective "we," including some of you; these all have been collaborative multi-agency, multidisciplinary investigations -- we were never able to get an isolate in animals. These are all presumed to be tick-borne. These were asplenic patients. These were not.

This could be confusing. These are called CAs, but they behave like WAs. Keep that point in your minds. I'll come back to it momentarily. It's in pink, because this was the second in the series of what we now call *B. duncani*-associated transfusion cases. This was in California in 2000. The donor was infected either in California or Oregon. The recipient was a premature infant, whereas this recipient was an elderly patient.

Again, these are the initial WAs and CAs. There

have been more, and they aren't necessarily just restricted to these states.

This organism -- "this" meaning the WA type -- did reach species-level designation in 2006. Dr. Conrad and collaborators named the organism *Babesia duncani*. This was a description of the organism and its differentiation from other piroplasms. The differentiation was not on the basis of morphologic criteria.

It doesn't matter if you use light microscopy, electron microscopy, it doesn't matter if you carefully measure the organism in terms of all the lifecycle stages found in red cells -- and here is a tetrad, by the way -- it's morphologically indistinguishable. Of course, that's not a big issue for donor screening. But it is a point for clinicians and laboratorians to be aware of.

In general terms, when the species designation *Babesia duncani* is used, that's what we used to call the WA1-type parasite. Often when I write or speak, I'll say something like *Babesia duncani*, formerly the WA1-type parasite.

What was the result of the christening? Old name, new name. WA1, 2, 3 all became *B. duncani*; the CA5 and 6, associated with transfusion, *B. duncani*. These CAs are still called CA1-type parasites.

The only reason I'm belaboring this point is

because it's just so confusing. In the literature often the nomenclature is off-base.

So *B. duncani* is for these organisms. These may be part of the same species complex. We just don't yet know.

Now I'm going to briefly mention the *B. divergens*-like organisms. As I mentioned, these aren't the only novel agents that have been documented.

This is a placeholder term, "*B. divergens*-like." Again, confusion reigns in the literature. To our knowledge, *B. divergens*, *sensu stricto*, does not exist in people or in animals in the U.S. We could be wrong. And I'll tell you about *sensu stricto* in a moment. *B. divergens*-like, in the sense of some morphologic and serologic criteria and phylogenetic relationship at some base differences -- how many do you need, and which genes to constitute a different species? -- but not conspecific in the biologic sense.

Many of you have heard of the MO1 organism, the index case. It occurred in 1992 in Missouri in an asplenic patient. This was a fatal case, unfortunately. The reason we said "another piroplasm" -- again, this is in the same timeframe as the initial WA and the initial CAs. There have been MO1-type organisms that we and others have identified in multiple states. We are not numbering them

anymore. So we'll just keep referring them until we name the organism the MO1 organism.

Babesia divergens sensu stricto -- I'm just going to briefly go over that. In European foci and North Africa, this is a bovine parasite that occasionally crosses over into humans. This is thought to be the tick vector. *B. divergens* versus *divergens*-like -- I have already mentioned the first reported case that probably was caused by *B. divergens*. More than 30 tick-borne cases have been reported, to my knowledge. No transfusion-associated cases have been documented. Most of these tick-borne cases were in asplenic patients, and they were reportedly caused by *B. divergens*. But the type and quality of the evidence varied.

In 1998 and 2000, the index cases caused by EU1, for European Union 1, were identified in asplenic patients in Italy and Austria.

Now, we're not saying that none of these other cases were caused by *B. divergens*, but it gets back to not only what constitutes a different species, but also that the more tools you have in your toolkit, the more you can differentiate among organisms.

The only reason I mention this, even though it's not in the United States, is because it is on the theme of these different organisms and having to rethink what people

thought was fact before.

But back to Washington -- I'm going on chronological order -- in Washington State, a *B. divergens*-like organism was documented in Washington State. So not just *B. duncani*, but a *B. divergens*-like organism, in this case, occurred in the year 2002.

Now back to the basics. I'm totally shifting gears -- truly, basics, fundamentals about some clinical issues and lab issues. Again, you are going to think, why am I even saying these points, because they are so obvious? But these are actually bullets that address questions that I am frequently asked.

First of all, regardless of the route of transmission, *Babesia* infection can range from asymptomatic to severe. I'm not saying they are equivalent routes of transmission. For a lot of you this is obvious, but for some people it's not. Regardless of the severity of the manifestations, they are nonspecific. So diagnostic testing is always required.

Symptoms, if any, usually develop within several weeks or months of the exposure, whether it's a tick exposure or blood transfusion. But they may first appear much later -- for example, after splenectomy. This is not an academic point; this is a real point. It has affected multiple transfusion investigations that, through nobody's

fault, got off on the wrong path until it was recognized that splenectomy was relevant, which then made different blood donors and different transfusions relevant.

Risk factors for severe babesiosis, as you have often heard, include -- and I want to say this five times -- asplenia, asplenia, asplenia, asplenia, asplenia -- first and foremost, if you don't have a spleen, that's not good -- advanced age -- I think everybody in here qualifies -- and then various other causes of immune dysfunction. "Immunosuppressed" is a catch-all term. What does it mean to be immunosuppressed? What does it mean to have advanced age?

I'm not at all minimizing immune dysfunction or immunosuppression. But again, this is not an academic point. Immunosuppressed patients may be afebrile and may have subacute versus acute remitting, relapsing clinical courses. This also has affected multiple transfusion investigations. People have said to me, "Oh, this person is immunosuppressed. He's going to become sick really soon after transfusion or after X or Y." But no. Often -- I say often -- you're on corticosteroids or you're on chemotherapy. You may be afebrile.

I'm not at all saying that people who are immunosuppressed can't have fulminate infection. Absolutely they can. I'm making the other point, just so

it sticks in people's brains, because it is highly relevant to investigations.

These points are self-evident to people in the room. Even persons who have asymptomatic infection or whose symptoms resolve may have low-level infection -- of course, how low do you go? -- for weeks to months, sometimes longer than a year. Of course, we wish we knew what factors influence chronicity.

The bottom line is, people can meet all of the criteria for donating blood despite being infected and infective.

This is a slide from Peter Krause and colleagues in the *New England Journal* that relates to persistent parasitemia after acute babesiosis. Now, that's just one permutation. Some people are asymptomatic the whole way through. This was back in the era when therapy was particularly reserved for moderate to severe cases, and not necessarily for mild cases.

If I recall correctly, he had about 20 people who were treated and about 20 who weren't. I remember they all had acute babesiosis initially. Of course, there was a difference in terms of documented parasitemia. Again, if I recall correctly, he tested them by PCR twice, three months apart, and if they had negatives twice, then he no longer monitored them. So that doesn't exclude the possibility

that they were parasitemic longer. But here you have someone who was not treated, with demonstrable parasitemia up to 27 months. That particular person had an unfortunate story, where he was being monitored every three months up to 18 months and then said, “Enough. No more monitoring.” Nine months later, at 27 months, he became very ill, was hospitalized, fortunately did well with treatment, but a renal tumor was discovered.

Briefly, lab evidence of infection -- just generic points. I’m not going to talk about particular tests, just generic points that again are self-evident to you. And I’m lumping molecular with parasitologic. So basically we have direct and indirect.

Some cautionary notes. Again, they seem so self-evident -- laboratory approaches in different settings, diagnostic testing versus donor screening. And I’m not even talking right now about having high throughput and having all the characteristics you want for a screening test. I’m talking about some fundamental issues that seemed to be difficult to grasp and convey at the FDA meeting in December 2008. For example, a clinical setting is very different from what you might expect in terms of PCR positivity than donors. Again, it seems obvious. You could have a sensitivity that seems wonderful for PCR, if you are testing patients with clinically manifest illness

and/or if you are testing preselected patients who meet various criteria.

Of course, what's the gold standard when you try to compare tests, for a positive and for a negative?

More cautionary notes, again just generically. As most of you know, some commercial labs for diagnostic testing offer serologic and molecular testing, but there are no FDA-cleared test kits for *in vitro* diagnostic use, even for *B. microti*. Testing is done with -- I purposely have this in quotation marks -- reagents that are assembled and evaluated by the individual laboratory. Not surprisingly, there can be inter- and intra-laboratory variability with respect to sensitivity and specificity and in terms of the patient data results, the reliability and validity. I'm not going to dwell on this point, but I think it's important to keep in mind.

Diagnosis, again in quotation marks -- again, I'm not talking about any particular technique, just generically. Direct -- you are detecting the parasite or DNA, but generically, not sufficiently sensitive to reliably detect low-level parasitemia.

Serodiagnosis, indirect -- and, for the most part, we are talking about IFA or immunoblot, in terms of what's available. But I'm not talking about the specifics of the test. Just generically, assuming you are testing

for *B. microti* or whatever, big picture -- overly simplistic, highly sensitive, but may be negative, as you know, very early in infection. We are not worrying right now about the recipients, who may be immunosuppressed, quote/unquote, and may not seroconvert for a while. In donors, we can talk later, if there is time, about how long the window period might be. But I don't, frankly, think that's our biggest concern at the moment. But it does not reliably distinguish active from past infection. Now, of course, that's an advantage in retrospective transfusion investigations, but it's not an advantage in donor screening.

The sensitivity of parasitologic methods, again just generically -- you might think microscopies about here, animal inoculation, assuming you have an animal host, and PCR. Maybe it can become more and more sensitive with some of the approaches that Sanjai mentioned. But the bottom line is that the results are helpful if positive, and negative results don't exclude low-level parasitemia, however you want to define that, with the low concentrations of parasites in DNA and the small volumes tested. That's not just the size of the specimen, but it's the aliquot that's tested, whether it's concentrated or whatever, versus what's transfused.

In transfusion investigations, which, of course,

are very different than donor screening, blood donors typically are implicated on the basis of seropositivity, with or without PCR positivity, and also epidemiologic plausibility, along with the seropositivity.

In our experience -- no surprise -- the likelihood of PCR positivity is higher for segments than for follow-up post-donation specimens, but does not approach 100 percent. Again, it's a generic statement. It wouldn't matter if -- type of PCR, whatever. But I think everyone would agree that it's not 100 percent. Whether it's 70 percent or 80 or 90, depending on the technique and the level of parasitemia -- but it's not 100 percent.

I'm going to end with case count issues -- surveillance, biovigilance.

Again, cautionary notes. Just like people like to see maps, people like case counts, and so do I. How many tick-borne and transfusion-associated cases have been reported to date? I'm purposely putting "reported" in quotation marks so that it covers the waterfront.

Of course, there are issues of case criteria and also how, where, when, and by whom or what the cases were detected, diagnosed, investigated, reported, and tallied. So who is doing the counting, and with what methods?

I like basic tools, so let's get out our abacus and let's think about the log scale. For tick-borne, we're

up in 10^3 in terms of what has been documented, several thousand. Even though babesiosis hasn't been officially notifiable -- and it will be -- we have already had cases for years voluntarily reported to us, and we are in the thousands. But this is without a standard case definition. We are in the 10^2 range for the documented transfusion-associated cases. For these other species that I mentioned and other agents that I mentioned, we are down in here, in terms of what has been documented.

There is typically a story. Whenever I'm called, which is frequent, for consultations about whether it's tick-borne or transfusion-associated, I ask, why was this case detected? Why did someone manually review a smear? Even severe cases often are serendipitously detected.

When we think of the "tick-burg" -- and, by the way, these are not *Ixodes scapularis* ticks, I apologize -- down here, not only are the asymptomatic cases or the cases with mild symptoms that never get diagnosed, but there can even be some people with severe infection, some of whom are never diagnosed, some of whom are diagnosed postmortem, unfortunately. But these people down here, including the donors, then lead to a blood -- so the "tick-burg" and then we have the "blood bag-burg." This is in no way meant to trivialize this problem. This is, everyone here knows, a very, very serious issue. We don't know how many are down

here. We just know, obviously, what has been detected. But what has been detected? There have been too many cases detected, obviously. I think all of us agree that there is a problem.

To date, it's over 100. I'm purposely doing this in big-picture senses, because it depends on how you -- and what you can and strength of evidence, et cetera. So to date, among these, quote/unquote reported cases, most have been caused by *B. microti*. Several were caused by *B. duncani*. Actually, a disproportionate number of the total, when you think about it, were caused by *B. duncani*, and a disproportionate number of the *B. duncani* cases have been associated with transfusion. Now, I'm not saying that that represents reality. I'm just saying that this is not just a theoretical issue. Is it an issue that can be solved tomorrow? No. But it's not just academic.

Blood components: Most cases have been linked to red cells, several to platelets. These are whole blood-derived platelets.

Incubation period: weeks to many months.

As I close and we talk about how to count, this multi-donation cluster of cases in Minnesota emphasizes multiple key points that are relevant, not only to counting, but to a number of the other issues we are discussing. Here is one donor, multiple recipients, multi-

donation cluster. The first donation: The red cell recipient became infected; the platelet recipient did not. The second donation: The red cell recipient became infected. It was the third donation that was associated with the index-case patient. The platelet recipient did not become infected. By the time the index-case patient was diagnosed as having babesiosis and the transfusion investigation was initiated, the donor had already donated again, and the platelet recipient became infected.

This donor was still PCR-positive in April, four months after this fourth and final donation, 10 months post his exposure during a camping trip in Minnesota, in June of 1999.

By the way, when we talk about these endemic states, he lived in Minnesota, but not in an endemic area. He traveled within Minnesota and became infected.

So unrecognized protracted infection in some donors -- am I saying this is the norm for all donors? Probably not. Donor travel to and from foci of endemicity -- it can be within your state, it can be to another state. Intra-regional distribution and inter-regional shipment of blood translate into the potential for transmission anytime, anywhere.

Four-season transmission definitely is the real world. By transmission, actually I can broaden it to

donations, transfusions, symptom onset, and diagnoses -- all 12 months. Yes, there is a seasonal peak, but in relative terms.

For the spatial distribution, undoubtedly the bulk have been in or near the traditional *B. microti*-endemic states, in terms of what has been documented. I think that reflects increased risk. It also has some component -- no pun intended -- of increased awareness. But I think it does, obviously, reflect increased risk. But in this era of planes, trains, automobiles, et cetera, we do have babesiosis without borders. But it's very important to emphasize that it's not equal risk.

Because I was talking so much about Washington and California before, just to give you a sense of what types of species are there naturally and have ended up there, as I close, red cells from Maine were shopped to California; a *B. microti* transfusion-associated case in California. A different type of example, which would not have been picked up by screening in this area: A donor from the Northeast went to train in Washington State and donated, and *B. microti* was exported.

Am I saying that Washington State should have donor screening? I'm not saying that. But we do have evidence of transmission in quite a few different states.

So what we are here to discuss is, whither

Babesia? Obviously, we would like it just to go away, whether it's with pathogen-reduction techniques or other methods. But it's not going away. I think we all agree that we have a problem, and something needs to be done. Counterpoint: We may not agree with which way to go, what methods, where, when, but I think we need a way to go forward.

Thank you very much.

DR. HOLLINGER: Thank you.

Any questions for Dr. Herwaldt?

(No response)

Dr. Herwaldt, can you tell us a couple of things which I haven't been able to see in the literature -- two things. On that multi-donation cluster of cases, what was the outcome of those four cases?

DR. HERWALDT: The index-case patient was the only one, quote/unquote, who became clinically ill. I say "quote/unquote" because often the information about other recipients besides the index patient is secondhand and may not completely accurate. I had surgery or I went through something that required a transfusion. Would I be feeling great? Probably not. But some of these people -- now, in this particular situation, we have information that both people who became infected and did not.

