member center, Blood Systems, performed an anonymous survey of donors in an attempt to estimate the donor loss related to the vCJD travel deferral.

This work is being presented at the upcoming meeting of the American Association of Blood Banks, and the authors are Doctors Murphy, Connor, McEvoy, Hirschler, Busch, and others. They have allowed me to present their major findings. They distributed 10,000 anonymous questionnaires to donors earlier in 2002.

Two thousand surveys were allotted to each of five geographic groupings of blood centers. They received 84 percent responses, or 8,400 responses. Overall, 3 percent of the donors responding to the survey met Phase 1 or Phase 2 vCJD criteria for deferral. However, there were marked differences in predicted deferral rates between northern California, in which they reached 7.7 percent, and other geographic

areas combined of 1.8 percent.

Northern California deferrals were for U.K. travel and European residence or service at a military base, and the other geographical areas deferral was almost exclusively for service at a European military base. This survey documented our major surprise with the travel deferrals.

In the past we focused our concerns on the impact on donors in large U.S. cities on both coasts. We now realize that military deferrals constitute a major component of the deferrals. This should not have been a surprise because traditionally military personnel and their families have been dedicated contributors to the civilian blood supply.

Some ABC centers are seeing substantial deferrals of high school and college students because they were military dependents and were born or lived in Europe for extended period of time. Other blood

centers have told us that they have seen significant attrition of donors at corporate blood drives with multi-national companies whose employees and families routinely are posted in Europe for extended periods of time.

I'd like to go to the next slide and talk now a little bit about the status of blood supply among ABC member centers.

The implementation of new deferrals came at a time when almost one-third of the ABC blood supply has been at critically low levels, as shown by the results of what we call our Stop Light Program. In the Stop Light Program, ABC member centers report their red cell inventories daily via an internet program; an ABC posts its daily summary report on its web site. It's not nice looking, but it's informative. The web site is www.americasblood.org, and it's public.

This figure summarizes the current

data, and what we have seen agrees entirely with the picture that has been shown by both Dr. Page for American Red Cross and Dr. Jones for New York Blood Center. That is, we classify -- or, the centers classify their inventories as green when they have three days or more of their average distribution in inventory and are comfortable with that; the yellow is two days of inventory; and red is one day or less of inventory. So, as you see, in the weeks of June close to 30 percent of our centers had one day or less of inventory.

So that there is no confusion with the nice but complex graphs that Dr. Nightingale showed to us, what most blood centers tend to do is to shift their inventories to the hospital to have the inventories available at the hospital level, so the complete picture is more difficult today. But the way the inventories get supplied is by requests to blood centers.

So blood centers with one day or less of inventory have fulfilled the hospitals. The hospitals may be doing okay, but that's where the situation at the blood center is more complex.

The next slide, please. It's clear -- and this is the percent of the ABC blood supply at the code red, one day supply or less, as of June 19th. It is clear that the current supply is less than optimal and that the trend is for a continuous decline.

This past Tuesday, all donations, blood banking, and public health leaders have joined together to issue a call for eligible Americans to give blood this summer. I'd like to extend this appeal to the members of the committee, and please donate blood. The appeal comes in face of increasingly significant blood shortages. Last in a one-day supply in certain parts of the country. The appeal was issued by the American Association of Blood Banks,

America's Blood Centers, American Red Cross, the American Hospital Association, and the American Public Health Association.

Now, I'd like to make a request to the committee. We all share a common purpose -- the availability of a safe blood supply. Concerns about the possible transmission of vCJD by transfusion have led us to implement strict donor deferral policies. They are having an impact, a serious impact on the blood supply.

They are not the only cause for these shortages, but I believe they are an important element of the shortages. While we recognize that the data about the potential for transmission of TSEs in animal models continue to develop, we still lack evidence of such transmission among patients that receive transfusions in the U.K. -- and I would like to hear more from our U.K. colleague -- or even more elsewhere and patients that have received multiple

transfusions and ---- with concentrates for many, many years since the beginning of the BSE epidemic traced back to 1980.

The number of cases of vCJD has increased slowly. However we continue to add and maintain measures waiting for definite proof to determine whether TSEs are or are not transmitted by transfusion. We respectfully request that FDA and this committee discuss mechanisms and criteria for review of those decisions.

Our questions are how do we balance the theoretical risk of transmission with the real risk of blood shortages. Or, more specifically, what would be the criteria or the requirements, or what would be needed for a decision to lift some or all of the vCJD- related travel deferrals. We certainly are ready to participate of such effort and provide you with as much data and information we can gather.

Thank you very much for the

opportunity to comment.

DR. BOLTON: Thank you, Dr. Bianco. Questions? Comments?

I have one. It's curious to me that the south was a -- 48 percent of the southern centers were at one day, yet that didn't seem to correlate with your vCJD deferral.

DR. BIANCO: Actually, Dr. Bolton, you hit the nail on the head. That was a surprise. Those are the centers where the military collections, where the military deferrals affected. I realize only now that, for instance, one of our members is the large center in San Antonio, Texas. About 30 percent of their collections come from military bases, and that's true from several other regions. I believe that you saw that in the Carolinas, Peter, and in other areas.

DR. BOLTON: Did that also show up in the 8,000 surveys that came back from

that region for example?

DR. BIANCO: From that region? The blood system is an interesting system that is more mid-west and West Coast. They cover -- they have 23 centers, is our largest members. But they do not have centers in the south.

DR. BOLTON: So, that survey was really a very remarkably good response, and even with what appeared to be very good data, you still could not tease out this effect in advance of the actual implementation.

DR. BIANCO: Well, it really emphasized the importance that the military have had as part of our donor base. We never -- we always called them volunteer blood donors. But now we are seeing the impact more clearly, and I hope Ron is going to help us more with that.

DR. BOLTON: Oh, Jay? Microphone, please. It's still not on. Why don't you

use one of these? Use the table mike.

MR. EPSTEIN: Again, I don't have the data in front of me but my recollection is that we did predict approximately a 2 percent national loss due to the DoD-related deferral. What was not predicted was the fine geographic distribution, because the survey wasn't capable of that.

I think the surprise has been the extent to which the military-related donors contribute at certain centers. But it's not that we were particularly off in our estimate of magnitude.

DR. BOLTON: Right. I know that as the committee deliberated on this, we were well aware that the military personnel on active duty, as well as retirees, are regular and willing blood donors as part of the military culture. And I certainly, for one, expected that they would have a significant impact in this way.

The good side of that is that we

can look to that as a model for the change in culture that we need to establish to begin to increase the percentage of all citizens that donate, because I think within the military there is a recognition that blood is important. It's sort of a part of your duty to be a donor, and we just need to spread that throughout the rest of the population.

DR. BIANCO: I think that I tried to say that. It was a surprise, but it shouldn't have been a surprise.

DR. BOLTON: You did say that. Dr. Piccardo?

DR. PICCARDO: Well, I think that we knew that the military contributes substantially. But the point is that they did not respond or the percent of response to the survey was very low. So, maybe the numbers aren't there, but we knew that they contributed substantially.

DR. BOLTON: Well, I think what

Dr. Bianco said is that the survey didn't reflect that southern region, so even if they had responded in the mid-west and what have you, it didn't really reflect that some of the southern centers would be hit in that way, and I guess Jay was saying the same thing, that the regional distribution or the spottiness of the effect wasn't teased out.

DR. PAGE: Page, American Red Cross. I just have to correct a statement that Dr. Bianco made about the American Red Cross. The Carolinas region had a deferral percent related to CJD in March of '02 of .4 percent, which is less than our system average of 0.6 percent. So, they were not amongst the highest.

DR. BOLTON: Yes, Dr. Gambetti.