But to answer your question a straightforward

way, only the index-case patient had what most people would consider clinically manifest infection.

DR. HOLLINGER: And the patient recovered completely?

DR. HERWALDT: Yes, this patient did.

DR. HOLLINGER: Of the fatalities, the ones that were discussed before -- about nine fatalities in 2008 or 2007 or 2009 -- where did they occur, and what was the underlying condition of those fatalities?

DR. HERWALDT: There have been others besides the ones that were officially reported to FDA. Again, I have nothing to do, of course, with that reporting. Some of them, probably understandably, weren't reported to FDA because a donor wasn't necessarily identified, though not all the donors were tested. Some of them were in people who had seemingly recovered and they had been discharged an went home, et cetera.

But I looked very carefully at the fatality data that has been provided to me, and it has been very difficult to come up with a particular pattern. For example, they have included premature infants. They have included people in their 90s. They have included asplenic patients. They have included people who weren't asplenic. Some of them were people, understandably, who had a poor prognosis even without the compounding effects of

babesiosis. But there is no doubt that babesiosis, even in someone who doesn't have, for example, a relapsing lymphoma, can in and of itself cause multi-organ system dysfunction and death, but particularly in people who have some of these sort of generic risk factors. For example, I'm not aware -- it doesn't mean it hasn't occurred -- of, say, a 20- or 30-year-old who was transfused because he or she broke his or her leg, and then died.

There has been a spectrum in terms of interval to death. Quite a few of them have been relatively shortly after the diagnosis of babesiosis.

It's also tricky because some of the physicians have had disagreement about how much the babesiosis did or didn't contribute. But there's no doubt that babesiosis has been a major contributing factor for a number of the deaths.

DR. HOLLINGER: The reason we ask that is because one of the questions we are being asked at least to discuss has something to do with whether one should test blood only for those that meet these particular -- asplenic patients, for example, much like we do CMV-negative patients in some cases. There is so much of this that we don't have the information on, these fatalities, which would be really critical.

Do we know the viral burden of that patient in

the multi-donation cluster that was then tested in April of 2000? Did someone do a viral burden on that, even though it may be different when they transmitted? Do we know the viral burden?

DR. HERWALDT: The parasitemia level, you mean?

DR. HOLLINGER: Yes. I said viral burden. Please excuse me. I made the mistake of what you put up there: Don't assume. Please forgive me.

Anyway, the parasitemia of that individual in April of 2000.

DR. HERWALDT: Not in a quantitative sense, other than that a blood specimen from the donor was positive by hamster inoculation, and then a couple of months later, a blood specimen was positive by PCR. This was not quantitative PCR.

DR. HOLLINGER: Dr. McComas?

DR. MCCOMAS: When you talk about the geography of the risk -- and I'm looking at your maps -- I see Vermont jumping right out. Can you explain why Vermont would not have cases? Does it have to do with topography or cases of reporting? I guess I'm trying to understand the vectors of this disease and how it may be spreading into other areas. Maybe there are other natural barriers or things that are impeding that.

DR. HERWALDT: Vermont has had some tick-borne-

associated cases that they reported, but I would have to check my records to see whether they thought they were all imported cases. I was in communication with them recently.

To my knowledge -- and this is just what has been documented, which again may be very different than what has occurred -- none of the donors and none of the recipients -- so none of the donations and none of the transfusions that I'm aware of have been associated with Vermont. On the other hand, I would not discount parts of Maine and maybe parts of New Hampshire.

Again, I don't want to exclude certain states. I'm not an entomologist, and I honestly don't know, one way or the other, if there have been studies in terms of local transmission, like infected ticks in Vermont, forget traveling donors. But, for example, in Maine, I think there probably is transmission, to some degree, on the southern coast. But I don't want to speak in particular about Vermont, because I don't -- if Sam Telford were here, he could give more perspective about whether he thinks the ticks are there and whether infected ticks -- I wouldn't doubt that there have been some documented, but I can't really speak intelligently to the issue.

David Leiby may speak about screening donors there -- not screening, but you know what I mean. But again, that could be confused by the issue of donor travel.

MS. BAKER: Given that so many have been identified in the northeastern states, have you been in contact with our Canadian colleagues?

DR. HERWALDT: There has been a documented case in Canada that was associated with a blood donor exposed in Massachusetts. I'm not aware of more recent cases. This case was a number of years ago. It could be that there have been some others, but I'm aware of just that one -- again, distinguishing documented versus occurred.

Also I didn't mention Japan, for example. The very first case of babesiosis documented in Japan -- and there haven't been many -- was transfusion-associated. So I do want to make the generic point that transfusion transmission can be the first way that you become aware of the fact that *Babesia* present in a locale.

DR. HOLLINGER: Any others?

(No response)

Thank you very much.

We'll move on to the next speaker, David Leiby, with the American Red Cross, who is going to talk to us about the experience with testing blood donors for *Babesia*.

Agenda Item: Experience with Testing Blood Donors for *Babesia*

DR. LEIBY: Thank you, Blaine.

As my title suggests, and I was asked by Sanjai

to present, I'll talk about the Red Cross' experience with testing blood donors for *Babesia*. By and large, I will keep the data restricted to our studies in the state of Connecticut.

What I will try to do today in the 20 minutes allotted is, first, discuss our *B. microti* seroprevalence studies that we have conducted in Connecticut. I'll briefly touch upon a 2009 NAT pilot study looking for window-period infections. I'll spend quite a bit of time on some longitudinal natural history studies, which were actually done initially with Dr. Herwaldt and the CDC, and we continue to this day. Lastly, I'll talk with one slide about look-back results.

We have been conducting seroprevalence studies in the state of Connecticut since 1999. We have primarily been focused in Connecticut, but recently expanded our cities to include Massachusetts, New Hampshire, and Maine. We have also tested in Vermont as a control population. So we have looked at many parts of the Northeast.

Donors are tested by IFA for antibodies to *B. microti*. We are using a cutoff titer of 1 to 64 for measure of positivity. IFA-positive donors are deferred indefinitely. At this point there is no mechanism for reentry, but I'm going to bring this issue up a little bit later. During the first two years of our study, we

deferred based on PCR positivity only. It was about that time that we began to recognize a relationship between being IFA-positive and the potential for being parasitemic. So we erred on the side of being conservative and deferred donors who are IFA-positive alone.

As you will see, our studies initially targeted highly endemic areas in the state of Connecticut -- and I'll show you a map -- but then we subsequently expanded statewide.

This is a summary of data from 1999 to 2007. You can see the number of tested donors per year. It ranges anywhere from about 2,000 to as high as 5,200 donors. This is all by IFA testing. In the third column, the numbers are not that important, but if you look at the percentages you will see that roughly we average 1 percent per year, each and every year. There are fluctuations. As Dr. Herwaldt alluded to, there are seasonal variations. The whole tick lifecycle is greatly dependent upon weather. Perhaps last winter was a very good winter. Ticks like snowpack, because the snowpack keeps them from becoming desiccated and dying, whereas a dry, cold winter is much more harmful to ticks and the survival of the parasite.

We also tested donors each year who are antibody-positive by PCR. You will see rates anywhere from zero to as high as 53 and 56 percent.

This slide often gets comments because of the high rates in 1999 and 2000. At that point we were testing only in the highly endemic areas of Connecticut, so we were much more likely, I think, to find repeat donors who are infected, had repeat infections. Also from the beginning of that time, we were deferring donors. So all the extremely chronic carriers are being weeded out of the population in Connecticut over time. As I will show you later in the last slide, it has had some impact upon our look-back investigations in Connecticut as well.

If we break Connecticut down by county -- and there are only eight counties in the state of Connecticut -- you will see that we found IFA-positives during that nine-year period in every county of Connecticut, but Middlesex and New London Counties had the highest rates, 1.4 and 1.7 percent.

This is a map looking at seroprevalence per 10,000 donations for the state of Connecticut. This is from a paper just published last December by Stephanie Johnson in the December issue of *Transfusion*. You can see there are two spatial clusters, high areas of endemicity in Middlesex and New London Counties, the southeastern part of the state. You can’t see it here, but that’s not far from where Long Island exists, Block Island, and all the other highly endemic islands of the state of Connecticut. So,

really, an endemic focus in the southeast corner of Connecticut, as Dr. Herwaldt alluded to. But I think the take-home message is, while that may be highly endemic in that area, you can find other areas of endemicity throughout the state. So as you move distally from that highly endemic area, you can also find other hotspots. There is probably no area in the state of Connecticut that is free of babesiosis.

In the summer of 2009, we began asking questions about nucleic acid testing, what use nucleic acid testing might have, because, as you look, all our studies using IFA were actually prospectively done -- we waited until we had a donor and we tested them. We really didn't know what their parasitemia levels were at the time of infection or how many acute infections we were actually seeing. So we targeted about 1,000 Connecticut donations. We wanted to get it done early in the tick season to pick up acute infections. Because of some logistical issues, we weren't quite so successful. But we collected donations from August through October in 2009 from Middlesex and New London Counties. Again, we specifically targeted the highly endemic counties because those were the ones we thought we would have the most success in.

With the help of Immugen, who actually did the testing for us, the PCR testing, we tested 1,002 donations.

Twenty-five, or 2.5 percent, were IFA-positive. That has been very consistent with the rate of IFA positivity in those highly endemic counties. Of those 25 that were IFA-positive, three were PCR-positive, two were IFA-positive, and one was IFA-negative. So, in essence, we identified one potential window-period infection.

I would also like to point out that all three of these PCR-positives were identified by the first week of September. Once we got into later September and October, we did not detect any more PCR-positives. This tends to go along with the appearance of the tick season in acute infections, although, as Dr. Herwaldt pointed out, you can virtually find these things year round.

The one window-period infection -- we feel the number is probably likely low. In some cases, acutely infected donors are probably too sick to donate, although in some cases they are asymptomatic and they do not have any knowledge at all that they are infected, and they do show up and donate. So this suggests, perhaps, a role for nucleic acid testing during the tick season.

One could look at this somewhat like West Nile virus. It could be seasonally triggered, using May through September. This would be quite important for targeting acute or window-period infections. But I think, as Sanjai alluded to, there are a number of technologic hurdles that

remain with PCR or NAT:

· First of all, PCR sensitivity is sufficient -- it’s a very sensitive assay for *Babesia* -- but parasitemia tends to be very low compared to viral infections. Keep in mind that these are not viruses; these are parasites.

· It requires whole blood. From the standpoint, I think, of companies developing NAT assays, there has been some reluctance and difficulty to develop whole-blood assays. It’s much more difficult than existing assays that are used for viral infections, because they don’t need to use red blood cells.

· There is also a limited volume available for testing. Remember, you are dealing with a very low-level parasitemia. As Dr. Herwaldt pointed out, that’s quite different than transfusing a whole unit of blood.

· Lastly, there are some considerations for concentration techniques that may allow the concentration of the parasites from larger volumes.

I'm going to spend quite a bit of time on the natural history studies, because I think, in some respects, these are perhaps the most interesting.

These are long-term, ongoing studies in the states of Connecticut and Massachusetts, where we have enrolled the *B. microti* seropositive donors from the study

you saw previously into the natural history study. Our attempt here is, every 30 to 60 days, to test the enrolled donors by serology to antibodies to *B. microti*. We looked at blood smears. We did PCR, and with the cooperation of the CDC, for the first three years, we looked at hamster inoculations as well.

We also asked the enrolled donors risk-factor questions. We gave them a rather extensive initial risk-factor questionnaire to determine how they may have been infected initially, what their medical history was, what their practices were, whether they did gardening, whether they played golf, whether they did other things that might expose them to ticks. Each time they came back and donated again for testing, we also asked them a shorter re-exposure questionnaire to see if perhaps we could measure reinfection. So in the end, we were actually investigating infection/resolution patterns over time.

Over about 10 years now, we have 227 seropositive donors who have enrolled in the study to date. We have 184 up to the year 2008 that we can actually evaluate and present some kind of concept and data on. Eighty-six, or 47 percent, of those seroconverted to baseline negative. I'm going to show you two examples. They completely cleared the infection, no longer have antibody titers -- for all practical purposes, appeared to have cleared the

infection. These are the donors who potentially could be reentered if there is a donor reentry protocol at some point. I'll show you some examples.

Half of the donors we looked at remained seropositive. But if you look at their patterns, you can see that a full 38 percent of those are also resolving infection. Actually, most donors who become infected resolve infection, clear antibody titers.

Thirty-one percent of the subset appear to have chronic infections, which I'll talk about briefly. A smaller subset, 12 percent, had reinfections. Keep in mind that our Connecticut donors are living in an endemic area, so they are constantly being re-exposed, and it's not uncommon for them to be reinfected. Lastly, 19 percent, or 19 of the subset, we really can't evaluate at this point.

This is the first example I'll give you of one of our study subjects that appears to have resolved infection. We picked them up for the first time in July of 2000, had an IFA titer of 512. We invited them to enroll in the study, and they did. They came back, filled out the questionnaire, gave us another sample, this time a whole-blood sample that could be injected into hamsters, as well as tested by PCR. Note that this is less than a month later. Antibody titer has dropped. But they are parasitemic, both by PCR and hamster inoculation.

A little bit later, about a month, in September, they came back again, gave another sample. Their titer dropped again. They were still PCR-positive, but we could no longer detect them by hamster inoculation.

Three months later, in December, the titer is still low. We can no longer detect parasitemia.

This is a classic example of what an infection looks like. You get parasitemia, you get IgM antibody -- and I'll show you some pictures of those -- you get IgG antibody, which clears the infection, and hopefully you return to baseline. As you can see, in subsequent tests, this donor dropped to below 1 to 64 -- completely negative. So this donor appears to have cleared the infection completely.

With the Immunogen and Victor Berardi -- he did some Western blot analysis for us. I'm going to do this real quickly so you don't spend a lot of time. The top ones are IgM antibodies for *B. microti*; the bottom are IgG. This is the same donor you just saw. Initially they had IgM -- you can see the bands; this is a positive -- which cleared. They also had intense IgG bands, which also cleared over time. So evidence of clearance of infection.

The first example I threw in there because the donor was PCR-positive when we tested them. But many of the donors we probably pick up after they have begun to

clear the infection or we can no longer detect it. In this case, we have a similar donor who was 1 to 128. They enrolled, came back. They were PCR-negative and hamster-negative. As you can see, their antibody titers over time, a little bit over a year, disappear and become negative as well. So in this donor, while they were IFA-positive, we could never demonstrate parasitemia. It doesn't mean they weren't parasitemic; it just means that we didn't detect parasitemia.

But the end result is the same. The donor cleared the infection and appears to be completely cleared -- again, a candidate for reentry.

What we do see in probably about 15 to 20 percent overall of the donors enrolled in the natural history study are people who appear to be perhaps chronic carriers. This is a donor identified in August of 2000, had a titer of 512. Similar in follow-up. Upon enrollment, positive by PCR and hamster. They were treated for the babesiosis, which sometimes is successful -- not always; sometimes patients require retreatment. Shortly after treatment, they were no longer parasitemic, but for a full three years, every time we tested them, with the exception of this one test, they had a titer of 1 to 512. So they remained with an elevated antibody titer, which I think, to Dr. Herwaldt and me and others, suggests they have an

underlying infection that is stimulating their immune response.

If you look at the Western blot analysis, very little IgM, which disappears, but the IgG response stays strong the entire time -- very high antibody titers.

We have also seen donors that are suggestive of re-exposure. This is a donor in July of 2007. You can see that these titers fluctuate all over the board -- 1 to 64, up to 256, then back down to negative, back up, and down. But what's interesting in this donor is, with the exception of some early infections in PCR and hamster, we were not able to demonstrate their being parasitemic, even though they have every suggestion of being re-exposed and reinfected.

You get some similarities in the Western blot early on. They had IgG, which disappeared. Then you have some fluctuations in here, if you look at the banding patterns of the IgG. But again, it maintains high levels of IgG antibodies.

I throw this one in because it's probably the most extreme example we have of an asymptomatic carrier. This is a 79-year-old male. So if anyone over 50 is considered elderly, then this person is extremely elderly, you could say. We picked him up in July of 2003 -- 1 to 1024. He came back the next month, 1 to 1024, positive by

PCR. Later on we tested by real-time PCR, positive, and also hamster-positive. Of note, of all the donors, the 227, that have been in our natural history study, this was the only donor that we ever identified positive by blood smear. You need significant numbers of parasites to pick up by blood smear, and this guy had them.

He was treated, 10 days of treatment, came back. He still had a high titer. It dipped shortly, but from then on, he remained relatively high for three years thereafter. By PCR, we only identified him as being positive once, but once we developed a more sensitive real-time PCR, we could pick him up several times.

However, that's not often the case. Many times we tested donors and we never detected them as being parasitemic. As I think Dr. Herwaldt stated, a negative PCR does not really clear them of being parasitemic. It just means that we couldn't detect infection.

I like to use this example. I'm sure the NIH folks in here remember this case very well of a chronic carrier who infected several people. This was a donor in the NIH blood program. We got involved because he was implicated in a transfusion case. This guy was an asymptomatic chronic carrier. He was actually a hereditary hematochromatosis patient who came into NIH routinely to be bled. This guy was a heavy marathon runner. He ran a lot

of marathons. The guy was in excellent health, no symptoms. But he had an implicated donation with his transfusion case. He had an antibody titer of 1 to 1024, and he was PCR-negative.

So here's a guy who felt perfectly fine, by all measures was physically fit, had an asymptomatic infection, which transmitted.

In follow-up studies, he was actually implicated in an additional transfusion case. The NIH, because he was a long-term hereditary hematochromatosis patient, had samples in the freezers, I think, over five to six years. We tested every one of those samples. He was 1 to 1024 every single time. But by any test that was ever done, he was never shown to be PCR-positive. Yet he transmitted infection twice.

My next-to-last slide is a summary of look-back results in Connecticut. We no longer do look-back in Connecticut. This stems from the years 1999 to 2005. If you remember this data -- you saw this in my first or second slide -- about 1 percent of the donors are seropositive for *Babesia*. From those seropositive donors, we conducted look-backs. It involved about 474 look-back donations, 656 products, and we were able to test 63 recipients, of which eight, or 12 percent, were found to be infected with *Babesia* from their blood transfusion.

But what's interesting is that most of the look-backs were early on. Remember those years when we saw high levels of parasitemia? Five of those eight actually were in 1999 and 2000. As we go through the years, look-back became less and less successful.

Our only explanation -- and we don't have a whole lot of data supporting it -- is that, because we are deferring donors, the chronically infected ones, as well as other donors, they are not there to transmit infections anymore, like that last NIH donor you saw.

In summary, seroprevalence among Connecticut donors is about 1 percent per year. A portion of donors -- it could be anywhere from 50 percent down to zero -- is parasitemic. It changes. There certainly is focal endemicity in Connecticut, but statewide distribution. A NAT role is indicated, perhaps, for seasonal, acute infections, but I think that's what we here today to discuss -- what role it might have.

The natural history of *Babesia* infection -- the majority of donors resolve infection. I would say probably 60, 70 percent of our donors that we have looked at resolve infection completely and perhaps should be considered for reentry.

Also I think -- we have heard this a couple of times today -- the chronic carriers pose a significant

transmission risk, along with acutely infected donors.

Perhaps, unlike *T. cruzi*, which sometimes has been less successful, look-back investigations have certainly demonstrated transmission risks for *Babesia*.

Thank you.

DR. HOLLINGER: Thank you, David.

Yes, Jeanne?

DR. LINDEN: Could you expand on your reasons for not doing look-back? Do you think the yield is very low or you are only picking up asymptomatic infections that aren't significant anyway?

DR. LEIBY: Somebody else in the system, the Red Cross, could correct me if I'm wrong, but we have gone to more of a market withdrawal. There is no recommendation or way to do look-back within the Red Cross, because many of the hospitals don't want to follow up and do the look-back. It's kind of a hospital thing. We provide case reports to them, that their patient has received a positive unit. In many cases they don't test them and provide information to us.

But we do do market withdrawal when there is a positive unit. The donor is deferred. The present donation and past donations are removed from the system and destroyed.

DR. HOLLINGER: Dr. Glynn?

DR. GLYNN: I just wanted to know, how long did it take, on average, to resolve the infection in the 70 percent of donors?

DR. LEIBY: I would say generally anywhere between six and nine months. Part of the difficulty is that we don't know -- we are picking them up as being antibody-positive -- we don't know in the infectious curve, often, when we are picking up the donor. That's an average. It could be longer; it could even be shorter.

DR. GLYNN: So if you were thinking about a reentry algorithm, would you wait nine months, 12 months?

DR. LEIBY: I'm not about to formulate policy, I don't think. But I think the way to do it -- my opinion only -- would be to wait some period of time, six to nine months, as you suggested, or even a year and retest to see if they are negative. I think time is less an issue as opposed to the result of being seronegative and at the same time, being NAT- or PCR-negative as well.

DR. HOLLINGER: Dr. Bower?

DR. BOWER: Is there anything in the literature or through your work that might suggest how long someone is parasitemic before they develop a detectable antibody response?

DR. LEIBY: I think that would generally be within weeks to months. There is evidence in some cases

that that really varies. I think one of the things Dr. Herwaldt was trying to point out is that, depending on the patient, sometimes that varies widely.

Keep in mind that we are looking at, at least in recipients, in these cases -- many of the cases are in asplenics, compromised, or elderly. Quite frankly, that's where a lot of them are. So there is a really wide range, looking at the incubation period and when it arises. It is different in each case.

DR. KAZMIERCZAK: You gave the example of your NIH person who was PCR-negative, seropositive, and yet transmitted to a recipient. Are there any other examples or is this the only one that you know of?

DR. LEIBY: No, that's not the only example. There are other examples of people being PCR-negative.

There are a couple of issues there. First of all -- and going back to what Dr. Herwaldt said -- the best opportunity to detect a donor as being PCR-positive is that you have segments, because the segments are closest to the original donation that transfused the infection. Many times we get reports from a recipient who has become infected, and it has been, let's say, a couple of months since their donation. Then an investigation of the transfusion case will be initiated. By the time we get around to testing the donor, it might be one of those

donors who has cleared infection. So the result is, you test all the donors and they are all negative. And while you assume it's a transfusion case, you don't know, because the donors are negative. If you do find a donor, in almost every case they are IFA-positive, but PCR-negative.

DR. KAZMIERCZAK: So it's really unclear, because there is no segment left to the test by the time the investigation starts, whether the donors actually would have been PCR-negative at the time of donation or not.

DR. LEIBY: That is correct.

PARTICIPANT: (Off-mic)

DR. KAZMIERCZAK: So there are cases where there is an existing segment still that was PCR-negative and yet seropositive, and yet we have seen transmission.

DR. LEIBY: Absolutely.

DR. HOLLINGER: And would that have been positive with the real-time PCR?

DR. LEIBY: I would think so. Keep in mind that the volume of blood in the segment is not great. Again, it's a numbers game. It's like *T. cruzi* and it's like *Plasmodium*. The infection levels are extremely low. I think Sanjai would agree, PCR for *Plasmodium* would not pick up these infections either because the infections are so low. That' the difference between transfusing a segment as opposed to transfusing a whole unit of blood. When you

test the whole unit of blood, perhaps you can find it. But if you test a segment, you probably won't.

DR. HOLLINGER: Dr. Baker?

DR. BAKER: Do you do any confirmatory testing on the donors you defer on your IFA positivity?

DR. LEIBY: The true answer is no. We test them for PCR, but that's not really a true confirmatory, because it's really a different kind of assay. I think one of the topics here today is, unfortunately, with *Babesia* testing there are very few options for tests. Whether we like it or not, IFA is considered the gold standard, so to speak. There are not commercial ELISA tests available to back up and do testing on. Blood smears are too insensitive. The alternatives for doing confirmatory tests are really very slim.

DR. BAKER: Yes, I appreciate that. Do you have any sense, is there false positivity in your testing?

DR. LEIBY: With IFA?

DR. BAKER: Yes.

DR. LEIBY: We have asked that very question. To get back to somebody's question about Vermont, for a control population, we tested 1,000 donors from Burlington, Vermont, to see what the specificity of the IFA was. We found one positive in Burlington. It turns out they were infected with *Babesia* in Massachusetts. I'm not saying

that the IFA is 100 percent specific. That's a small subset of results.

DR. HOLLINGER: David, back to some of your questions. You seemed to imply that perhaps an IFA test at 1 to 128, 256, or so might be indicative of an infection. You mentioned a patient at 1 to 512, and you felt it was a patient who was infected. But the question is, have you taken any of the blood from patients like this, had them come back in, concentrated a whole unit, and retest by an RT-PCR to show us whether or not this blood has any parasitemia or anything like this?

DR. LEIBY: No, absolutely not.

DR. HOLLINGER: That would be important, I think, in the whole area.

The only other thing I wanted to ask -- you did a look-back and, if I read this correctly, you had something like the possibility of looking at 650 individuals. You looked at about 10 percent, about 60-some. Eight of them had antibody, I believe. Were any of those looked at also for chronic infections? What was the outcome of these eight? Did any of them have any symptoms at all that you could tell?

DR. LEIBY: Yes, they did. Rich Cable and Stephanie Johnson and I are in the process of writing this up for publication. Hopefully you will see the results

soon.

DR. HOLLINGER: The question is, did any of them have a persistent infection?

DR. LEIBY: Did the recipients have a persistent infection?

DR. HOLLINGER: The eight that were positive by, I guess, antibody.

DR. LEIBY: Are you asking if they were persistently infected or if we picked them up by PCR?

DR. HOLLINGER: If they were persistently infected.

DR. LEIBY: Prior to transfusion?

DR. HOLLINGER: No. I'm talking about the recipients now and the look-back. You said you looked at 63 recipients, of which eight of them --

DR. LEIBY: Eight of them were positive.

DR. HOLLINGER: Positive for antibody, I take it.

DR. LEIBY: Correct.

DR. HOLLINGER: And were any of those PCR-positive?

DR. LEIBY: Yes, there were a couple that were PCR-positive. Primarily they were antibody-positive.

DR. HOLLINGER: Thank you.

Hira?

DR. NAKHASI: The question is, what's the

sensitivity of your PCR? You mentioned that 1 to 1,000 antibody was also PCR-negative, 1 to 128 was also PCR-negative. So the question is, what's the sensitivity of PCR? Is it very low or very high? That may be the reason you are not seeing some of these PCR-positives. You said twice this NIH person donated blood, and it was implicated in the transmission.

DR. LEIBY: We are still doing determinations, but I think it's on the level of the *Plasmodium* PCRs as well -- fewer than 20 parasites per ml.

I think, if you go back to those donors -- I mean, that's one explanation you could have, that you are not picking them up because the PCR is not sensitive enough. That certainly is a possibility, and hopefully we will do additional studies to figure that out. But I still think that the true measure is that the PCR -- it's not so much the sensitivity of the PCR. It's the sample size that you can actually test. When the infection levels are low, you just may not have a parasite in the sample of blood you take.

DR. NAKHASI: How many aliquots did you do, one, two times, three times?

DR. LEIBY: We do in triplicates, and we have a whole algorithm that I can share with you.

DR. HOLLINGER: Dr. Alter.

DR. ALTER: Two things, David.

One, I would imagine it's very hard to do look-back in a endemic area. It has certainly been true of dengue. Is your saying that these are transfusion-transmitted cases just a difference in the rates of antibody?

DR. LEIBY: In some cases we had samples before they were transfused, so we are assuming that's how they got it.

You make a very important point. When you are measuring look-backs of an actively transmitted agent in an endemic area -- so we have to compare 8 percent versus 1 percent. In many cases, when we talked, we also did follow-ups on recipients as questionnaires. Some of these were older recipients who had been in their houses, hadn't gone out for years and years, so their likelihood of transmission of natural infection was very low.

DR. ALTER: The other point -- this is a debated point -- I question whether the presence of antibody always means an ongoing infection. At least in hepatitis C, although this is debatable, people who spontaneously recovered and people who have treatment-recovered, for the most part, do not have detectable virus by very sensitive techniques in the blood, the cells, the cultured cells. A few groups have found it, but most people do not find it.

They really look like they have cleared the virus, but still have persistent antibody, which eventually wanes, over decades.

DR. LEIBY: That's a virus?

DR. ALTER: It is a virus, yes, which is much better than parasites.

DR. LEIBY: That is a good example, Harvey. Parasitologists might look at it a little bit differently. Maybe some of the other parasitologists here will agree with me. For many years, leishmaniasis was thought to clear and provide sterile infections. It wasn't until they developed mouse models in which they could actually immunosuppress the mice -- by every measure they had, mice were completely clear of infection, but once they immunosuppressed the mouse, the *Leishmania*, which are in lymph nodes and other locations, came out and they got whopping infections.

It's a good question. That's something we could talk about. There could be other issues involved here, too, as Barbara alluded to. There could be subspecies of *B. microti*, some that cause chronic infections and some that don't. There's a wealth of things that we can do research on and take a look at.

DR. HOLLINGER: Thank you, David.

The next speaker is Mark Walderhaug from the FDA,

who will give us an analysis of the risk of *Babesia* infection in U.S. blood donors.

Agenda Item: Analysis of *Babesia* Infection in U.S. Blood Donors

DR. WALDERHAUG: Thank you, Blaine, for the invitation to speak here.

I'm going to be talking about a different way of trying to get at the rates of illness and where we need to do our testing, based on using data other than the CMS data that has been provided by Dr. Herwaldt.

Here's the CDC data based on transfusion-transmitted *Babesia*, the number of cases. As you can see, the leading cases are from the endemic areas of New England and some of the Middle Atlantic states, with California being one of the ones that has relatively few numbers of transfusion-transmitted. Of course, these numbers need to be updated by the 2009 and perhaps 2010 cases as well.

But what you are looking at is a relatively sparse amount of information on which to base a decision on where to do testing.

So we were confronted by these limitations. Many of them have been talked about by Dr. Herwaldt and Dr. Leiby. It's hard to get good information on these diseases. They are only reportable in a few states, with passive reporting. Of course, there are misdiagnoses of

these particular cases as well. Our main question is, how many of them are going undiagnosed and are the asymptomatic carriers which are providing the risk for our transfusion-transmitted cases?

We created a model where we tried looking at asymptomatic carriers who we believe are responsible for these cases. We thought of them as being a fixed ratio of the symptomatic incidence. I understand that those of you who are epidemiologists are saying we are mixing prevalence with incidence. But, in a way, we are trying to just use the incidence as a model for the amount of asymptomatics that are out there. Obviously, from Dr. Leiby's presentation, we know that asymptomatics can go on for a couple of years. So they don't necessarily resolve in that year, but perhaps, as he mentioned, when we get more data, we might be able to have a better idea of what the prevalence is, based on the amount of symptomatic incidence.