DR. GAMBETTI: I assume that all of these data are based on the FDA recommendation for deferral rather than the Red Cross criteria.

DR. BIANCO: That is correct. Our

centers have adopted the exact recommendations of FDA for Phase 1 and Phase 2. The only thing that I should note again is that about 60 percent of the centers decided to implement both Phase 1 and Phase 2 at the same time. But they are the exact FDA criteria.

DR. GAMBETTI: So, there is no room for correction.

DR. BIANCO: No, there is room for collection, not correction.

DR. BOLTON: Dr. Williams.

DR. WILLIAMS: Thank you. A very brief comment for the record on how the deferrals related to military went into the calculation. We were not totally dependent on the survey for those numbers.

We, in fact, got figures from DoD on the estimated proportion of military and ex-military residents in the states, did ---- calculations on the likelihood of donations by that cohort. In fact, ----

has 2 percent. In fact, in one site we did try to specifically survey the military population to see the proportion that it spent, the appropriate times in Europe and the U.K. that would result in deferral, and we got a less-than-10 percent response rate.

So while they like to donate blood, they don't like to do surveys, so that was a difficult measure. But I think the overall impact nationwide was probably on because of the way the data were obtained.

DR. BOLTON: Very good. Okay, I think we'll move on now. Our next speaker is in fact Maj. Ronnie Alford from the United States Air Force. Maj. Alford.

MAJ. ALFORD: Good morning. Can I have the next slide, please?

We in the Department of Defense implemented the additional vCJD deferrals in October of last year. We implemented the deferrals as recommended by the FDA with one

notable exception, and that is we combined the north of the Alps and the south of the Alps recommendations and applied the more restrictive south of the Alps recommendations for all of Europe.

One note of interest here is that from our assignment histories, we knew that we could expect 18 percent of those forces currently on active duty to be deferred based on the new deferrals. That on top of the historical 25 percent deferral rate that we experienced prior to vCJD deferrals, our No. 1 deferral was for piercings and tattoos. Of notable interest here, we do not include time aboard ship for naval forces that were on Mediterranean cruises. Only time ashore is accounted.

Slide, please. I put this slide in just to give the committee members an appreciation for where our donor centers are located. We only operate 18 donor centers in the continental U.S., and you can see

where they're concentrated with the huge void there.

Slide, please. In addition to our CONUS operations, we also operate six donor centers outside of the continental U.S., and you can see the locations. So, total of 24 donor centers worldwide.

Slide. Our donor population. Our donor centers within DoD. We do not go outside of the gates of our installations, if you will, to collect blood. So, we're totally dependent upon our active duty population and family members and civilian employees on base. The lion's share of our collections comes out of that 1.38 million active duty personnel. Ninety-two percent, in fact, of our donations come from those people.

We only get 2 percent of our donations from family members. Even though that family member number is fairly large, two-thirds of those are children.

Our total donor operations within DoD are very small in comparison to the other speakers this morning. We only recruit about 130,000 donors a year, and we only recruit off of about one-tenth of major DoD installations worldwide. We only recruit from 32 DoD installations worldwide.

So, we're getting about 9 percent penetration into our active duty forces. They're actually getting out to donate. Major interest here is that the distribution of that 18 percent is not even. We have seen deferrals as high as 50 percent in some of our forces -- heavy armor units at Fort Hood, Texas, for instance, because of huge rotations to Bosnia over the past several years and also huge rotations to Germany.

Slide, please. It's very difficult to accurately determine the impact of the deferrals that we implemented. This may be news -- it certainly is big news within DoD and certainly I think it goes

along with the normal course of business for our civilian counterparts. We have not traditionally had access to donor recruiters.

We were successful in gaining funding and placing recruiters out into all of our donor centers this past year in October alone. So, as we implemented the deferrals, we gave the donor centers recruiters and we have had a tremendous -- they've given us a tremendous boost in our collections.

Again, we've seen self-deferrals at command briefings, and that's -- command briefings are when we actually get a battalion or a company, a large group of soldiers, sailors, airmen, marines, together and we would generally get a small five- or ten-minute time frame to brief those people. We've seen deferrals as high as 50 percent among those units. Again -- and we've also some additional educational things from the

DoD level going down.

Although operation Enduring Freedom is not front-page news in The Washington Post every day, it's certainly front-page news at places like Fort Bragg because those are the places where those troops are coming from, the family members there, the soldiers remaining behind.

That's very much on the forefront of their minds, so we've seen recruitment being eased because of forces being pushed forward into a theater of operations. We have pushed about 15,000 units into southwest Asia and European evacuation routes as well.

Another complicating issue is that because our donor centers are typically staffed with the laboratory technicians from the hospitals, a lot of those lab techs were forward deployed, so our donor centers lost staffing. We did have reserves -- some reserve forces come in, but of course you

lose productivity with bringing them up to speed and training and you lose one of your valuable trained staff to do the training. But we have seen a major bounce-back.

Then probably the most important thing that we've done is expanded our collections at our basic training commands.

Slide, please. In a comparison of the year prior to implementation of the new vCJD deferrals to the six months afterward, we've actually increased our whole blood donations by 9 percent. We are averaging 12 percent deferrals for vCJD, and that's, again, across DoD, again, with the greatest impact being with units with heavy European rotations. We're seeing less than 1 percent in some of our basic training units. That's actually basic training throughout all of the services.

So, our total deferrals for fiscal year '01 was 25 percent. Now we're down to 16 percent total. That is for

everything, including piercings and tattoos. So, we have actually -- we've made great strides here.

Next slide, please. Then just a graphical representation of our collections. Again, you can see the 9 percent increase.

Slide, please. So, in summary, although we did implement the additional deferrals in October, we took corrective steps to alleviate the increased deferrals by adding the recruiters and shifting collections to trainees. Again, we went to war, which I think definitely made recruiting easier, although we really did not see a way of quantifying that.

We've increased our eligible donations and the actual whole blood donations by 9 percent. The range of deferrals, again, as low as 1 percent, as high as 50 percent. Tremendous swings even though we do not see a clear geographical distribution, if you will, for those

deferrals. It's really more toward the mission of the units, and we pretty much have a good feel for the types of units that have heavy European rotations.

Again, we have decreased our total deferrals by 40 percent. That's clearly as a result of actually having donor recruiters and having effective donor education programs to let the people know -- the potential donors know -- if they are eligible to donate. So, in regard to CJD deferrals, put a checkmark there. We have certainly met that challenge and in fact increased our donations.

Any questions?

DR. BOLTON: Thank you, Maj. Alford. Questions.

I guess I have one. I'm the only one who has lots of questions today. I've completely forgotten about the piercings and tattoos, so, I mean, this is a little off of our subject but can you inform me is that a

lifelong ban or is it a year period of time from the time that the piercing or tattooing is done?

MAJ. ALFORD: It's a year.

DR. BOLTON: It's a year, okay.

MAJ. ALFORD: One of the things that we've noticed is that now that we're focusing on trainees, I guess it's the culture of the military I guess that many young men and women feel that you graduate from basic, "Let's go get a tattoo." So, we're collecting them before we let them go get the tattoos.

DR. BOLTON: It's a very good strategy. I was just going to ask -- not getting the tattoos so that they don't have to give blood, though, is that the --

MAJ. ALFORD: No.

DR. BOLTON: Other questions or comments for Maj. Alford?

Very good. Thank you very much. It's nice to see something that can be done

in a positive way to deal with the situation.

Our next speaker is Kay Gregory, Director of Regulatory Affairs, the American Association of Blood Banks. Kay?

MS. GREGORY: I think the mike's on now. I want to begin by explaining who the American Association of Blood Banks is. We're a professional society, so we don't actually collect blood or transfuse blood but our members all do, and we represent 8,000 individuals involved in blood banking and transfusion medicine both, and approximately 2,000 institutional members, and these include blood collection centers, hospital-based blood banks, and transfusion services as they collect, process, distribute, and transfuse blood and blood components and hemapoietic stem cells.