We are looking at the number of reported cases in each state as being a measure of their potential for the asymptomatic carriers. Again, that's a big assumption, as opposed to the fact that states have differing levels of incidence within the state, and, of course, there are also instances, in many cases, where babesiosis is acquired in one region of the country and appears diagnosed in another

region. We are also estimating around a one-to-one ratio of infected units to asymptomatic donors, meaning that a donor can donate an infected unit multiple times. Dr. Leiby's data shows that it is definitely the case where that can happen.

For those who like to look at Venn diagrams, this is our vision of the model, where we have blood donors, who are a large group, and then intersecting with them are the *Babesia* carriers. Of course, the symptomatic cases are people who are too sick to donate, so they would not be part of the group that will be at risk for causing a transfusion-transmitted babesiosis. But those asymptomatic ones that intersect with blood donors would form that small group among the blood recipients who become transfusion-transmitted victims of babesiosis.

In this model we have two large areas of unknowns. The first unknown is what fraction of *Babesia* carriers are the asymptomatics -- again, cases conflated with carriers. There is some ratio in there where, if we knew what that ratio was, we would have a pretty good idea of what the numbers would be.

Another big unknown is the infectivity of the blood from asymptomatic *Babesia* carriers. We get the impression that it's probably very close to being one hit/one transmission, but we just don't know at the present

time. In the model that I'm going to be showing in this particular case, we are going to assume that if the person is an asymptomatic carrier, that unit of blood will be causing a transfusion-transmitted case of babesiosis.

In most cases, in this particular model we are only going to be talking about red cells. You have heard instances where babesiosis has been transmitted by platelets, but we won't talk about them in this particular model. In this particular case, we have simplified things to think of just one donor/one unit of red cells that could be causing the case of babesiosis. Again, that's an assumption in this model.

Where are we going to get the symptomatic babesiosis carrier data? As Dr. Herwaldt mentioned in her presentation, there are thousands of cases over a period of time. We are limited on where we are going to get that particular data. It's hard to use that data on a basis for decision making. AHRQ has a nice resource, known as the Healthcare Cost and Utilization Project. They have regional reporting of inpatient discharges. But a really difficult limitation of their data is the fact that not all states have reported data. Some states do have individual data, but other states don't. In one particular case, a critical state doesn't have individual data on which to base a nationwide estimate.

So we looked at CMS data. CMS data is a limitation because it's a fraction of the total population who were exposed. It's only the elderly. But there are some younger, disabled beneficiaries included in that list of people. It has the advantage of being nationally reported. In this case, what's helpful is that we can get billing data. The billing data means that we are getting data from individuals who are being billed for a diagnosis of babesiosis, as opposed to perhaps looking at a medical record where you would see the case of babesiosis listed as being ruled out. So this was a useful database for us to go look at.

Here is the data for which we have good data to compare with, CDC's, from 2006 to 2008. There is an interesting decrease of cases in 2007. I'm sure that's probably a cold, dry winter in the Northeast in that particular instance. It's interesting, although it may not be significant, that there was a decrease in transfusion-transmitted babesiosis cases in 2007, as reported by CDC as well.

It's 11 percent of the population. As you can see, these numbers are a lot larger than the sort of numbers we had been seeing from these other reporting databases. Part of the reason for that is the fact that, as you look at the location of the recording diagnosis,

very few of these cases are taking place in inpatient facilities or even outpatient facilities. In reality -- and we had had this earlier; we found this out relatively lately -- over 80 percent of the cases being diagnosed are from a physician recording the diagnosis, meaning they are diagnosed by physicians being reimbursed by Medicare.

I should point out that this data is from a SafeRx contract that FDA has to look at this data.

I would also like to mention that just last week we got data from another source, which is HealthCore. It's limited also by the fact that it's an HMO reporting group that has limited as opposed to national exposures. The preliminary data that we got from them is very similar to the CMS data as well. The advantage of the HealthCore data is that it's not just elderly, but a representation of all their beneficiaries in their particular health service group.

This is actually the reported cases of babesiosis per 100,000, according to states. If you look at the list of states here, the ones in the endemic areas are the ones that are hitting the very highest rates overall. As you can see, there are variations over the three-year period as well. Interestingly enough, Vermont does make this particular list of states.

I should explain that the stars that we have here

are due to the fact that the cases in that particular state did not exceed 10 for that particular year. According to some potential privacy reasons, we are not reporting the actual numbers. We have to sort of censor our own data.

But you can see that in small states with higher prevalences we don't get levels high enough, but larger states that have lower prevalences do have enough cases for us to share with you.

The model is very, very simple. We take a state symptomatic rate. We find a rate of asymptomatics to symptomatics, multiply that times the adult population, then the general fraction of the donating population, the average number of units donated by a donor, and we get the number of asymptomatic units donated. From that, we are assuming that the number of cases of transfusion-transmitted babesiosis is the same number as the units donated.

Another large uncertainty that I want to talk about is the asymptomatic-to-symptomatic ratio, which we are using to normalize the results based on reported cases, to reach the same number as the average number of cases reported by the CDC for those three years of transfusion-transmitted babesiosis. If those rates are actually higher, then we would have a different ratio that we would like to have to use, to bring the predicted numbers in line

with the CDC results. That's a key uncertainty that we were not able to get an estimate for.

The state babesiosis rates -- we were able to do analysis, by David King, to give us a certain measure of uncertainty for how many true cases of babesiosis there are, based on the data we received. I should also credit Mikhail Menis for getting the data from CMS for this particular analysis and Dr. Forshee for his contributions to this model as well.

Here are the model results for the predicted number of cases of babesiosis based on states. As you can see, they are pretty similar to what the CDC results were in terms of which states had the most cases. One of the big exceptions is that Rhode Island is much lower in the list of states compared to what CDC was reporting. Other states that aren't normally mentioned but still had cases of transfusion-transmitted babesiosis, like Maryland and Virginia, were higher than predicted by the CDC methods as well. The numbers in parentheses are the 90 percent confidence intervals based on David King's uncertainty analysis of the data.

We have this factor of 1.75 percent as the ratio from asymptomatics to symptomatic. Again, that number, as I mentioned earlier, was derived in order to get the number of predicted cases to be 15, which was the average number

of cases for the transfusion-transmitted babesiosis cases from 2006 to 2008. If that ratio was actually 10 times higher, you would see 10 times the number of cases observed. So it could be a much bigger problem if it were underreported to that degree.

Here is a comparison of the states predicted -- if we did testing in those states, it would encompass 95 percent of the transfusion-transmitted babesiosis cases. Many of them are similar, but some of them are different. That has to do with the fact that, whether they be due to unrecognized endemic cases or to imported cases, there are differences between what the FDA model predicts and what the CDC data says.

Another interesting example of a difference is the cases for the Midwest, in Minnesota and Wisconsin, rate lower in the CMS babesiosis cases compared to the transfusion-transmitted babesiosis cases in CDC data. So there are interesting differences there.

This gives you an example -- I apologize for the fact that you can't see this unless you are really up close -- I have here the FDA predicted numbers of transfusion-transmitted babesiosis cases by state on the x-axis, and the average number of cases on the y-axis, with a measure of, I think, an understatement of the uncertainty of those numbers. This is strictly based on the

uncertainty of the CMS data, which is nationally reported data. It shows that New York has the highest amount. The blue line that you see traveling up from here is the cumulative number of cases. As it accumulates up to 100 percent of the cases, this is the 95 percent cutoff level right here. You can see that at this particular point, when you get to Georgia, in this model it would be 95 percent of the cases, with cases all the way over here.

I should mention that Wyoming and Montana were the only states in the CMS database that did not report cases of babesiosis.

So the model shows some concordance, but not identity, with observed data. It can be improved with more complete observational data. As we get, perhaps, better, more representative data from across the country, we might be able to get a better estimate of transfusion-transmitted cases. If we had better understanding of the infectivity, which seems to be pretty close to 100 percent -- but again, because, as Dr. Herwaldt and Dr. Leiby mentioned, there may be subspecies which are more capable of causing chronic cases than others -- certainly if we had a better understanding of the overall prevalence and how long people stayed asymptomatic, we could probably get a better asymptomatic-to-symptomatic ratio for making our estimate as well.

Thank you. I'll take questions.

DR. HOLLINGER: Thank you.

Questions for Dr. Walderhaug? Dr. Kazmierczak?

DR. KAZMIERCZAK: One of the statements you made in your earlier slide that reported cases of babesiosis for a measure of the state's potential for asymptomatic carriers -- I see a problem with using the CMS data, simply because it does reflect a much more elderly population, a population that is more likely to have symptomatic babesiosis as opposed to the asymptomatics, who are the ones most likely to donate blood. Can you comment on that?

DR. WALDERHAUG: Yes. We have thought about that. We would expect that certain states that had higher fractions of elderly, like Florida, would be ones where we would have more trouble relative to the others. Given the fact that Florida shows a reasonable rate, based on where we were, we thought perhaps that -- although your point is certainly an important limitation of this assumption -- the differences probably aren't that great. But it's certainly an area of future research, to make certain that it's not a real limiting problem.

But, yes, it's a possibility that elderly would have higher rates of symptomatic cases.

DR. TROXEL: Related to that, did you assume that that ratio of asymptomatic to symptomatic cases is constant

across all the states?

DR. WALDERHAUG: Yes, that's how the model was done, although if we had extra information, we could probably change that to perhaps reflect the changes in the ratio of elderly to the regular population.

DR. TROXEL: Related to this -- I'm sure you feel this way also -- it's a little unsatisfying to use as your basis for that estimate the thing that makes the model match the data. I understand the reason for that and the motivation for doing it, lacking other data. But my question is, isn't there some way you could use perhaps the data that we just heard about in the Connecticut natural history study to try to get an estimate of what that rate actually might be, based on something sort of substantive rather than something mechanical?

DR. WALDERHAUG: It would be great if we had that level of understanding of babesiosis prevalence in all the rest of the states other than Connecticut. But because we don't have that data in the other states, we really couldn't apply it in this particular instance. We are hungry for real data, as opposed to using this mechanical estimation to normalize things the way they are.

What we wind up doing is, we are probably just looking at the level of risk, based on the cases of babesiosis, rather than looking at actual numbers. It's a

relative ranking of states based on babesiosis rather than an actual number of cases.

DR. TROXEL: But would it be instructive, at least within the state of Connecticut, to do the same model, but using the estimate based on the natural history data, to see if, at least within Connecticut, you are approaching -- or how discrepant it might be from the reported transfusion-transmitted cases?

DR. WALDERHAUG: I guess I can sit down it with Dr. Leiby and maybe we can have a discussion about that. But it's hard to do, based on the fact that we are trying to compared state to state. We could probably get a model looking from county to county, but I'm not sure how we translate that into a national model. But we'll think about it.

DR. HOLLINGER: Dr. Trunkey?

DR. TRUNKEY: This morning, the very last presentation by Dr. Le Grice from the NCI showed that we can get much better testing and probably more accurate testing. We are going to be asked in a few minutes to answer question 1. I'm not sure that we don't have it backwards. Wouldn't it be better to have NCI come up with better testing for this and then do number 1?

DR. WALDERHAUG: You're asking me that?

DR. TRUNKEY: Yes.

DR. WALDERHAUG: I would love to have better testing, personally speaking. I can't speak for FDA, but they probably would like better testing, too.

DR. TRUNKEY: It's not going to come from industry. So why don't you go back to NCI?

DR. WALDERHAUG: I guess if we could get babesiosis tied in with cancer, then we could probably get a good buy-in with NCI.

DR. TRUNKEY: I'm sure, if they live long enough, it can cause cancer.

DR. WALDERHAUG: I think you're probably right on that.

DR. HOLLINGER: Dr. Linden?

DR. LINDEN: I would like to see if you can better explain to me how you got the ratio of asymptomatic to symptomatic, and also just clarify, since the state of transfusion and state of donation are two entirely different things, for the CMS data you are talking about patients residing in that state, for the donors you are talking about donations in that state, regardless of what state they wind up going to.

DR. WALDERHAUG: Right. I believe the data that I was trying to show from CDC was not the location of where the transfusion-transmitted babesiosis case occurred, but where the blood was donated. We tried to account for that.

As far as that ratio is concerned, the way that was determined was to just run that model and then look to see what ratio arranged the data to get to the level where CDC's data was. It was done by just deriving that ratio. It's not necessarily data. It's more a way of scaling the relative numbers of cases to equal the number of cases that CDC reports.

DR. HOLLINGER: Yes, please.

DR. KRAUSE: My name is Peter Krause. I'm from the Yale School of Public Health.

I wanted to mention that there has been a study actually looking at the ratio of symptomatic to asymptomatic cases. That was a study we did in 2003 and reported on. This was an epidemiologic study where we looked at cases on Block Island. We documented fairly carefully the symptomatic cases, and the asymptomatic cases we were able to define through a serosurvey, where we looked at antibody at the beginning and the end of the season, and then as those people had been diagnosed or had symptoms. What we found was that about a quarter of the adult patients were asymptomatic and about half of children were asymptomatic.

I wanted to point that out.

DR. WALDERHAUG: Thank you for that data. We'll check that out.

DR. KRAUSE: I don't know if this is okay, but I had a point to make about Dr. Leiby's excellent presentation. Is that okay to do or should I wait to talk about that at a later point?

DR. HOLLINGER: Go ahead and ask it now to Dr. Leiby.

DR. KRAUSE: Thank you. I wanted to know your definition of reinfection versus relapsing infection and how you were able to distinguish that. Or is that not so easily done?

DR. LEIBY: Not so easily done. It was very arbitrary. It was only by looking at antibody titers. If we saw someone who consistently had pretty much a flat-lined antibody titer, he appeared to be more chronically infected, whereas someone whose antibody titer fluctuated from being 256 down to negative, back up, suggested a reinfection. Both of those could be wrong.

DR. HOLLINGER: Thank you.

DR. KUMAR: Can I make a brief comment?

DR. HOLLINGER: Dr. Kumar.

DR. KUMAR: I just wanted to respond to Dr. Krause. I just want to mention that the study was not lost on us. We did make a note of this in the issue summary. The problem that we had was that we could not base our entire premise of determining asymptomatic-to-symptomatic

ratios -- probably that is the most important question we were confronted with. But we had to have a study which is more largely representative, not based on a few hundred samples at the most. That's why we refrained from using the whole basis. That's all.

DR. HOLLINGER: Thank you. Yes?

DR. CABLE: I just want to point out something, to sort of follow up on David's comment about deceasing rates of look-back. I was the medical director in Connecticut for most of the years this was being done. I noticed, very interestingly, that the Connecticut TTB cases are about half of the New York and Rhode Island cases, and yet the tick-borne cases are about double the New York and Rhode Island cases, suggesting that the TTB-to-tick ratio is about fourfold less in Connecticut than in those two states, which I think is compatible with our hypothesis that our limited testing of only 2,000 or 3,000 donors a year, focused in endemic areas, has done some significant good in reducing the rate of TTB in Connecticut. If that's the case, it opens up the possibility for alternative strategies to control the disease to testing every single blood donor.

DR. HOLLINGER: Could you give us your name, please?

DR. CABLE: Richard Cable, C-a-b-l-e, with the

American Red Cross.

DR. HOLLINGER: Thank you. Yes, Dr McComas?

DR. MCCOMAS: In relation to the CMS data and the physician recording diagnosis, 81 percent, I'm wondering, has any study been done on how accurate those diagnoses might be? We have heard that in many cases it's sort of hard to diagnosis this. Was there independent verification or any sort of assumption built into your model to take that into consideration?

DR. WALDERHAUG: Our assumption -- and it is a limitation -- is that if it was in billing data as opposed to diagnosis data, it was more real, more likely to be valid. We have not requested any chart review to validate that data. That's something that we probably should do eventually. But it's the best we could do at the present time.

DR. KAZMIERCZAK: I have a question about your red cell calculation model. The first box says, "State symptomatic babesiosis rate." I guess my question is, is that based on the same number as -- Dr. Kumar showed a map in his presentation that showed CMS beneficiary claims.

DR. WALDERHAUG: Yes.

DR. KAZMIERCZAK: It is?

DR. WALDERHAUG: Yes.

DR. KAZMIERCZAK: That raises an issue. In that

map that he showed, Florida had 40 cases -- it's difficult to read -- a lot of cases --

DR. WALDERHAUG: Those aren't cases. He had the total number of cases, but what we are doing right here is the rate, which is the number of cases divided by the total number of beneficiaries in the state.

DR. KAZMIERCZAK: But your numerator is based on the same number that Dr. Kumar had.

DR. WALDERHAUG: Right.

DR. KAZMIERCZAK: So my question is, from what I know about other tick-borne diseases for which *Ixodes scapularis* is a vector, Florida ain't got that much of a problem with ticks. They don't have that sort of incidence of high incidences of Lyme disease or anaplasmosis or ehrlichiosis. So how does that jive with what we know about other tick-borne diseases? Why is Florida so high?

DR. WALDERHAUG: It's probably because people spend time in New England and then go down to Florida.

DR. KAZMIERCZAK: So we're talking about snowbirds, essentially.

DR. WALDERHAUG: Maybe. But the CMS data is the CMS data.

DR. KAZMIERCZAK: But if we are looking at the risk of -- the basic question is the risk of a blood donation. The real question is, where do these donors come

from, not even necessarily where they donate? I know in Wisconsin we had a snowbird who donated in Florida that wound up infecting a Florida resident.

I would just question using that figure as your numerator for your rate. That kind of starts off your whole model.

DR. WALDERHAUG: I think one of the nice things about the CMS data is that it captures both the endemic and the travel cases of babesiosis, to some degree. We don't know where those diagnoses of babesiosis are coming from. All we know is that they have been diagnosed and tested and confirmed and then billed to CMS, based on a state basis. That's our limitation.

DR. HOLLINGER: I think we'll go to the final presentation. Dr. Kumar is going to come back and talk to us about the FDA perspective.

Agenda Item: FDA Perspective

DR. KUMAR: I'm back with giving the FDA perspective and present the FDA proposal to the committee.

FDA is proposing a broad-based, region-specific approach to blood donor testing for *Babesia* infection rather than a national testing program. The FDA risk analysis suggests that implementation of a donor screening test in 20 states and the District of Columbia would address an estimated 93 percent of the current risk in

donors.

Most of this is based on the way we have identified the risk. The risk is identified based on the model that Dr. Mark Walderhaug presented. That was a culmination of the inputs of data from the CMS data and TTB case data. Then we tried to set our marks where the inclusion of the number of states would cover around 93 percent, 95 percent of the total risk at the national level.

By using that criterion, we have come up with these states here: New York, Connecticut, Massachusetts, California, New Jersey, Maryland, Virginia, Florida, Pennsylvania, Rhode Island, Texas, Michigan, Minnesota, and Wisconsin. These are the states identified based on the risk. Some other states were included because cases of transfusion-transmitted babesiosis were identified. So the risk -- sole basis for the inclusion of 20 states here where the cases of transfusion-transmitted babesiosis were identified -- for example, Ohio and Indiana -- or some of the states because of geographic locations. If the state which is -- high-risk states but did not make the cut per se based on the risk model, they were included here, for the matter of being pragmatic, really, and to not overlook how the risk is in the surrounding areas, and all the travel back and forth. We had to be cognizant of that.

Plus we included the District of Columbia because of the number of cases seen in the CMS data set.

This is a simple -- the proposed 20 *Babesia* risk states and the District of Columbia represent approximately 60 percent of the total U.S. population ages 18 years and older.

Here, just putting them geographically. Again, here are the states, mostly in the Northeast here, the Mid-Atlantic states here, upper Midwest. So all the suspected states where the transmission is known to occur -- most of them are. Plus there are some high-population states here -- Florida, based on the data you just saw from Dr. Walderhaug's talk, Texas, and California. These are the states we are proposing for testing today.

Just more -- assuming that blood donation rates are approximately equal across the states, approximately 60 percent of the U.S. blood supply would undergo testing for *Babesia* under the proposal that we are making today. Given the current s of *Babesia* detection tests, we are proposing a two-phrase testing strategy.

The first phase, donor testing in the proposed 20 states and the District of Columbia by nucleic acid test. However, we are also saying, simultaneously, if an investigational antibody-based test for donor screening is available, then both NAT and/or antibody testing may be

applied. So we are not hung up on one approach here.

In Phase II, testing might be extended to include donors in lower-risk areas or in areas where natural transmission of *Babesia* is not known to occur, but clinical cases and/or infected blood donors have been reported. So basically that is to cover *Babesia* risk wherever it might exist within the country.

Also we would like to emphasize that high-throughput antibody tests might be more applicable for donor screening in Phase II testing.

Having said presented the proposal today, the questions for the committee today:

· Do the FDA risk analysis and the available CMS and CDC data sets together support the concept of regional testing of blood donors for *Babesia* infections?

· Given the current sensitivity limitation of NAT for *Babesia*, please comment on whether the public health benefits of NAT testing warrant consideration of broad-based regional testing of donors by NAT.

· Considering the current technologies, please comment on the suitability of antibody testing for *Babesia* infections in blood donors.

I will stop.

DR. HOLLINGER: Thank you, Dr. Kumar.

We’re going to take a 15-minute break right now.

We will go until about 4:30.

(Brief recess)

DR. HOLLINGER: Before the open public hearing, at which two people have asked to speak, I need to again read the announcement on the open public hearing for the record.

Both the Food and Drug Administration, FDA, and the public believe in a transparent process for information gathering and decision making. To ensure such transparency at the open public hearing session of the advisory committee meeting, FDA believes that it is important to understand the context of an individual's presentation. For this reason, FDA encourages you, the open public hearing speaker, at the beginning of your written or oral statement, to advise the committee of any financial relationship that you may have with any company or any group that is likely to be impacted by the topic of the meeting. For example, the financial information may include the company's or a group's payment of your travel, lodging, or other expenses in connection with your attendance at the meeting.

Likewise, FDA encourages you, at the beginning of your statement, to advise the committee if you do not have any such financial relationships.

If you choose not to address this issue of

financial relationships at the beginning of your statement, it will not preclude you from speaking.

Having said that, we have two people who have officially asked to speak from the floor. The first is Andrew Levin, with Immunetics. He has asked for 15 minutes, which we will give him.

Agenda Item: Open Public Hearing

DR. LEVIN: My name is Andrew Levin. I'm president and scientific director of Immunetics in Boston. We are a developer and manufacturer of test kits for infectious diseases and blood screening. Immunetics paid my way.

First, I would like to thank the committee for allowing me to make this brief presentation at today's meeting.

Assays which have been used historically to detect *Babesia* have included the four on the slide here -- serology, microscopy, and nucleic acid testing, which I think have been discussed enough earlier. It's somewhat apparent that all of these techniques have some limitations, but especially in throughput. They are not terribly automatable or scalable, and there are some performance limitations, based on specificity or sensitivity.

We at Immunetics are attacking this problem

through a consortium effort with two other organizations, Creative Testing Solutions and Blood Systems Research Institute. Immunetics is engaged in assay development, Creative Testing Solutions operates several high-volume testing laboratories where assays can be evaluated and implemented, and BSRI will guide clinical studies and ongoing research involving the *Babesia* assays under development.

The consortium's intention is to be able to work towards the approval of *Babesia* tests which can be used in high-volume blood screening in a cost-efficient way.

Furthermore, we are currently in the negotiation stage with National Heart, Lung, and Blood Institute for funding under an SBIR contract for which the solicitation issued last November. I guess the intended award date would be September 30.

Our paradigm for testing is based on well-proven testing algorithms in blood screening and clinical diagnosis, in which screening is carried out by ELISA and confirmation by immunoblot. This paradigm has served for testing diseases, including HIV and Lyme disease, as fairly well-known examples. However, as in these examples, first-generation *Babesia* ELISAs relying on whole cell antigens have proven rather inadequate in both sensitivity and specificity. The roughly 95 percent values for sensitivity

and specificity reported for a whole cell *Babesia* ELISA illustrate the challenge. But the traditional advantages of ELISA in cost-effectiveness, objectivity, and easy adaptation to automated operation for high-volume screening make it very desirable for this application.

With respect to confirmation of *Babesia*, immunoblot has previously been developed by our group and published. As typical for an immunoblot, this is a low-volume/high-accuracy assay.

To address the challenge of relatively low specificity, which many ELISAs suffer from, we have developed a patented immunoassay format, which is shown here in schematic. It's a little difficult to see. This is a capture assay, in effect, which makes use of direct antigen conjugates. In the assay we mix a sample containing specific antibodies, a serum sample, with two direct antigen conjugates, one with antigen-streptavidin, the other antigen-enzyme. In the presence of a specific antibody, a ternary complex is formed which is bound to a biotinylated microplate. That's shown on the right, by comparison with the conventional indirect immunoassay on the left, which makes use of a second antibody-enzyme conjugate. Our format eliminates this antibody-enzyme secondary conjugate. As a result, it eliminates much of the nonspecific reactivity that goes along with

conventional indirect immunoassays.

We have been successful in adapting other ELISAs, such as for anthrax, to this format, with a net increase in specificity in that example from about 95 percent to greater than 99 percent, with no loss in sensitivity. Based on that experience, we are optimistic that highly sensitive and specific *Babesia* ELISAs can be developed in this format.

The second major issue in ELISA development is the choice of a suitable antigen or antigens. We have experience over a number of years with a family of antigens which were originally generated by researchers at the former Corixa Corporation, David Persing and others, using a *B. microti* expression library which was screened with human babesiosis sera. This led to the identification of a number of immunodominant peptide epitopes, some, but not all, of which have homologies with *Plasmodium* surface antigens. Some first-generation ELISAs were developed using both recombinants and synthetic peptide versions of the BMN1 family of antigens, but none of these assays were truly optimized, either at Corixa or subsequently. We at Immunetics are now the licensee for these antigens.

Some preliminary work which we did showed encouraging results in terms of separating *Babesia*-positive sera, on the right -- this is a histogram, so absorbance

values -- from blood donor control sera, on the left. We have a pretty good, but not perfect, separation, with a reasonable cutoff range right around here.

A similar scatterplot showing about two dozen *Babesia* sera clinically confirmed cases, on the left, shows that the DO values again are generally well separable from blood donor sera, down here.

This was fairly encouraging, but this is a conventional ELISA format, not yet our capture format.

Looking at results from an ROC analysis, we find a cutoff, in pink. I have an ROC line showing that at a cutoff value here we achieve 100 percent sensitivity and 96 percent specificity, which appears to be close to optimal performance for the conventional ELISA. From this point, we are optimistic that the new direct antigen conjugate capture assay chemistry we have should enable an improvement in specificity, which ideally would bring us above the 99 percent level that we have achieved in previous examples.

Secondly, our proposition to use immunoblot as a confirmatory assay following ELISA would make use of the immunoblot that we published previously. This is a *CDLI* 2001 article. This assay made use of whole-cell *Babesia* lysate producing the type of banding patterns seen in the photo here for IgG and IgM blots. We identified 10 antigen

bands as criteria for interpretation. As shown in our table, use of a single band as a criterion for a positive blot gave a sensitivity of 100 percent and a specificity of 96 percent. Use of two bands gave a sensitivity of 96 percent and a specificity of 99 percent.

If we combine ELISA and immunoblot in a serial algorithm, similar to the algorithm used for HIV testing, for example, then the combined sensitivity is equal to the product of the individual sensitivities, and the combined specificity is shown in the equation on the table. If we model the predicted sensitivity and specificity of this two-assay approach using the previous data, we find using two immunoblot bands as criteria for positive should lead to an overall sensitivity of 96 percent and specificity of 99.96 percent, while use of one immunoblot band to define a positive should lead to overall sensitivity of 100 percent and specificity of 99.82 percent.

These values for sensitivity and specificity are likely to be more compatible with blood screening requirements than those of the original ELISA assay or immunoblot assay independently.

If we compare the ELISA and immunoblot model against the *Babesia* assays mentioned at the beginning, the ELISA and immunoblot would be predicted to come in at just slightly less than the 100 percent specificity reported for

some of the other assays, but would improve on them at 100 percent sensitivity.

Of course, this is just a model, based on our previous data, which did not extend to a very large number of *Babesia* serum samples. We have yet to show that this will be possible with the new generation of assays which we are currently developing.

More importantly, though, if performance levels in this range can be hit with the ELISA and immunoblot combination, which we are optimistic should be possible, that would make possible the application of high-volume screening in a cost-effective manner for *Babesia*.

Finally, the availability of ELISA and immunoblot assays for *Babesia* should make possible a range of further studies, including some listed here. These go beyond the basics of assay development, which is my focus here, but it’s worth mentioning these possibilities for ongoing research that these tools could open up.

Thank you.

DR. HOLLINGER: Thank you.

Any questions of the speaker?

DR. TRUNKEY: What is your N?

DR. LEVIN: We had in those slides about two dozen *Babesia* sera.

DR. TRUNKEY: What happens, as we were shown

earlier, in regards to the drop-off of titer in these patients? Were these known positives or known possible carriers?

DR. LEVIN: They were positives confirmed largely by IFA.

DR. HOLLINGER: Any additional questions?

(No response)

Thank you.

The second person who has asked to speak is Ilene Grarcar (phonetic) from AABB. Oh, I guess it's going to be Dr. Leiby.

DR. LEIBY: I'm speaking for Ilene. I have nothing to disclose, and I paid my own way here.

This is a statement coming from the AABB entitled "Risk of *Babesia* Infection by Blood Transfusion and Potential Strategies for Donor Testing." I'm presenting this as the chair of the AABB *Babesia* Workgroup.

AABB is an international not-for-profit association representing individuals and institutions involved in the field of transfusion medicine and cellular therapies. The association is committed to improving health by developing and delivering standards, accreditation, and educational programs that focus on optimizing patient and donor care and safety. AABB membership consists of nearly 2,000 institutions and 8,000

individuals, including physicians, nurses, scientists, researchers, administrators, medical technologists, and other health-care providers. Members are located in more than 80 countries.

AABB has taken steps to address the issue of transfusion-transmitted *Babesia*, beginning in July 1989, with a recommendation to indefinitely defer donors who give a history of babesiosis and to recall and destroy associated components. This recommendation was followed in 1991 by a standard in the 14th edition of the *Standards for Blood Banks and Transfusion Services* that remains in effect today.

The current AABB donor history questionnaire contains the related question, but limited available to date indicate that this question is only marginally effective at preventing cases of transfusion-transmitted *Babesia*, TTB.