Our members are responsible for virtually all of the blood collected and over 80 percent of the blood transfused in

this country. For over 50 years the AABB's highest priority has been to maintain and enhance the safety and the availability of the nation's blood supply.

The AABB believes that lack of appropriate data is a major barrier to determining the state of the blood supply in the United States. Although public health experts and the transfusion medicine community recognize the need for such data and committees such as this one routinely ask for this information, no agency is willing to fund a comprehensive ongoing collection and analysis of data about the blood supply.

Because there is no systematic, scientifically valid routine collection of data concerning supply and usage, there's no established baseline and thus it's impossible to measure the effect of policy changes, such as the new vCJD deferral. Any attempt to quantify the effect of the new

vCJD deferral will be difficult if not impossible.

At this point in time, it certainly is not possible to gauge the effect of a policy that was required to be implemented only on May 31st, 2002. Any decrease in donations is compounded by the well-known summer slump in donations. Moreover, the same difficulties in measuring the effect of new donor policies that were discussed at previous meetings of this committee with regard to the in initial round of vCJD deferrals are also applicable here, and you've heard some of these.

It may be possible to measure how many donors appear at the blood center and are deferred because of vCJD criteria. However, we cannot measure how many donors self-defer because of advanced publicity, including significant efforts on the part of many blood centers to notify donors about this change.

The committee should also keep in mind that the number of deferred donors does not equate to the number of blood components that are lost. Apheresis donors can donate more often than whole blood donors, and deferral of such donors increases the number of blood components that cannot be collected.

That said, AABB appreciates this committee's interest in the state of the blood supply. The National Blood Data Resource Center, an independent subsidiary of the AABB, continues to collect data that may be of interest. NBDRC captures data on a monthly basis from 25 blood centers in the United States. This geographically and statistically representative sample accounts for 32 percent of allogeneic, whole blood, and red blood cells collected by U.S. blood centers annually.

This slide illustrates the allogeneic collections for the most recent

six-month period from December of 2001 through May of 2002. Although not apparent from the graph, December monthly collections were the lowest reported for the entire year of 2001 -- only 319,000 units. Thus far in 2002, monthly collections have averaged about 342,000, and that's not significantly different from the average for the same period in 2001, which was 338,000. The 3.1 percent decline in collections experienced in May, however, is statistically significant when you compare April and May.

We also collect inventory data twice monthly, and until recently we had not seen any significant fluctuations in 2002. However, between the third Wednesday in May and the first Wednesday in June, the sample experienced a 5.6 percent decline in whole blood, red blood cell inventories. That is statistically significant.

The NBDRC's monthly analysis of collection data provides an important first

element of determining the true state of the nation's blood supply. However, as we have stated to various government agencies and advisory committees, additional data on blood usage are also needed if we are to understand long-term trends and forces affecting the nation's blood supply. The AABB strongly urges the Department of Health and Human Services to support this essential data collection and analysis.

Thank you.

DR. BOLTON: Thank you, Kay. Questions? Steve?

DR. NIGHTINGALE: Thank you. I would like to make a follow-up comment on what Ms. Gregory said -- and if you'll show the -- it's slide No. 2 I want showed, if I could, please -- because I think we are on our way to getting to the point that Ms. Gregory and the AABB would like us to get to, and that is on a line that I neglected here.

I want you to look at this line here, at the bottom. This is the total usage of blood at the hospitals that are served by the three community-wide blood centers in Seattle, Pittsburgh, and Tampa-St. Pete. It is not representative of the United States but it is representative of a lot of hospitals. What I want to show you is that there is a lot of regularity in here where you see the two dips here. That's a Thanksgiving dip; that's a Christmas dip.

I can't tell you how many of these blood units went for obstetric care, how many went for cancer chemotherapy, how many went for trauma. But I can tell you over almost a year period this has been quite stable. I personally think it's up in the air, and it's going to be discussed when we review our program in September just how much more detail we need to get into, but we may be a little closer to it with efforts

like this than the people appreciate.

That leads me to the second of the two points. This stability -- this constancy of blood use -- this demand for blood has persisted over a time when the price of blood has gone up very, very substantially, and I think the committee should have an immediate appreciation for the fact that at least with the current supply and demand, the demand for blood at current prices is very, very inelastic.

Blood is somewhere between $115-$200 a bag right now, substantially more than what it was before and probably not at its peak. The question is how much more are hospitals willing to pay for blood and how much more are hospitals willing to pay for additional resources necessary to bring blood to the market.

A question before you is -- before us is: Whether or not the federal

government should embark in a $10 million campaign to promote blood donation. I will speak as an individual here just for this sentence, is that the 10 million that has been proposed seems to me to be a low ball estimate of the cost that it's really going to pay. Ten million is less than one dollar per additional bag of blood, and I think it's going to take more than that, and when you make your recommendation, a little bit of economics should go into it.

DR. BOLTON: Steve, while you're up there, let me ask you this question. What would it take -- not thinking in terms of dollars but in terms of organization -- to get all of the major blood collection organizations and centers and possibly the hospitals across the country to participate in a data collection system as you have established?

DR. NIGHTINGALE: I think that at this point if we wanted to -- I want to use

this word carefully -- bang on the door real loudly as opposed to kick the door down, I'm sure that ABC and ARC would cooperate. The information is available through back channels.

We talk on the phone all the time. I think we have to respect the fact that the responsibility for blood collection in this country is in a private sector and not in the government, and we would be much more aggressive if that were not the case.

We do not operate a Canadian system, and we try to get feedback from Red Cross and the ABC but how hard can we lean on you on this. Sure, I'd love to have the daily numbers from the Red Cross and the ABC for my particular project. The figures would look better.

But the reason for this project is not to get a better paid ---- transfusion. The reason for this project is to try to support the private sector -- do a very

difficult job better. That's why we tap on the door gently.

DR. BOLTON: Right, and my question comes from the assumption that the organizations that are actually doing this job in the private sector, and in the military as well, would benefit from having that global -- national picture to analyze and to really understand, for example, the effects of not only policy decisions that we might recommend, but other events that may occur.

DR. NIGHTINGALE: There are some realities in the business that we have here that are different, say, from Britain. We have a competitive environment, and an ongoing philosophic, political, even economic question is how much of that is good and how do we keep it from, you know, competition for the same high school students, for example. That's an area where I think a knee-jerk government response

could very well be counterproductive.

DR. BOLTON: Right. Well, I know there's a balance between how much that information would be useful and how much it would be scary in a sense to competitors sharing their information. But I would certainly ask the various blood collection organizations to think about this and see if there isn't some way that -- I don't know if we can preserve the proprietary nature of some of the data and yet still make it useful not only for us but for them as well.

Yes.

MS. GREGORY: If I could just add something. The difficulty is not necessarily getting the data from the blood collection centers. The difficulty is getting the data on the other end of it, and that is: The transfusion data is much more difficult to get than the collection data.

There currently -- as far as I know, Steve is the only one collecting any

kind of data, and as he -- we don't know whether blood's being used for cancer patients -- exactly what it's being used for. We would like to be able to have that information, but right now there is nowhere to get that from.

DR. BOLTON: Well, let Steve respond and then Dr. Bailar.

DR. NIGHTINGALE: Just last -- last -- I mean, one of the reasons, obviously, that we might like to have that information is to identify how much waste there is in the system. I presented one piece of data: In our system there is essentially not waste at all in the centers.

We cover about 8 percent of the blood supply. We see 10 units of blood outdated a day in 8 percent of the blood supply and, anecdotally, that's all AB positive that you can't get rid of. That situation is different in Britain for several reasons.

One possibility is the economic pressures are different. Blood's expensive here, and we don't outdate it.

The second question is how much education till we recommend to change physician practices. I don't think it should be taken as received wisdom, but there is massive wastage of blood in the system right now.

Harvey Klein, Director of the NIH Blood Center, and for that, my first resident when I was an intern -- said something that I feel is very wise and needs to be repeated here, which was HIV scared a lot of the slack out of the blood system right now, and the thing that I would follow up from Harvey is that the price of blood where it is right now -- if this hasn't scared the slack out of it, when blood hits 250, that's going to scare the slack out of it.

DR. BOLTON: Dr. Bailar?

DR. BAILAR: I'm pretty convinced. As a committee member, I'd like to make a strictly informal request to FDA to work with the private agencies in considering whether FDA should actively promote a substantially expanded and improved data collection effort, related to blood supply and usage and, if so, to return to this committee with any specific questions or proposals where we might help.

DR. BOLTON: Okay, Dr. Wolfe?

DR. WOLFE: When Dr. Scott made her presentation this morning, she pointed out -- and this is, for starters, just a question -- that back when this committee made the recommendations for deferral, there was also some recommendation for a national recruitment campaign. Neither Dr. Bailar, who has raised this issue, nor I were there then.

Was there specifically a proposal to try and do something about the so-called

summer slump? I mean, I'm all in favor of more data collection, too, but the data is unequivocal that year after year there's a summer slump, and it happens this year to coincide with the initiation of the deferral program so it makes it look worse.

I would have liked to have seen seasonally adjusted data for some of the presentations that were made, but was there specifically a recommendation to try and do something about the summer slump? Dr. Linden has pointed out that New York creatively initiated this motor vehicle registration reminder at the beginning of the summer, and it seems like to focus more on campaigns at the time when there is a predictable slump would at least take some of the edge off. Was there that recommendation, and what has been done by all the agencies that are involved?

DR. BOLTON: You're testing my memory, but my recollection is that there

was not a request to specifically address the summer slump, but there was a request to move to improve recruitment by a national education campaign, and there was also a suggestion that this data collection system be instituted -- some means of trying to track the blood collections and usage.

DR. WOLFE: I would then add to Dr. Bailar's suggestion in addition to getting more data that we should use existing data and here and now request that all of these agencies that are involved come back with a very specific plan for dealing with the summer slump. If, as suggested earlier, it will take yea number of months -- 12, 18 -- before that's implemented, we've lost already a year or whatever since the committee made a general recommendation but apparently did not focus on doing something about the summer slump. It seems like that would make it much more tolerable to implement this policy.

DR. BOLTON: Let's get Jay's input. Jay?

MR. EPSTEIN: Well, first, it's correct that there have not previously been proposals specifically directed to the summer slump. However, the concept of the government getting more involved with efforts to both monitor and increase the blood supply do date back several years.

When we first discussed the potentially large impact of precautionary policies directed toward variant CJD, it was recognized, particularly by Dr. David Satcher, then the Assistant Secretary and Surgeon General, that we would need to accompany any such policy by a program to monitor and increase the blood supply. The department did adopt in November '99 an element of what we called our blood action plan, which is a plan that dated back to March '98, an element specifically to address monitoring and increasing the blood

supply. We did build on previous efforts.

There was a period -- I think it was three years -- during which the NHLBI funded the NBDRC; you heard their report from Kay Gregory -- to produce monthly data. Up until that point in time, there were no periodic reports. There were only surveys that were done basically as retrospectives covering approximately every other -- I'm sorry, once every two years there were these reports that were looking back several years. And instead, effective roughly, the fall of '99 we had monthly data with a one-month lag.

It was then recognized that those data were based on the collectors and didn't give us the other side of the equation which was used, and after much dialog and debate over responsibility in funding and so forth, the department took on the challenge of creating daily monitoring of hospital inventories coupled with shortage reporting.

That's the system that Dr. Nightingale has been presenting.

Now, you know, are we where we want to be in terms of data collection and analysis? Well, I think the discussion today, and discussions that have gone on, you know, within the government and the blood industry, do suggest that much further progress could be made. But we are where are because of the deliberate effort to do the very thing that you're talking about recommending.

Now, there has also been a lot of talk about the relative role of the government versus the private sector in initiatives. It's not a trivial issue. There have been efforts to engage government officials in public service announcements coordinated with urgent blood drives. Some of that has been done. I mean, there actually were videotapes of public officials and there have been efforts to coordinate

the voice of the blood industry so that there are unified rather than competitive donor campaigns.

We have also taken on the scientific challenge of trying to remove unnecessary barriers to donation by re-examining the deferral criteria, as well as the use of tests. We've done that largely with the Blood Products Advisory Committee because many of those issues are not TSE related. So, for example, we've reviewed the deferrals for body piercing, tattoos, and acupuncture. We've reviewed the exclusions for males who have had sex with males. We've reviewed whether we should continue testing for syphilis and so forth. So we have been moving on all these fronts, but I certainly share the sentiment of the committee that more is needed in the current situation, that we're not yet on top of the problem, and I'm certainly appreciative of the remark that we need to

focus in particular on this periodic summer slump. Let me just say that December is no picnic either.

DR. BOLTON: Other questions or comments? Very good.

Well, that concludes our presentations on the blood supply of the revised guidance, and at this time I think we'll move to the open public hearing.

Bill, do you want to --

OPEN PUBLIC HEARING

DR. FREAS: Yes, Mr. Chairman. We have not gotten any requests to speak at this morning's open public hearing. Is there anyone in the audience who is not on the agenda who would like to address the committee on topics on the committee's agenda? If not, this will be a very short open public hearing.

I turn the microphone over to you.

DR. BOLTON: Okay, well, we're running about even with that short public

hearing. We are still running about 20 minutes behind time. I would like to ask the committee members, sort of informally, would you like to take a 10-minute break and come back? Okay, that will put us a half hour behind but I think we'll still be able to conclude, so let's meet back here at 11 o'clock.

(Recess)

DR. BOLTON: Would the members of the committee please take their seats and we'll resume our meeting -- as well the members of the public? I was obviously too lenient. I gave you a 15-minute break instead of a 10-minute break.

Okay, the final part of our meeting we'll have two presentations, also updates on some experiments with variant CJD and filtration. So, the first presentation is by Dr. Larisa Cervenakova, and she will be speaking on studies of the variant CJD infectivity in blood of experimental mice.

Dr. Cervenakova?

STUDIES OF vCJD INFECTIVITY IN BLOOD OF EXPERIMENTAL MICE

DR. CERVENAKOVA: Good morning. I would present studies in variant CJD infectivity in blood of experimental mice.

Slide, please. What I'm going to do -- I would like to compare our data, which are still in progress. We didn't finish experiment yet, but we have a sufficient amount of data to give you the idea what's going on with use of blood infected mice with variant CJD, and I would do comparison to our previous experiments, which were done in also mouse model, using Fukuoka strain.

At the end I will briefly show you one slide when I will compare these data with experimental data collected in hamsters infected with 263K strain, derived from scrapie in sheep.

Next, please. This is just to

show you how the experiment was set up. The brain tissue from the variant CJD 10 percent brain homogenate was inoculated in R3FADK (phonetic) mice by ICNAP route of inoculation, at the Institute for Animal Health in Edinboro. We get brain tissue material from a mouse which developed the disease at 336 days after inoculation from ---- and as well we got from the strain of mice susceptible to variant CJD, and we actually breed these mice in-house and inoculated them with 1 percent mouse brain homogenate, inoculated intracerebrally in group of mice.