In 2008, the AABB Board of Directors established the *Babesia* Workgroup to provide leadership, with the goal of analyzing risk to the U.S. blood supply and developing scenarios to mitigate the risk. The workgroup reports through the Transfusion-Transmitted Disease Committee and is composed of representatives from blood-collection facilities in *Babesia*-endemic areas of the U.S., as well as members of public health and liaisons from the FDA and CDC.

The initial focus of the *Babesia* Workgroup was to draft an Association bulletin to address current safety issues posed by *Babesia*. In August 2009, AABB released Association Bulletin Number 0906, "Transfusion-Transmitted Babesiosis," to provide, one, information about the potential for TTB; two, educational materials for clinicians about the epidemiology of *Babesia*, along with descriptions of the signs, symptoms, diagnosis, and treatment of babesiosis; three, information on the processes that have been used by blood centers in endemic areas for investigating TTB cases; and four, a summary of the development of interventions to reduce transfusion transmission of *Babesia*.

The experience of blood centers operating in highly endemic areas was included to provide insight into management of patients, donors, and blood components in the event of known or suspected cases of TTB.

The current project of the *Babesia* Workgroup is to develop a system to tabulate ongoing clinical cases of TTB by requesting and reviewing case reports that have been forwarded from the transfusion services. Much time has been spent developing the data parameters. Developing and funding the database and a mapping system are the next steps.

Today the BPAC is asked whether the available

data support the development of regionally selective donor screening/testing strategy to reduce the risk of TTB. Additionally, the committee is asked to comment on the suitability of donor screening either by nucleic acid test, NAT, an antibody test, or both, given the current technology limitations.

AABB would like to restate and update some of the information provided in the August 2009 Association bulletin prepared by the task force.

The increased prevalence of TTB has provided impetus for the development, evaluation, approval, and implementation of interventions to reduce or eliminate TTB. Currently, there is no FDA-approved blood donor screening test available. Pathogen reduction methods are under investigation, but feasibility in red cell products remains to be demonstrated.

In the absence of FDA-approved blood donor screening tests, one American Red Cross center in a highly endemic area, using serologic testing -- i.e., IFA -- performed within a research protocol not currently under IND, has implemented interventions consisting of selective testing based on geographic areas and season of the year. This center has policies that require the deferral of donors with reactive or positive test results and, when

appropriate, withdrawal of other identified components by the same donor. Some blood establishments restrict collections during tick season from geographic areas known to be highly endemic for *B. microti*, but the efficacy of this approach has not been rigorously established.

Research studies should be conducted and expanded, investigational tests should be developed, and associated protocols should be designed to prevent or reduce TTB. Ideally, testing strategies should focus on the detection of infectious donations. It is necessary to determine donor prevalence and incidence of *Babesia* infection in areas with reported cases of TTB prior to the consideration of an intervention. In areas found to be highly endemic by research prevalence and incidence studies, testing under IND protocols should be considered prior to the availability of an FDA licensed test. Potential testing strategies that should be considered by manufacturers, FDA, and blood centers in such highly endemic areas include, one, testing annually or seasonally, or two, the screening of components designated for selected groups of patients who are at risk for severe clinical outcomes if infected with *Babesia*.

Of note, the Rhode Island Blood Center implemented this second approach under IND on July 8, 2010, using both IFA and NAT through IMUGEN, Incorporated, on a

limited number of collections.

AABB continues to believe that a selective testing approach to *Babesia* is warranted. While it is difficult to determine which geographic areas have sufficient risk to warrant testing, we are not convinced that the inclusion of 20 states representing 60 percent of the blood supply is the appropriate catchment area in which to begin testing. In part, the FDA proposes testing in several non-endemic states -- examples, Florida and Texas -- based solely on isolated cases of TTB. Such cases are usually found to be associated with imported blood or recent donor travel to a highly endemic area. AABB feels strongly that regionalized testing should be defined by *Babesia*-highly endemic areas identified through sound scientific studies of seroprevalence or locally acquired incident infections.

AABB also believes that there are insufficient data to establish whether NAT screening would be a superior approach to antibody testing and that this issue requires careful evaluation before a conclusion is reached. Longitudinal studies presented today by the American Red Cross suggest that chronic carriers need to be considered as important contributors to TTB, in addition to acutely infected donors. In most instances, these chronic carriers cannot be detected by NAT, but can be identified through

serologic testing, which is almost exclusively performed using IFA. While generally considered highly sensitive, NAT is limited by its ability to detect low levels of infection, sample volume issues, and in the case of blood screening, difficulties in adapting NAT to whole blood. Consensus opinion is that NAT would not be effective in screening for *Plasmodium*, the causative agent of malaria, and this should be taken into account when projecting whether NAT would be effective for *Babesia* screening.

Antibody testing by IFA has been established as the gold standard for *Babesia*, and has been used in virtually all seroprevalence and transfusion case investigations published to date. Accumulated data suggest that donor screening using IFA would capture most infectious donors, with the exception of acute window-period infections that could be detected through seasonally administered NAT during the tick season. For example, in one endemic area, recent evidence shows that approximately 1 percent of donors were IFA positive, with fewer than half of these donors being identified as positive by NAT. Certainly, antibody screening may unnecessarily defer non-infectious, antibody-positive donors, but, as discussed today, many of these donors resolve infection and could be reentered upon development of an appropriate protocols.

The issue of TTB is a critical blood safety

issue, and AABB applauds the FDA's willingness to address the issue in today's committee meeting. It must be recognized that the IFA methods used in previous research studies are not suitable for large-scale donor screening. We think it is crucial for companies to develop assays that can be used to screen the blood supply in highly endemic areas. These assays must use methodologies that are compatible with the high throughput and cGMP required of a donor screening test. Further, AABB urges that testing begin in the most highly endemic areas rather than in more widespread locations and that the debate about the merits of antibody versus NAT should continue following the collection of additional data.

Thank you.

DR. HOLLINGER: Thank you, David. Why don't you just stay there for one minute?

Any questions of Dr. Leiby, speaking for the AABB?

DR. KAZMIERCZAK: The position statement talks about the fact that you are not supportive of this sort of widespread, 20-state testing, that you prefer to see it focused down. On what basis, though? What data would you like to see used to determine what states should implement screening?

DR. LEIBY: My personal opinion or the AABB

opinion?

DR. KAZMIERCZAK: Both.

DR. LEIBY: I'll stay with the AABB opinion. In looking at the data, the feeling among the working group committee, as well as with the TTD Committee, which reviewed this statement, was that we need to focus on where the issue is the problem. The problem is in the endemic areas. We certainly recognize the fact that some people become infected and move to other locations and donate blood as well. But I think the pushback from other blood-collection groups in non-endemic areas, if you ask them to test, would be rather extreme.

DR. HOLLINGER: Any other questions for right now?

(No response)

Thank you, Dr. Leiby.

Is there anyone else in the public section that would like to provide any more information on this topic?

(No response)

If not, we'll close the open public session and begin the open committee discussion.

Agenda Item: Open Committee Discussion

Dr. Kumar, do you want to put up the questions?

The first question to the committee, and the only one that we are going to vote on today: Do the FDA risk

analysis and available CMS and CDC data sets together support the concept of regional testing of blood donors for *Babesia* infections?

I'm going to open up the discussion. I would like to hear from all of you about this issue, since this is the most important issue. Then we'll amplify on that as it goes along.

Yes, Dr. Rentas?

DR. RENTAS: I just want to clarify what the FDA means by regional testing. Are we voting on 20 states here if we answer yes?

DR. HOLLINGER: I was going to say, I think that's part of the discussion. That's specifically it. Do you think there are benefits to doing regional testing? If so, do you think you need to do it for 20 states, for four states, for five states, or what?

Am I correct in that, Jay?

DR. EPSTEIN: Yes. I think we have to separate the concept of regionalized testing. We don't have that in our country. Yet we know that there are some transmissible diseases that are highly focal regionally. So, first, we would like to be advised whether we should or shouldn't support scientifically a concept of regionalized testing, recognizing, as has been said many times, that blood moves and that donors move. They can get infected in one area

and go donate somewhere else.

Be that as it may, should we pursue regionalized testing?

I think we would very much welcome the discussion by the committee about the 20-state model or the seven-state model or the 14-state model. Or, more generally, how should one decide where to test if one were to regionalize testing?

DR. HOLLINGER: Dr. Ragni?

DR. RAGNI: Another question is, what do we mean by testing? We have heard about a number of tests today, for which I think there is some interesting data. But one could argue, hearing some information about the immunoblot test and NAT testing and ELISA testing and the IFA -- I'm not sure how some of these tests compare. Should there be a question about a prospective comparison of some of the tests if something is implemented? I don't think we have enough data to actually answer what the testing profile should look like.

DR. HOLLINGER: What do you think? What do you think we need?

DR. RAGNI: I don't know. I think it would be very interesting if we had a chance to look at the comparison of some of these assays in a prospective way. It's hard to make decisions with limited data.

I know that's not a good answer, but that's my answer. And I'm sticking to it.

DR. HOLLINGER: You're sticking to it.

Yes, Dr. Glynn?

DR. GLYNN: I agree completely. I think we have very limited data. Going back to that very first question, I just want to make sure that our response does not mean that we agree with the particular model that was presented by the FDA, which was the 20 states doing NAT testing in the first phase and then going to a second phase. This is not what you are asking us. You are just asking us whether we think it would be a good idea to consider regional testing, whatever that means. It could be a few states -- I just want to make sure we're clear on the question.

DR. EPSTEIN: Yes. In addressing the first question, we want the committee's opinion on the concept of regional testing. It will then open the door to a broader discussion of how one would decide what region. In no way are you specifically advising the FDA on the 20-state model. Of course, we would appreciate your opinion on how to refine the model or any alternatives.

DR. HOLLINGER: Yes, Dr. Trunkey?

DR. TRUNKEY: I'm concerned about the sensitivity of these tests and the ability to pick up all of the people at risk. We have heard, at least from industry, that there

is some possibility that we might have better tests out there. I think eventually you will have to do a regional test program. When you do that, you have to do a cost/benefit analysis. I think there are some states that probably would never benefit from having donor testing. On the other hand, I think the rationale that's being used here, to use the highest 20 -- it could be a different number -- is not irrational. But I don't think we are going to learn that much if the tests are flawed and don't pick up all of the carriers.

DR. HOLLINGER: Let me rephrase it. If we have a test that is highly sensitive and specific, documented appropriately, then you might favor -- if you are going to have a test like that, then you would favor regional testing selection at that point?

DR. TRUNKEY: I think the regional testing is logical. It has endemic areas. I think it would be worthwhile, when you do that, to do a cost/benefit analysis. You may be able to reduce the 20 states or you may want to expand it, based on even lower ratios of infectivity.

DR. HOLLINGER: I feel that the model is not very robust. I think it has a lot of assumptions put into it that make it difficult to decide about 20 states -- many assumptions, and then predictions from those assumptions,

using data, as you mentioned, that may be flawed because of biases, like the CMS data, and so on. So at least for me, it's difficult for me to accept the model for the 20 states. On the other hand, I could accept it for four or five states, which I actually at one point put down, such as New York, Massachusetts, New Jersey, and Connecticut, and maybe even California. It's nice to go across to the other side of the United States, too, every once in a while -- so something like this as a regional thing. But to do 20 states without having some actual data in terms of the benefits, the changes in incidence, and so on -- and then also the test, as you mentioned, has real problems for me.

Yes, Dr. Rentas.

DR. RENTAS: And that's exactly what my concern is. If you look at these 20 states in here, we have quite a few states in here with only one case in the last four or five years. We were not provided any information here to show whether that case was someone that had traveled to Connecticut or New York or Rhode Island. So I have some issues with that.

The other thing is that we do have some national testing labs out there that may not be part of these 20 states, but they may be testing donors that belong to one of the 20 states as well. North Carolina, with the ARC

testing lab, comes to mind. They are not included within the 20 states, but I'm sure they are testing people from Virginia, maybe the District, and even Maryland.

DR. HOLLINGER: Dr. Troxel.

DR. TROXEL: I first want to say that I think the concept of regional testing is eminently reasonable and clearly warranted in a situation like this, in which there is such geographic variability.

I want to preface the second part of my comments by saying that I commend the modelers for the work they have done. I'm not intending to pick on you or criticize. I know it's a very, very difficult task, and especially with such limited data as exists here. In many cases all you can do is make assumptions and hope that they are as well informed as possible. So I certainly appreciate all of that and the work that has been done, because I think it has been very good.

Having said that, though, I'm concerned about, as you said, the number of unknowns in the model, and I'm a little bit worried by the fact that the concordance between the observed CDC data and the model predictions is not so great, both in terms of the actual states that are listed and the ordering of the states. Rhode Island, which tops the list in the observed data, is pretty far down in the modeling-based list. So I think the question of what

states to choose, and how many, is a very difficult one and absolutely relies on tradeoffs and cost/benefit-type analysis. The cost has to do with real economic costs, in terms of how much we have to pay to accomplish the testing, and also blood supply costs, in terms of how many donors might be potentially deferred and so forth.

Without better information on any of those costs, it's very, very difficult to make a judgment as to where we should draw that line and say, in this group test and in that group don't test. Obviously, at some point somebody has to make some kind of decision about where to do that. But going back to the comment that we really need more information, we don't know what the costs are of the testing, and we don't really know what the characteristics of the tests themselves are. There is really a lot that's very unsure.

DR. HOLLINGER: Dr. Kazmierczak.

DR. KAZMIERCZAK: As usual, I have more questions than answers. I guess I just have a question about the process of putting this in place. For instance, we're talking about screening in an individual state, based on where the donations are occurring. Is it possible or impossible, just for logistic purposes, instead of basing it on the state where the blood drive is occurring, to base it on where the donor has been? Is that even doable, from

a blood bank perspective? If you have a snowbird from Rhode Island that's donating blood in Georgia, that's really the crux of the risk, rather than the fact that this blood drive is occurring in Georgia and that's where the donation occurred. So is it logistically even possible to base testing on whether the donor is actually from one of those states, as opposed to where the particular site of donation is?

DR. HOLLINGER: And I would really wonder how many people go from one place to another and then donate at that other place. Obviously it happens, but does it happen very often? I suppose if they are a snowbird going down to Florida, where they are going to be six months, that's a different story. Otherwise, they probably would not. So it would be somebody who would be in transition from one place to another, don't you think?

DR. KAZMIERCZAK: Yes, and I have no idea what the numbers would be. But it just seems like, if you are talking about, realistically, where the risk is, it's where the donor has been rather than where the donation occurs.

DR. RAGNI: So couldn't you just capture that by a question? I'm sorry if that adds to all the questions that are already being asked? But that might be one way around that issue.

DR. KAZMIERCZAK: You are already asking whether

the patient has ever had babesiosis. Just find out where they are from.

I do have one other question, though. This is regarding testing and what test to use. And I'm certainly no expert in these tests, but it seems like the test deficiencies are such that the serological test will pick up, possibly, low parasitemia that's not detectable on a PCR assay, except during the window period. That seems to be a weakness in the antibody assay. Is it even possible, from a logistics standpoint -- and I guess maybe Dr. Leiby would be the best to answer this -- to say, within the states that we decide to test in, seasonally the PCR would be the preferred test, to eliminate that window? Then, once tick transmission season is over -- like once you are into October -- at that point, could you move to serologic testing? Or is that just logistically too complex to do?