In one group of mice, we euthanized at 17 weeks after inoculation, in order to see if we can produce the disease clinically, and later on we saw, after 157 days, first thing was started to develop the disease. At this point we euthanized the rest of the animals, and we collected from both groups of animals blood, pulled it

together, separated it into components and inoculated the components. We also collected brain tissue and spleens for later detection of the presence of abnormal prion protein in brain and spleen.

Next, please. These data compare actually the incubation period in mice infected with BSE and variant CJD and secondary transmission from the same strain of mice, and you can see the shortening of incubation period was shrunk for both strains -- for BSE and variant CJD.

Next, please. Because we wanted to compare our data to Fukuoka strain, it was important for us also to see if Fukuoka strain would take in R3 mice and what will be the incubation period for R3 mice and for Swiss mice, in which experiments with Fukuoka strain were done. And you can see the incubation period for Fukuoka strain shown in yellow and for variant CJD is shown in blue and actually the incubation period

for Fukuoka strain in both strains of mice is shorter than for variant CJD.

Next, please. This is by chemical ----, if you wish, or from PrP 27-30 in both strains of mice for Fukuoka strain and variant CJD, and you can see that there are differences in the profile, and the profile stays the same in both strains of mice.

Next, please. This is data on the presence of abnormal PrP in spleens of mice infected with the Fukuoka strain and variant CJD as a clinical stage of the disease. And right now I would like to say that we did for clinical animals as well, we haven't done every animal, every single animal, but we actually can detect the presence of abnormal BRP in spleens of variant CJD infected mice at 17.

We're accepting inoculation at 23 weeks after inoculation and very recently we inoculated another strain of mice with variant CJD strain just to propagate as the

infection in animals and have animals ready for other blood infectivity studies. And at 79 days after inoculation is 10 minus 4 infectivity in inoculate, we detected the presence of abnormal PrP in spleens of these mice, and this is the answer probably to the questions which you addressed yesterday.

You can detect the presence of variant CJD agent 3 in no particular system clinically and, yes, we can but it is not very much different from what we know from other strains that in half the incubation period, approximately, we can detect the presence of PrP infectivity in spleens of the animals.

Next, please. This shows how the blood was separated into components, and we inoculated all of these components, but today I'll talk about buffy coat platelet rich plasma and buffy coat platelet poor plasma, because this is part of the experiment which is almost done, completed.

Next, please. Here are the pulled data from mice infected with Fukuoka strain and with 310V mouse adopted BSE strain and the level of infectivity in blood components of these animals and you can see that this data correlates nicely together.

Next one. This slide is very busy, but it gives you the idea about the incubation periods in mice which were inoculated with Fukuoka 1 strain by intracerebral intravenous route of inoculation. Buffy coat plasma, buffy coat in R3 mice inoculated with Fukuoka strain and various CJD inoculated R3 mice. And as well, you can see different routes of inoculation.

If you look at the slide, actually you cannot see significant differences between incubation period in mice infected with GSS or in mice infected with variant CJD. This last blue across here shows that this animal was detected to be positive when

all animals were euthanized at 560 days after inoculation by Western Blot resulted in clinical science of the disease.

Next, please. This table represents the groups which we inoculated with different dilutions, a clinical phase of the disease, and this is for buffy coat, and here in yellow you can see that actually we completed this group and conformed -- did the Western Blots on the -- these animals were actually euthanized. All of them were euthanized at 560 days, and we were not able to perform Western Blot analysis on all of them, but this gives you a pretty good idea about what's going on.

Here you can see that the incubation period when the first animal showed signs of the disease. And here, as I told you before, the animal which actually was confirmed to have the disease only by Western Blot.

Next, please. This is data for

inoculation of plasma, platelet rich plasma, platelet poor plasma for 23 weeks and ---- phase 17 weeks. These animals were already euthanized, these two groups, and this is still in progress. They will be euthanized very shortly, and these animals are still incubating. We saw a couple of those in both groups but as the animals were not confirmed yet to be positive for infection by Western Blot.

Next, please. This is data from the study which originated from Dr. Brown, and as you may remember that inactivity in buffy coat was found to be present during the incubation period and raised dramatically at the clinical stage of the disease. Almost zero infectivity was found in plasma, but, again, that's a clinical stage of the disease. You can see increase in the infectivity are present in plasma.

Next, please. This is data for variant CJD. This is not completed yet, but

I still put zero here right now. But you can see, again, the infectivity titers in animals, you know, if this goes to the clinical stage of the disease. But still you can see the difference, that the clinical stage shows infectivity is a little bit high, compared to the clinical stage of the disease. And suddenly this is present in buffy coat, and we found this infectivity to be present in plasma at the clinical stage as well.

Next, please. These data compare the level of infectivity between Fukuoka strain in Swiss mice and R3 mice, because we did this comparison again, and you can see that there is pretty good agreement between the infectivity in buffy coat of Swiss mice and R3 mice infected with Fukuoka strain at the clinical stage.

Next, please. This is data comparing variant CJD and Fukuoka strain. Buffy coat and plasma inoculated by

different routes of inoculation by IC and IV. IC is shown in yellow and IV is shown in red, and you can see that intracerebral route of inoculation is most efficient for both buffy coat and plasma compared to intravenous route of inoculation, but we were able to show that it is possible to transmit the disease by both routes, and this is in comparison to Fukuoka strain.

Next, please. This is combined data for Fukuoka strain in mice, variant CJD strain in 263K scrapie strain in hamsters, and in all three rodent models you can see the highest level of infectivities present in buffy coat, and this is per ---- buffy coat or per ---- plasma, and as well in plasma rich infectivity present as well at the clinical stage of the disease.

Next, please. This is experimental data on transfusion studies. You know about the experiments which were performed at NIH many years ago when

chimpanzees were transfused and no transmission of the disease occurred. This is data collected by Paul Brown in cooperation with us, and one transmission was achieved upon transfusion of 20 animals.

This is data from Dr. Rowher's lab and, as well, he got three animals out of more than 100 transfused, and this is data from Fiona Houston from the United Kingdom when actually 24 sheep were transfused from 18 donors and 2 animals developed the disease, and this is our data with variant CJD. We did also close to 100 transfusions, and we right now have three animals which developed the disease upon transfusion.

Next, please. Finally, I would like to acknowledge people who were my collaborators or my technical staff. Thank you.

DR. BOLTON: Thank you, Dr. Cervenakova. Questions? Steve?

DR. DeARMOND: We see tons of data

on mice and hamsters but absolutely nothing about human blood transmitted into any animal model. What's going on with that? It's now 7 to 8 years, 120 people, lots of blood available for analysis. What is the result of human blood into an appropriate animal model?

DR. CERVENAKOVA: Well, first of all I would like to say -- I haven't seen any published data, at least on blood transfusions from human to animal. Suddenly it is very difficult to address this issue because we know that conventional mice are not very susceptible to TSCs -- human TSCs.

In this case, what we have to hope for is that we have susceptible, maybe transgenic, mouse. Even so, Dr. Brown is saying that maybe it is over-representing something. But still probably it be valuable in order to test and inoculate if it proves to be a susceptible .

The problem is, in my opinion,

that when we were getting this sporadic CJD, in general we have very low level of infectivity in blood of animals. If you have some infectivity present in blood of human, it probably also is at very low level. If you look at the studies which were done -- and were done very recently -- by Moira Bruce in the United Kingdom, when she inoculated buffy coat lymphocytes from human infected variant CJD, the problem is that she inoculated a very small number of animals.

Even if there is infectivity in the sample, she will be not able to detect it, because I forgot to mention that when we inoculated buffy coat only into five animals, we were not able, even with dilution 1 to 4, to 2, we were not able to see any infection. In this case, it is really necessary to have a large group of animals to be inoculated, and I have to say that Bob Rowher is completely correct about

it, that all his study points to the necessity to do these studies on the large scale.

There is no way how to do it otherwise because if you have, let's say, 10 animals out of 100 to show the infection, it means that it will be one animal out of 10. And it's always a question: Is it a true result, or it is just some kind of contamination?

This is what you have to take into account, that once you work in the lab, you handle a lot of material, infectious material. You have to really have worked out very well all the profiles which I showed you, because we are aware that if you Fukuoka, variant CJD, or some other strain, you may get cross-contamination.

In this case, this is one of the way to prove that this is true result because the strain stays the same. It doesn't change during the time.

But I think that it is necessary to do transfusion transmission studies using small animals and probably try to use different transgenic mice and see how they will perform.

DR. DeARMOND: Well, everything you say is absolutely true and that's the way you would run the experiment, but the right experiment hasn't been done yet. And we keep getting this stuff which implicates blood, but we don't know the real answer in humans.

As far as I know, the 11 variant CJD patients who gave blood and was transfused to other people, those individuals haven't come down with the disease. Intracerebral inoculation is certainly different than intravenous inoculation.

So I don't understand, and Stan Prusiner I know is very upset because we haven't been able to get the blood for

animal models that we know are susceptible to variant CJD from humans. So, this is nice data, but does it have any relationship to the human condition, is what I'm saying?

DR. CERVENAKOVA: Well, there is probably -- what we can take from this, that we have to have our open minds, that if there's a possibility that low levels of infectivity are present in blood of people --

DR. DeARMOND: Well, our minds are open. For the last two years, we voted to ban any import of blood because of this.

DR. CERVENAKOVA: Yes.

DR. DeARMOND: We believe that there might -- that there's a real risk --

DR. CERVENAKOVA: Yes.

DR. DeARMOND: -- but no one has shown it yet. This kind of data doesn't help.

DR. CERVENAKOVA: I believe the group which actually had better good

opportunity to do that is Dr. Prusiner's group, because he has susceptible transgenic mice to do the experiments, and --

DR. DeARMOND: Well, put some pressure on the British government and European government to send us the samples.

DR. CERVENAKOVA: Well, I don't know, actually, if this is British government, because right now there is a problem from our government to import even antibodies from Prionics. You know, our antibody was kept on the border last time for three weeks without noticing, as if we have to have some kind of permit in order to get them.

In this case, it is really difficult situation, but what I'm trying to say that experiments -- some experiments are in place, and probably is the best experiment right now which is going on it is the best experiment using squirrel monkeys, which originated from Dr. Brown.

The experiment -- and I don't remember if it was presented here, but the experiment is set up in the ways that actually blood from patients with variant CJD patients infected with variant CJD was collected, separated into components, and buffy coat and plasma were inoculated in squirrel monkeys.

But the number of animals is so low that I even question if we are going to see the infection, because you have three animals and if you don't have any of them developing the disease, what are you going to say? There is no infectivity. In my mind, no, you are not able to draw this conclusion.

There is no money to expend this study to have significant number of animals, which will be sufficient in order to show that there is really nothing there. This is very susceptible animal model to use for these kind of studies.

DR. BOLTON: Sue.

DR. PRIOLA: I have two really easy questions for you, I hope. What's the infectious dose that you used? Do you have an idea of the titer you inoculated, both IC and IV? This was presumably a very high dose.

DR. CERVENAKOVA: Well, when we inoculated originally, we compared actually the dose which we inoculated and it is 7 LD50s for both, for Fukuoka and variant CJD, because this was important for us to show that it is.

DR. PRIOLA: Seven LD50s?

DR. CERVENAKOVA: Yes.

DR. PRIOLA: That's not much.

The second thing is -- you alluded to this in part of your answer to Dr. DeArmond's question: Controlling for lab contamination when you have -- even in the positive experiments you have, you know, just a couple of animals coming up, and it's

obviously very low titer. I assume you look in all the brains to see if you have the shift in size between the GSS, the Fukuoka, and the vCJD?

DR. CERVENAKOVA: Yes.

DR. PRIOLA: Did you do a parallel mock infection going -- doing exactly the same thing but using --

DR. CERVENAKOVA: Yes, we did.

DR. PRIOLA: Those were all conclusive.

DR. CERVENAKOVA: They're negative. They're negative.

DR. PRIOLA: All negative. Same numbers of animals, etcetera.

DR. CERVENAKOVA: Well, we haven't done the same number of animals, certainly, but we do inoculate them, periodically, to have this control, because it is very difficult to produce a sufficient number of animals for this particular study, and this is why we switched to another animal, which

was showed, actually, is susceptible to variant CJD and has the same incubation period. And this is FVBn strain.

DR. BOLTON: Let me clarify. The inoculated dose was 7 LD50s or 7 log 10 LD50s?

DR. CERVENAKOVA: Seven logs.

DR. BOLTON: Okay. There's a little difference there.

DR. CERVENAKOVA: Yes.

DR. BELAY: The BSE agent transmission in the experimental sheep model that you showed. There were two positives, right?

DR. CERVENAKOVA: Yes.

DR. BELAY: Now, the one positive that was published received blood obtained in the incubation period of the experimental sheep.

DR. CERVENAKOVA: Yes.

DR. BELAY: How about the second positive? Did it receive also blood

collected during the incubation period or at the clinical studies?

DR. CERVENAKOVA: I'm trying to remember it. I believe it was also from pre-clinical but very close to the clinical phase. Actually, I didn't include this data on transmission of scrapie from sheep which naturally develops the disease. And it was recently shown that they achieved transmission in four cases of natural scrapie. This data was submitted for publication and were present at the meeting in Bergen which I attended, but they are still not readily available.

DR. BELAY: How about the mouse transfusion study?

DR. CERVENAKOVA: Yes.

DR. BELAY: Was it also collected in the incubation to pre-clinical phase?

DR. CERVENAKOVA: No. When we did our transfusion studies, these were animals which developed the disease. At this point,

we euthanized them, and they are actually coming even from different strains, but I don't think so it is important.

What we did, we transfused into the same just to see because we tested a couple of strains and when the animals developed the disease we took the blood and transfused. This data will be presented later in a more subtle way.

DR. BOLTON: Would you introduce yourself from the floor?

DR. EGLIN: Yes. I'm Roger Eglin from the National Blood Service in England. If I could just make a few comments about the human cases of variant CJD we have: Although we've had 121 or 122 cases now, there are only 8 still alive, and it's proved very difficult to persuade the earlier cases to give reasonable amounts of blood.

Their families have been very protective of the cases. The cases tend go

on to die, and they really don't really want to give blood donations or even sizable volumes at all. So, that's proved a very difficult thing to do. There is a natural experiment going on in the blood donor's in England, in that some blood donors have subsequently developed variant CJD, and there are 20 or so recipients of those under those transmissions as well.

So, there's a natural experiment going on there with the longest incubation period of about 9 years so far. No signs of any of those implicated recipients going down with illness.

Thought I'd just make a comment on the sheep experiment as well. Although it's 2 out of 24, in total it's actually 2 out of 2 of the ones that you would expect to be showing clinical signs at the moment, because the experiment was set up sequentially, they weren't all infected as a batch. So, that makes it somewhat more

impressive, and I believe there are some more suspects in that experiment. They'll becoming ill, too.

DR. DeARMOND: But while you can't get blood from the patients during life for various reasons. How about at autopsy? I know that some of them denied having autopsies.

DR. EGLIN: A lot of them -- most of them do, yeah, and they die at home, and it's just proving very difficult. They're not experimental animals.

DR. DeARMOND: There must have been at least a couple that will donate blood. Do you know the numbers who have allowed blood, or is it zero?

DR. EGLIN: I think it's very few, and I think some of the earlier cases, the blood was not stored in the most appropriate manner.