DR. HOLLINGER: Actually, I think that was one of the points that the working group made, the possibility of doing NAT testing during the months of May to September or October, something like this, but not in the months -- am I correct in that, David? That's one of the things you commented about, I think.

DR. LEIBY: That's correct. But the concept wasn't that NAT would preclude IFA testing. As we discussed and as Dr. Herwaldt demonstrated, people can be

infected, chronically infected, year round. If we just went to NAT testing and not some kind of serologic testing, we would probably miss some of those people.

DR. HOLLINGER: Dr. Baker, you have a comment?

DR. BAKER: On the question with regard to local donors, the plasma derivative industry actually does employ a local residency requirement for plasma donations. I would say, for the Red Cross or for the volunteer segment, this would be very, very difficult to implement. You have questions of college students, who are a preferred donor population, who, of course, are often very far away from home, active military personnel or recently active military personnel. There are a lot of complications if you try to introduce a local residence requirement for your donor. So I wouldn't recommend that as an avenue to explore.

DR. HOLLINGER: Dr. Kazmierczak, do you have another question?

DR. KAZMIERCZAK: My point wasn't to require local residence. It was simply to ask the question of whether they have been in a high-risk area. That donation, then, would be targeted for screening.

DR. BAKER: Obviously you could do that. There are some questions that a volunteer donor might question about that. But that's certainly something that you could do. It would complicate, though, your donor management.

Given how complex donor management is anyway, there could be a very substantial unintended consequence of that, I think, for the volunteer side.

DR. HOLLINGER: Dr. Stramer, do you have a comment about this issue that we're discussing?

DR. STRAMER: Susan Stramer, American Red Cross.

I was just going to concur with Dr. Baker that logistically asking the donor if they have been to an endemic state -- it's terribly complicated. It does mean that you have nationalized testing implemented. Staff in every collection area that might do testing has to be trained, equipment validated, SOPs. It's just not logistically possible.

I would make a plea to the committee in their deliberations to listen to what the AABB said. We have a localized problem -- specifically, a concentrated problem. And perfect may be the enemy of good. Right now we're not doing anything for the most transfusion-transmissible agent we have. Some of the proposals are that we start small. They may not be perfect. They may not catch every window-period donor. They may not catch every convalescent donor, every reinfected donor, every recrudescent donor, or any definition that you want. We may not capture every state in the country that has a traveler or an imported blood product. But right now, as we talk about it and slice and

dice it every which way, the more complicated we make it, the less likely we will have introduced an intervention.

We have been looking at *Babesia* now for a decade, still without an intervention. You haven't even touched on the fact that we are only talking about testing *Babesia microti*. Dr. Herwaldt talked about all the other types. Right now all the assays are truly only focused on *microti*. So we are only going to pick up a portion of the problem.

If you do a comparison for West Nile triggering, we know that people travel to areas where West Nile may be epidemic and they travel back and donate. We may mistakenly trigger, for a donor and a state, because they may have traveled somewhere else.

So we have related problems in our industry. But right now what we really have to look at is going from nothing to something and then learning over time, through investigational studies and collection of follow-up data and additional look-back data, what really is the best paradigm for testing. But right now we're starting at zero.

DR. HOLLINGER: Thank you, Susan.

Dr. Bower?

DR. BOWER: I just wanted to verify what we are talking about here. My understanding from the comments are that the only logistically feasible test that we can do

right now is the PCR. I just wanted to verify that that is the case, that IFA is not practical to do for this testing. So there is only one real test that we are talking about, and when we are talking about serology, we are talking about something that has to be developed by industry.

I do want to ask the AABB or the blood banks how practical it would be to do individual unit testing for 60 percent of the population. I have always heard them complain about having to do individual testing.

DR. HOLLINGER: Would someone like to respond to that?

DR. STRAMER: I think one of the tests that was proposed as well was IFA, so let me first talk about that.

Although IFA you may think of as I did of IFA decades ago, where you are doing one slide at a time and there is human intervention, technology has progressed. There are automated, objective methods to do IFAs, as there are for ELISAs or any other technology. So serological testing in an automated, objective fashion is feasible.

The feasibility of PCR, as David has presented, will not pick up every chronic carrier. So there are limitations right now, probably inherent to just the primer pair selection, what sequences. There are inherent issues with the design of PCR right now. There are also sample issues, because the concentrations of parasites in a unit

of blood may be very small. Then there is the issue that this is an intraerythrocytic parasite. So there are other issues which we have never dealt with as an industry in dealing with whole blood or red cell lysis to then do PCR off that product.

So there are a number of issues before we introduce single-unit NAT to 60 percent of the blood supply. Again, I think we have to pilot things like that and really look at the upsides, downsides, how we perfect those techniques, before we make a massive change to implementing single-unit testing.

DR. TRUNKEY: Just a comment. Several years ago, when Tom McCall was the governor of Oregon, he put a billboard on I-5, on the California border. It said, "Welcome to Oregon. Enjoy your visit, but don't stay." I guess we'll have to put on there, "Don't donate" also.

DR. HOLLINGER: Dr. Linden.

DR. LINDEN: I just want to point out that a state-by-state approach isn't consistent with the pattern of what we have seen with the cases of these diseases. The FDA analysis did not consider communicable disease reporting data, for reasons that I'm not completely clear on. But when one looks at those, at least in our state, New York is far from uniform. It's a very diverse area of hyperendemic foci, and we know where those are. If you

look at 1,200 cases that occurred, over 90 percent of those are in one of three counties. One county is almost always implicated in our transfusion-associated cases. If there is a donor from that county, it's always that one. There is a higher-risk county, but it's such a low population -- an order of magnitude lower -- that it just doesn't come up.

But there are parts of the state with zero. There is a center up by the northern border with Canada. I that county and all the ones surrounding it, the incidence is zero, zero, zero, zero. Even in New York City, tick surveillance, as well as investigation of disease case reports, have shown that the risk for acquisition is zero. Every single case is linked to either transfusion or travel.

I'm not suggesting that travel would be able to be captured. Most of the hyperendemic areas for babesiosis are basically vacation spots -- the islands in Massachusetts, Long Island. I think even in Maine there are isolated foci, I think three counties or so on the southeastern border. But northern Maine, as far as I know, is zero. That's my understanding. I don't know the specifics on Maine.

This is certainly something of great concern to me. I would also point out that when you look at our total

state data, going back to 2004 -- this is community-acquired and transfusion-acquired cases, all cases -- they have more than tripled since 2004. This is getting worse, consistent with our tick surveillance data. Clearly, I think the time is now to try to pursue something -- not that I'm convinced that we have a test at the moment that would be useful. But I do agree with Dr. Stramer and others that it might make sense to sort of focus on your highest-risk areas. In our state we know exactly where those are. To paint the entire state with the same broad brush -- upstate is served by three different blood centers, and the number of cases -- there is not a single county that has more than three, and those are probably all related to travel anyway.

So I would make an argument to not go state by state, look at the pockets. One does need to recognize the blood service areas. It certainly makes sense for a given center to do all or nothing. But I think consideration should be given to the patterns that we have seen, which are very predictable with these organisms.

DR. HOLLINGER: Is babesiosis a reportable disease in New York?

DR. LINDEN: It has been 1986.

DR. HOLLINGER: So that's one of the states where it's reportable. Thank you.

Yes, Dr. Glynn.

DR. GLYNN: Talking about trying to get more data, I think, Dr. Herwaldt, you mentioned that it was going to be notifiable as of January of next year. Would you care to comment a little bit more on that? What does that mean exactly? It sounds like we are going to get more data, where we'll be able to evaluate more the actual number of cases.

DR. HERWALDT: Yes. I'm hesitating a bit because a common misconception is that when something is nationally notifiable, suddenly you hear about everything and the data are perfect. It's still dependent on detecting cases in the first place and it's still dependent on the accuracy of the diagnosis. Then in the notifying that would come to CDC, there would still be the tricky issue of pulling together and differentiating between the transfusion-associated cases and the tick-associated cases. The collective "we" -- and Jeanne Linden is one of the key persons in this -- have worked for months pulling together a case definition that the Council of State and Territorial Epidemiologists approved in June that would encompass trying to make some of these distinctions.

The other key issue to point out is that making something nationally notifiable does not necessarily mean that all states have to make it reportable. Just to give

you some sense, already there are around 16 or 17 states in which babesiosis is explicitly reportable. For a number of years, most of those states, and also some in which it's implicitly reportable -- a distinction that's not important for our purposes now -- have been providing surveillance data to CDC, but with different surveillance case definitions and minimal data.

I want to underscore that it's great news that it will be nationally notifiable. I can't go into the details of why it's actually a major coup, but it relates to this regional issue. To get state and territorial epidemiologists to take something that they might perceive as regional and agree to make it nationally notifiable is a major coup. It's a step in the right direction. But it's not as if in February suddenly we are going to have the perfect data.

Does that make sense?

DR. HOLLINGER: Let me just ask another question along the same lines. There are three other options. I want the committee maybe to deal with these, if they would, a little bit. Besides the broad-based regional testing -- and I'm taking into account what Jeanne has mentioned when I'm using that term -- there are three other possibilities. One is to do nothing, do no testing at all. The second one is to do universal testing everywhere. That obviously

doesn’t seem to be what the committee is favoring. The third one is some sort of a selective testing of blood, much like they do for CMV-negative individuals, where O negative blood is tested and used for patients who are CMV-negative. This would be selective testing for patients who are immunosuppressed in some way or another, asplenic, or neonates, prematures, and so on.

Could the committee talk a little bit about those areas? I would like to hear what you may have to say about any of that. Dr. McComas?

DR. MCCOMAS: I guess this is just a matter of comparison, since we are in sort of a comparison mood. I found myself trying to understand the consequences of babesiosis. Nobody wants it, but in the cases that we were presented, when somebody got it, it didn’t really describe what happened. Did they die? Was it a greater risk? I’m trying to understand this and put this in a perspective with what we do about Lyme disease or West Nile virus or some of these other procedures. Do we have regional screening for those in place? If so, why, and if not, why? And how does this fit within that perspective?

This is not going to be the first nor the last tick-borne disease that this committee will address. I would kind of like to understand where it fits within the picture.

DR. HOLLINGER: I think that's important. In several other articles, the coinfection with *Borrelia* and *Anaplasma* and *Babesia* can occur in the same ticks and be transmitted differently and so on. I think those are valid observations.

Any others?

DR. RAGNI: Just to sort of follow up on that, I think I read in one of these piece of paper here that somebody actually later on had a splenectomy and then had some problems. So I think selectively studying this or implementing testing is a little tricky, a little concerning, because you might miss some of them.

The other issue, of course, is that the people who are getting transfusions are already sick. I think we heard a great story about that.

I just think selectively picking them -- maybe we do need 20 states. Just to start somewhere and just to pick a few tests to compare might make some sense. I think this is really hard because we don't have enough data. Yet this is a problem the extent of which we have no understanding of.

DR. HOLLINGER: Dr. Herwaldt, you made a comment, and you seemed to emphasize it greatly: asplenia, asplenia, asplenia, asplenia. There must have been a reason that you said that. Can you tell us why you made

that so emphatically?

DR. HERWALDT: If you were taking your infectious disease board exam and there was a question related to *Babesia*, it probably would throw in something about Nantucket and asplenia or whatever.

But to get to the fundamental points, when I spoke, I wanted to make sure that people understood the spectrum. I said things like, regardless of the mode of transmission, the infection can range of asymptomatic to severe. But there is no question that this is a potentially life-threatening disease. If you don't have a spleen, that is, for sure, a major, major risk factor.

In studies -- whether they are surveillance studies or prospective studies, whatever -- when they have looked at risk factors for hospitalization or mortality or whatever, yes, there are higher risks for people of advanced age and higher risks for various types of immunosuppression. Those can be major risk factors. When I emphasized the importance of the spleen, I was not deemphasizing the others, other than to the point that I wanted to make absolutely clear the important function of the spleen. I think it's incredibly important not to lose the concept that this is a life-threatening disease, even though you can have people who are absolutely asymptomatic, the donors, and you can have some recipients who are

absolutely asymptomatic. When I talked before, when you asked me a question about fatalities, I said it could be very complex to figure out why people died. Did they die with this or because of this? Was this a proximate or contributory cause?

It's not a good thing to have babesiosis, especially on top of whatever else you have that is requiring transfusion.

On the other hand, if you are getting your transfusion and you are otherwise healthy, then it can be something that is asymptomatic.

DR. HOLLINGER: Dr. Alter?

DR. ALTER: From an audience perspective, just to answer the previous question -- Dr. Herwaldt has already answered it in part -- I think we know that this can be a severe disease. It's on the case fatality list for FDA. If you compare it to West Nile virus, it's similar in that you have a whole spectrum. We did something about West Nile because it caused encephalitis in some people and it killed some people. This does also. With West Nile, it was clear that something should be done. But West Nile had already spread across the country, so you had to implement a national policy.

I think here, to me, it's clear that something needs to be done, but you don't need to make it a national

test at this point because it hasn't spread that far. You clearly have hyperendemic regions. As Sue said, you start somewhere. I think it would be complex to do what Jeanne is recommending, to do parts of a state, but maybe that's possible. But I think you start out with the hyperendemic states, where probably 90 percent of the severe cases are coming from, and do something because something needs to be done. If IFA can be automated, then you have a way to do it.

So I would vote for regional testing with the test we have.

DR. HOLLINGER: Since you can't vote -- Dr. Linden?

DR. LINDEN: Dr. Alter, you used the West Nile example. Could you or Dr. Stramer comment? Wasn't that based on consideration that the perceived risk, based on frequency, would depend on what you are actually finding in a particular area, so it would not be uniform across the country? Might that not also apply here, potentially, as an option?

DR. ALTER: It is uniform across the country. It's just a matter of whether you do pool testing or individual donor testing. A certain number of cases per region trigger individual testing. But it's still a national policy. Some regions do pooled, some regions do

individual.

DR. KAZMIERCZAK: This may be a little off the topic, but since the idea of pooled specimens was brought up, in one of these references it talks about the fact that you don't want to pool specimens for PCR analysis because it's going to make the sensitivity still lower than it is. But one thing that I didn't see addressed -- can you pool specimens for serologic testing? I don't know how much detail we are going to be asked to go into as far as the mechanisms for testing, if the committee decides to recommend that. But I'm just curious to know whether you can pool serologic specimens.

DR. HOLLINGER: Let me hold that question if I can. I think it's a question we can deal with, but let's deal with this first issue here. Yes, Don?

DR. BAKER: As another nonvoting member, I would say I'm probably positionally closest to Sue Stramer and Dr. Alter and those folks. I think the data that we have today would support a very restricted pilot-type analysis.

However, one point that I did want to raise has not been raised yet. In this whole discussion, it has been surprising to me that we have been very silent on the impact on the donors of these kinds of test results. I would not consider it absolutely benign to tell an asymptomatic individual that they may or may not have an

active parasitic infection. I haven't heard any discussion on how the donor management goes after that. These are individuals who have volunteered to provide blood, and I think they need some consideration in terms of how we would manage them after this testing.

DR. HOLLINGER: Any other questions?

I think the question -- we might as well just finish this out -- with the dilution, I guess it would be sort of like detuning an assay. What you are really asking, I guess, is, if a serologic test was more likely to predict infection at a dilution of 1 to 512, and you are choosing that as a level -- or even 1 to 256 -- I suppose, theoretically, you could put four samples together, down to 1 to 64, which seems to be a cutoff, probably because of specificity and so on. Maybe you could do it that way. But you would need to know, I think, what the probabilities are of the antibody tests for predicting an infection. I think that might be the case.

Does anybody want to comment any further on that? Yes, Jay?

DR. EPSTEIN: This question arose decades ago with HIV testing and the fact that many countries of the world didn't have the resources to test individual samples. FDA was asked many times what we thought about pooled serologic testing. There was actually a pilot study that

was done cooperatively with Canada to try to advise PAHO whether this was a reasonable strategy for the Americas.

The underlying issue is that the specificity of tests generally goes down with sample pooling. So there is a tradeoff between the benefit of reduced costs of pool testing versus the increased cost of breaking down positive pools. There is also the potential loss of sensitivity, but that's relative to what titer you need.

The general feeling was that it introduces so much uncertainty, because the behavior of pools was also somewhat idiosyncratic. In other words, in some pools you had no loss of sensitivity and no aggravation of loss of specificity; in other pools, you did. Then there were other issues that were assay-specific, particularly for IFA. My recollection is that there was decreased sensitivity that was ultimately attributed to anti-isotype interactions. You can get immune complexes when you do serum mixing.

So the general feeling was that there were too many uncertainties, and so wherever it was feasible economically to do individual sample testing, that was strongly preferred.

But I think it's context-specific, and one could potentially show that it worked for *Babesia*. But I think we are nowhere near there right now.

DR. HOLLINGER: I think we should probably consider -- everybody is okay to vote on this topic? Are there any burning comments from any of the committee?

(No response)

If not, I think we will go ahead and vote on the question. Basically, what the FDA is asking is whether you all believe that, if there is to be testing done for *Babesia*, it would be as regional testing, without specifying where or how and so on, regional testing in endemic areas of blood donors for *Babesia* infections.

If that's your understanding, what we will do is -- we have these blinking lights here before you. If you believe that the data support at least regional testing in endemic areas, you will use "Yes" for your answer. If you don't, it will be "No." You can abstain by pushing the middle button. All of these are tied in to you individually. Once you vote, don't vote again. Up on the screen here will be your name and how you voted. Lieutenant Commander Emery will then read off the votes and how people voted.

Dr. Baker, I will not forget you. Once we have voted, then I will ask you also how you would have voted, since you are a nonvoting member of the committee.

Yes, Jeanne?

DR. LINDEN: Can I ask Dr. Kumar or Dr. Epstein,

does this mean regional versus universal or regional testing versus no testing?

DR. KUMAR: I think the question is only regional testing. That's how we are asking for.

DR. EPSTEIN: I think what we are getting at is, assuming that testing is needed as an intervention strategy -- putting aside whether we have the right tests yet, but assuming the need for testing -- the question you are being asked is, should it be regional or should it be universal in all areas?

DR. HOLLINGER: Thank you, Jay, for that clarification.

I'm going to ask everybody to place your vote. Before we read it off and get it up on the screen, I'll ask Dr. Baker also how he would have voted. So everybody vote now.

Dr. Baker, how would you vote on this?

DR. BAKER: Very regional.

DR. HOLLINGER: Very good.

Can we have the votes, please? Go ahead, Lieutenant Commander.

LCDR. EMERY: There are 12 yeses, there are no abstentions, and there are no noes. Yes from Dr. Hollinger, yes from Dr. Linden, yes from Dr. Rentas, yes from Dr. Edwards, yes from Dr. Trunkey, yes from Dr. Ragni,

yes from Dr. Kazmierczak, yes from Dr. Glynn, yes from the consumer rep, Judith Baker, yes from Dr. Bower, yes from Dr. Troxel, and yes from Dr. McComas.

DR. HOLLINGER: Thank you.

It's always bothersome when everybody agrees, isn't it?

So we have gotten through the first question. We don't have a vote on these next two questions. Really, what the FDA wants is some further input, if you feel there is some, for the next question. Let me read you the second question: Given the current sensitivity limitation of NAT for *Babesia*, please comment on whether the public health benefits of NAT testing warrant consideration of broad-based regional testing of donors by NAT.

Any comments about that?

DR. TRUNKEY: My concern relates to the sensitivity of the various tests that are being proposed. I support the concept of regional testing. I just don't think we are ready to do it.

DR. HOLLINGER: So what would you propose, Don? How would you handle this issue, then? Would you set up a research project, a research study, in an area with IND or for new tests? How would you go about doing this?

DR. TRUNKEY: As I stated earlier, I thought Dr. Le Grice's presentation this morning was superior. He took

tests, refined them, and got them down almost the nanogram level. It was amazing to me how much he improved the testing. I'm not sure that that can be done with *Babesia*, but I think it probably can be. And before we look at it, I think you have to at least make an effort to improve the testing.

DR. HOLLINGER: I think the concept that he put out was clearly exceptional. That, of course, was in laboratories that were doing HIV testing. All of these different laboratories, from protein expression and so on, were all set up to do this. This is a little different, obviously. It may be difficult for them to put in. But I think the concept that he had was exceptionally good, I agree.

Dr. Troxel?

DR. TROXEL: I think we weren't presented with the data that might allow us to really answer this question, which would be, I would think, some kind of a table showing us sensitivity of NAT in different subgroups of people -- recently infected, on the down-slope of clearing, and so forth -- or, combined with that, the antibody-based testing and, for people at a certain level on the IFA test, what the sensitivities are of the NAT. It's not clear to me that NAT is really a single test. There seem to be multiple different flavors of PCR that can

be used that have different sensitivities and so forth. So it's difficult to answer that.

Having said that, the picture that was painted by the Connecticut data is not terribly encouraging for NAT, because it seems to be missing all kinds of things all over the place.

That's more of a general impression than anything that's based on specific data, but it doesn't seem to be something that we can rely on at this point.

DR. BOWER: Unless I missed it, I didn't see any data that would show that NAT would pick up anything that the serology wouldn't pick up. It was just the speculation that there would be some cases that would be parasitemic prior to developing antibody. But we didn't see any data that showed any of that.

We have no information to base an answer on for number 2, except just supposition about what we know about infections, that you have the organism present before you develop serology.

DR. HOLLINGER: I think Dr. Leiby presented some data where they took 25 IFA-positive samples, three of which were PCR-positive, and in that group which were PCR-positive, two of those three were IFA-positive and one was negative.

David, could you comment a little bit more,

particularly on that IFA-negative? Did you follow the donor to see if they seroconverted?

These are questions which are critical to us, and we don't have the answers to them and haven't had them for two years.

DR. LEIBY: You're exactly right. In many cases the very data you are talking about doesn't exist.

To clarify what we did, we tested 1,002 donor samples by both IFA and PCR. Three of them were IFA-positive, two of which were PCR-positive as well. One was PCR-positive, but IFA-negative. But they were tested, all 1,002 samples, by PCR and IFA.

To your question about whether we followed up that donor -- exactly. The thing to do would be to follow that donor and get additional samples to see if they would seroconvert. The donor was unwilling to give us additional samples. So, in essence, we were screwed.

Those are the questions that need to be asked. We have talked about setting up additional studies to ask those questions. Those are the kinds of data we need.

DR. HOLLINGER: Yes, Dr. Rentas?

DR. RENTAS: Can we ask the FDA what the rationale was for picking NAT testing versus IFA?

DR. EPSTEIN: I'm going to recognize Dr. Kumar, but first I want to come back to this question about

clinical sensitivity of NAT versus IFA. To me, the most informative data have been the tests that were done on a limited -- albeit limited -- number of index samples from donors who actually transmitted babesiosis by transfusion. My understanding from those studies, although they are limited, is that it was no better than 50 percent positive by NAT. So I think we have in our hands a crude estimate of clinical sensitivity of NAT.

It's true that NAT is evolving, but ultimately we will always be up against this problem of sampling. We know that there is infectivity at levels below which one can have assurance of getting the parasite into the sample.

The thinking, though -- and again, I'll let Dr. Kumar comment -- was along the lines that although the sensitivity is limited, you are much closer to knowing that you have an infectious sample when it's NAT-positive. By comparison, the IFA or whatever antibody test you do is potentially over-inclusive. We know there are persistent antibody-positives who are no longer infectious. So the issue was whether there would be greater resistance to development of an IFA test because you will, at least for now, indefinitely defer the donors, because you are not sure how to reenter them. I think that's why Dr. Leiby was stressing the need for and potential feasibility of a reentry strategy. People are going to stay antibody-

positive for a long time, presumably much longer than they are infectious. When you can readmit them?

So the thinking was that perhaps the first-stage approach -- and this is why Dr. Kumar suggested Phase I, Phase II -- is, you go with the test that is closer to having a demonstrable value. I think we heard today that correlation with infectivity perhaps would be reasonably good at higher IFA titers. There are other ways to look at the problem. But FDA's thinking -- and, Sanjai, you can comment -- was that it might be best to start with a test that has direct parasite detection, albeit at lower sensitivity, which would result in much lower donor loss and would provide at least some benefit, certainly, in highly endemic areas. Then, as IFA matures, perhaps we add IFA or switch to IFA.

I just want to make one other comment. What underlies all of this discussion is trying to put forward a line of thinking that will motivate a test-kit manufacturer. The manufacturers are trying to figure out where this market lies. We are trying to do two things at the same time. We are trying to protect the health of recipients by adequately capturing risk in donors, but at the same time, doing it in a practical way where a test will come about. We just can't forget that. There are real barriers here. Individual NAT will probably never be

universally done for *Babesia*. I say never; who knows? But it's a barrier, at least at the present time.

From that point of view, you would rather see serology. But then you have the question of whether ELISA is up to the, quote/unquote, gold standard of the IFA. It's a much more amenable test with current methods.

Sanjai, do you want to comment further about the rationale?

DR. KUMAR: I think Dr. Epstein has very nicely captured the basic underlying thinking. We have agonized over this for long. The inferences are made with *Plasmodium*. We very aware of it. But we know some things about *Plasmodium* which we don't know. We know a lot about -- also what we know that 10 infected red cells are sufficient to cause fulminant malaria infection in the case of *Plasmodium*. We don't know that here. We do not know what is the minimum parasite burden to sustain infection in asymptomatic infections.

Also it's true that we have not seen the true analytical sensitivity. At least no one has shown us data for *Babesia microti*. We are waiting to say the data is still here.

Our underlying thinking was that it's possible that we will be able to have direct parasite demonstration with NAT in the sense of a complete antibody-based test and

have a way of going forward, actually. That's what we have been thinking. It is possible there is a test that is highly sensitive and can detect few parasites, five parasites, 10 parasites, per ml of blood. That may be sufficient. I think we should give NAT a sufficient chance also, because then we don't have to worry about antibody-positive donors which will not be infectious.

So that's the basic premise behind our thinking.

DR. HOLLINGER: Thank you.

DR. BENJAMIN: Dr. Richard Benjamin, chief medical officer, American Red Cross.

I would like to reinforce something that Dr. Epstein said. The blood centers are primarily interested in preventing transmission of *Babesia*. Dr. Leiby's data showed that of 1,007 tests they did from a high-prevalence area, a high-incidence area, they had 25 IFA-positives and three PCR-positives. You also showed that look-back -- about 12.5, or one-eighth, of the IFA-positives were infectious. If you do the math, these 25 IFA-positives would have prevented three transmission, and we had three nucleic acid test-positive. Essentially, the two tests -- very crudely, very small numbers -- would have prevented about the same number of transmissions.

Clearly we need a lot more data than those 1,007 cases. We need 10,000 or 50,000 tests. We need to

understand that. We need a lot more data on the tests before we make any decisions on this.

Thank you.

DR. ALTER: I definitely agree with what Dr. Benjamin just said. We always need more data. But based on what I heard today, if David Leiby is correct that the presence of antibody indicates persistent parasitemia -- although it's presumably dwindling over time -- then antibody takes on a different meaning. I'm encouraged by the fact that the antibody disappears eventually. You will be able to reenter these donors later on. So if I had to pick one test, IFA makes more sense to me, given the pitfalls of that particular PCR. You would miss an occasional window-period, but we know we're going to miss things. But you would capture the people who are infected for a longer time. You become positive relatively early in the infection. I think it's more doable on a blood-bank basis than would be PCR.

But it all hinges on that issue of antibody indicating infectivity or parasitemia.

DR. HOLLINGER: Any other questions?

It's interesting. If you go back to the 2008 workshop -- it's always amazing to me. You have major workshops. Things are discussed at the workshop and then not much is done in the two years or so since then.

I went down through that workshop and put down some things that were mentioned as topics that they wanted to resolve. It's amazing how many have not been resolved. For example, they talked about the carrier interval, based on serial testing, how long a carrier remains. There was some data that was presented today that suggested that about 30 percent of individuals who had an acute infection may be PCR-positive for more than 12 months. Most of them had lost their detectable parasitemia by 12 months. That was one thing that they thought was important. There is some data on this now.

We don't have much evidence about the pathogen burden, either the fatalities or in those with persistent infection. We don't know the minimum number of pathogens necessary to transmit disease.

I'm glad to hear that in January this disease will be a nationally notifiable disease. That's good.

There is not a lot of information about cross-reactive epitopes in assays to pick up the other *Babesia* variants.

There were questions before about how you distinguish between asymptomatic donors with active infection and those with resolved infection. We did have a fair amount of information on that in terms of the fact

that we do have PCR, and real-time PCR seems to be more sensitive. This can help distinguish an active infection from a resolved infection. Perhaps even high concentrations of antibody in some patients may provide that information.

We still don't know the frequency of acute versus chronic disease that occurs in populations or the incidence of disease that's occurring in endemic areas.

We don't know the duration of IgM antibody, how long it's present, and whether that would be useful in determining acute infections.

We don't know the percent of current infections that are non-*B. microti*.

The final one is, we did have data that perhaps the new IFA that is available, or the new technology that is available, would be adaptable to mass donor screening.

So while there has been some movement afoot, there are many questions we don't know. I think that's the problem that the committee is facing. Many of you here have expressed issues about testing, about the specificity and sensitivity of the testing, and so on. While I think we have given the FDA some information about -- if there is a test and if it's going to be tested, perhaps some sort of regional testing would be useful here. I think the blood banks are able, obviously, to do this; the blood

organizations are able to do this, and do it quite well.

Any other thoughts on the topics today? It's 6:00. We're only 15 minutes late.

Yes, Jay?

DR. EPSTEIN: Just to remark that FDA welcomes the possibility of INDs looking at different test technologies. The whole incentive here is to do something now. Everyone recognizes that there is more babesiosis, that there is at least the apparent trend of more transfusion-transmitted babesiosis, which would correspond to what we think is the general epidemiology, that test technology, generally speaking, is evolving in a positive way. We heard about RT-PCR. We heard potentially about automated IFA. We heard about ELISA that may be comparable, perhaps, to IFA -- small N, but still.

The FDA's position is, better to be doing something that, at least on a basic scientific consideration, is likely to have benefit than to be doing nothing.

We would strongly encourage candidate test-kit manufacturers to approach the agency so that we can expand the availability of testing under IND.

DR. HOLLINGER: Thank you. I hope that we will see some movement afoot on this, both with testing that comes in and how to approach this issue.

Anybody else have any burning comments?

(No response)

If not, we're going to adjourn. We'll see you tomorrow morning at 8:00. Thank you.

(Whereupon, at 6:04 p.m., the meeting was recessed, to reconvene the following day at 8:00 a.m.)