DR. BOLTON: Steve, Stan had a variant CJD patient at UCSF for treatment.

What about that individual?

DR. DeARMOND: That's being looked at. So, the patient was brought in and the blood was withdrawn from that patient and that's all under study right now. But we're talking about seven years of disease and no data yet.

DR. BOLTON: Bob? Would you introduce yourself, please?

DR. ROWHER: Yeah, Bob Rowher. Just to emphasize the last two points, that one of the biggest obstacles in making the demonstration which Steve is calling for is the lack of blood itself. It's not just at the level of variant CJD, but that's also sporadic CJD.

We have 300 and some cases of sporadic CJD in this country a year, and getting a unit of blood out of any of them has been extremely difficult. I think it's something that if this committee was going to do something useful, in terms of

advancing this idea and advancing the opportunities for these types of demonstrations, it would be to in some way facilitate, by virtue of a recommendation maybe, that some considerable effort go into making this possible. The logical place for it to come through is Dr. Gambetti's efforts as the CDC repository -- I mean, the CDC-sponsored CJD repository. So that's basically what I'm getting at.

The other point I wanted to make is that we also mentioned the Baxter experiment. This is an experiment that's going on at the cost of several million dollars a year, using squirrel monkeys, and it's main limitation was the monkeys were there and the blood wasn't. They inoculated everything they could get their hands on, but when you sit down and add it up, I'm not sure we could get a positive demonstration on the hamsters, using the volumes of blood that were available for that experiment.

The most promising aspect of that work, in my opinion, is that there is also going to be -- there have also been transmissions by the intracerebral route into squirrel monkeys and the blood from those monkeys will be used for subsequent transfusion experiments in monkeys, where there will be no species barrier.

I think it's important to realize that even humans to monkeys there are undoubtedly a species barrier effect also involved there, and you're not necessarily going to see it when you're looking at very low titers, such as those that are involved in blood, even with direct transfusion from humans into monkeys. So, the monkey-to-monkey experiment may be the most important one.

The last point is that at the EMEA meeting in London last week, Fiona Houston presented an update on her sheep transmission experiments. And she has now

seen four naturally infected, scrapie-infected sheep transmit the disease by transfusion to 90 sheep. This has been a missing link in this whole story.

All of the experiments that have been done to date have been done on experimentally infected animals. There's always been this question that was alluded to over here -- by -- I think it was Sue or somebody -- about whether we're just re-isolating the inoculum. That's what we're finding in the blood.

Those experiments that I showed you yesterday from our laboratory, we are now inoculating our animals and limiting dilution just to get around that effect. We know that that has to be de novo infectivity that we're seeing in those particular cases. But in the case of the natural infection, this is not a question anymore, and there is really I think very solid evidence now that the blood of naturally infected animals --

in this case scrapie-infected sheep -- does contain infectivity.

The BSE-infected animals are, by the way, experimentally infected, so they don't fall into that category.

DR. DeARMOND: Well, we seem to be successful at bringing a cohort of CJD patients to UCSF and getting blood from them, and for injection into mice you don't need a pint. You can use what you get derived from that -- unless you want to concentrate the protein. But we're taking multiple vials of blood from individuals and testing them for the CDI assay, which is the most sensitive -- at least that I know of -- immuno assay for the prion protein plus injection into our susceptible animals.

So, I think the experiment can be done with humans, and one doesn't need to have high levels or huge quantities of blood. But I still think the final -- we're judging policy right now on animal models

without any evidence from human disease, and we really need to study the human disease. That's all I'm trying to say.

I'm not sure that we haven't proven that there's an infectivity titer high enough to infect another human in blood. There may be some there but is it infectible? Is the infectious agent, whatever you want to call it at this stage, in a state in the blood that can infect another human? That's all the data that we need. It's that simple.

DR. ROWHER: If I can just respond to that. We do need that data, but I disagree with you entirely that we need small volumes. The thing is we need large volumes, because if we're going to make this demonstration --

DR. DeARMOND: No question, we'd like to have large volumes, but in the meantime if we can get smaller volumes, two or three vials, 10 cc's, 20, 30, 50 cc's,

even a hundred cc's, which we are able to get from volunteers, CJD patients -- that's at least a good start. I wouldn't stop the experiment because we can't get a pint.

DR. ROWHER: Across the species barrier, you're going to have difficulties with that.

DR. DeARMOND: We don't have difficulties with some of the animal models. For instance, the bovinized PrP model is highly sensitive to both BSE prions and to variant CJD prions, and the MHU2M also with modifications of amino acids in the helical regions are highly sensitive to sporadic CJD.

DR. ROWHER: I will make an offer that I've made several times in the past to the Prusiner laboratory: If they'll provide us with these mice, we'll do the experiments the way we do them in hamsters, and we will be able to answer that question one way or the other.

DR. BOLTON: Therein lies the problem. Okay.

DR. DeARMOND: Well, we can do them also, and that's a political problem between Stan and --

DR. BOLTON: I agree, and we won't deliberate further on that here.

Yes.

MR. BARON: Henry Baron of Aventis Behring. I just wanted to bring some clarification to some of Steve's questions.

We actually did the experiment with Stan, Steve, at least once. We have already inoculated bloods from 13 CJD cases into the transgenic mice ----.

DR. DeARMOND: Sporadic --

MR. BARON: Twelve sporadics and one familial. Now, this wasn't large volumes. These were groups of 8 to 10 mice, each of which got 30 microliters of blood from a case.

We also did plasma, red blood

cells, and whole blood, and all these experiments were negative. Now, one can use the caveat that we didn't do the whole blood volume on a given patient in that one or two infectious units for ML we missed because of the volume question, but nonetheless it's data, and it's negative data, and we want to do the same thing with the bovine transgenic mice with variant CJD cases.

At Aventis Behring, we have the mice, as you know, and unfortunately, as he said, it's difficult to get the samples. What we are doing now is we're doing this in collaboration with a U.K. group, which has a grant, which gives them access to samples. So, we're trying very hard to address this question with the best possible models.

DR. DeARMOND: Hank, that's exactly right, and I think the current wisdom is that sporadic CJD is different than variant CJD and there's a large amount of data from transfusion studies that argues

that sporadic CJD doesn't transmit through blood. The question is whether variant CJD, which is the whole basis of this, all these regulations, whether it does.

MR. BARON: Yes. I mean, there's data on variant CJD, too, but it's very limited. But for 10 years now, there have been 30 million transfusions in the U.K., and there's no evidence but it's early. You don't know what the incubation period would be --

DR. DeARMOND: That's right.

MR. BARON: -- in human-to-human transmission of blood. But, you know, data is continuing to be generated and I think that's all we can do, is work at it and generate data and hope that it continues to be negative.

DR. DeARMOND: So, what do you think of more mouse data, mouse transmission of Fukuoma and variant CJD?

MR. BARON: I think it helps, but

I agree with you that the real proof of the pudding is in demonstrating that there's something in humans, and that's why we've really got to use these highly susceptible models to human prions, whether it's variant CJD or sporadic CJD.

With respect to the sheep data, I'd just like to make one very brief comment, and I think we should all reserve judgement until the data is out and published. I think if we all had a chance to look at the data, we'd see some very unusual and paradoxical discrepancies in the incubation times by transfusion as opposed to intracerebral inoculation of brain homogenate from BSE. It's a study that merits some rigorous scrutiny I think.

DR. BOLTON: Point well taken. Dr. Piccardo will have our last comment or question on this subject.

DR. PICCARDO: Well, it's a question actually, Steve. Forgetting the

unfortunate human-to-human experiments, people have developed vCJD that donated blood, basically I don't have the update of what happened to the recipients. Are those -- what are we -- are they alive or dead? I mean, if they are dead, were they analyzed by an autopsy, etcetera, etcetera. So, it's an update; it's not a question.

DR. BOLTON: Microphone.

DR. SCOTT: I can give you what little information I have, and this is what I heard at the World Federation of Hemophilia from some people in the U.K. in January. Apparently there are 12 living recipients of blood products from the people who came down with variant CJD, and the rest of the people died. I think there were 20 or 22 recipients overall of non-CJD illnesses.

The other so-called "natural experiment" that appears to be going on is that there were recipients of factor 8 in

the U.K. in '96 and '97, which had a donor that had vCJD, and there are approximately 200 of those.

So there is a substantial amount of recipients that died. Did they specify what happened, how they were analyzed -- at autopsy or --

DR. PICCARDO: All we know is that their diagnosis was not CJD illness, and these were by death records I believe.

DR. SCOTT: So then the data is not precise.

DR. PICCARDO: Well, it's not precise, although we're assured from the U.K. that these people are believed not to have died of CJD, but they -- vCJD -- they could be incubating I suppose. I would just make the point that if transfusion is difficult, transfusion transmission is difficult or rare, this tiny number of people for this amount of time probably wouldn't be sufficient if detected and has

the same limitations in some of the animal studies that you're talking about. So, I think to rely on this kind of a look-back, unless we get a positive, isn't going to tell us anything that really helps.

So, the question is what happened with the people that remain alive? Is there a mechanism in place to try to get more information when they eventually die, or there's nothing going on --

DR. BOLTON: My understanding is the patients that are alive are being followed prospectively. Some of the recipients died, I think, before there was an awareness of a donation.

DR. SCOTT: Yes, the transfused people die a lot, and that was a limitation of the classical CJD look-back studies as well.

DR. BOLTON: I think we should move on. Do you have a brief comment, Dr. Bianco?

DR. BIANCO: I just wanted to place a number on Dr. Scott's statement that people that received transfusions die. Because of the underlying disease, our experience with HIV and other HCD look-back, over 50 percent of the people that received transfusions died within the first year because of the underlying disease.

DR. PICCARDO: There is important information that can come from that people, which is analyze the spleen, for example. I mean, if you're talking about people that died very shortly after they received transfusion, there is ---- which is not the brain, but it's the spleen, so that is important information.

DR. BOLTON: I think we'll move on. We can discuss this a little bit later.

Our final presentation for this morning and the meeting is retention of TSE infectivity by Planova nanofilters as a function of spike composition, and that will

be presented by Dr. Louisa Gregori.

RETENTION OF TSE INFECTIVITY BY PLANOVA NANOFILTERS AS A FUNCTION OF SPIKE COMPOSITION

DR. GREGORI: Thank you and good morning. This presentation will switch, I think, the topic of conversation to something completely different. I will be talking to you about our experimental data on the rotation of TSE infectivity by Planova nanofilters as a functional spike composition.

In the management of viral contamination for manufactured product, we have three classical methods of defense against contamination that's deferred for blood and sourcing for biologicals, screening of donated blood and testing of the raw material, removal and inactivation. Although all of these methods have provided to be very effective against conventional analysis, in the case of TSE they all have

presented challenge.

TSE is characterized by a long incubation time, which limits the effect of deferral. Also we don't have a rapid and sensitive assay to screen donated blood or test the raw material. Finally, the inactivation -- TSE inactivation requires a harsh condition so they are usually not applicable to biologicals. So, we're left, pretty much, with the removal methods.

This is a slide just -- Bob Rohwer has already talked about this yesterday. I'm not going to repeat, and basically what I'm showing here, in terms of inactivation that you see ---- we have either physical or methods that are rather harsh are also very harsh chemical conditions and usually we prefer to use a combination of both.

So, clearly inactivation is not a valid option in this case. As I said, removal -- methods of removal is where we are left for the TSE agent.

I have listed here just a few methods like in this chromatography filtration. Of course there are other methods, too. I just want to tell you that I'm going to focus on this filtration method. And whatever method we're going to use will have to be validated.

In the validation studies, the most critical question to address is the type of spike to use. If we are validating manufacturing product involved in blood or blood products, then we have two options: We can either start with infected ---- and infected blood like from animal models -- we have done this in ---- experiment -- or we can use brain spiked infectivity.

In the infected blood, the advantage is the high relevance. Of course we are removing the type of infectivity that might be present in the original material. The disadvantage is that we have low level of infectivity in blood, so we cannot

demonstrate many logs of removal by the process, and also the ---- is expensive because it requires animal assay.

If we use brain ---- infectivity, then the question, of course, is the relevance because brain infectivity might be present in a form and the characteristic that is different from the one present in blood. However, the advantage is that the we can spike with a very high level of infectivity and therefore we can demonstrate several logs of removal by this study, and also we have in vitro alternatives, and this with I'm referring to the assay involving the biochemical measurement of the TSE ---- PrPs. Also, those are less expensive.

In terms of brain-derived spike, what we can see is that it's rather arbitrary. Each laboratory prepared their own spike in this large variation and often we cannot even compare the same study from two different laboratories, because the

spike is different. And also in a brain that are spike we can use whole brain.

This is, of course, we have probably all form of infectivity present there, in terms of size distribution. The question is: Is the distribution appropriate for what we want to validate.

Same thing is for clarify supernate (phonetic) and microsomes and --somes. They might be more homogenous, but it's still maybe not appropriate as a spike.

Finally, we can use purified fibrils. Fibrils are abnormal sectors that are present in the affected brain, and they can be purified and during the purification, basically, we prepare fibrils. The majority component is the PrP, the prion protein. The problem with the fibrils is that they are insoluble and, again, they might be not even exist in the brain or in blood.

As I said, we're going to be looking at filtration. So, the advantage of

filtration are the relatively independent from buffer composition, the flexible placement in the process. The removal can be rationalized from particle size. Of course, if you look at particle size that you want to remove, then you can design a filter that will remove only that size. Therefore, a list of principles could provide an absolute barrier.

Of course, TSE presents a challenge also to filtration. The problem is that we don't know unit size of the TSE agent. However, we know the operational size is probably this purse. What I mean by that is that there is a wide distribution inside of the TSE infectivity and list in the brain homogenate. So, the question is what is the appropriate pore size?

This cartoon here represents, in scale, a ---- fibril. There's a sector, as I said, that's found in the brain, and they are associated with TSE infectivity by

electromacroscopy (phonetic). The size of these fibrils can go from 100 to more than a thousand nanometers. The one depicted here -- it corresponds to 810 nanometers. Just to give you orientation, this bar here corresponds to 20 nanometers, which is the size of a small virus, like a pavel virus.

In contrast -- I don't know if you can see this dot here -- but in contrast the prion protein, which is believe to be the TSE agent, is this dot here. That's drawn in scale with the fibril. This protein is at 27,000 dot molecular weight.

So the question now is: When we do a filtration study, what is it we're trying to filter? Are we trying to filter prion protein or are we trying to remove fibrils, or are we try to move something in between.

Even more important, what do we think is the -- what form do we think is present in your sample? This is just a

cartoon to introduce you to the Planova nanofilters that we used. There was a 75 nanometer filter, the 35 nanometer filter, and a 50 nanometer filter. This is how they would behave with conventional viruses.

Large viruses are blocked by the 75 nanometer filter; the medium size virus by the 35 nanometer filter; and the very small viruses, like the pavel viruses, are blocked by the 15 nanometer filter. Of course if we would have a molecular product, we would expect this to go through all filters and to be found in the 15 nanometer filtrate.

This is a slide showing you the filter size versus the log removal for three conventional viruses -- the pavel virus, the BVD virus, and the HAE virus. I also added the Viax 174. This study was done by Elaine Elosikoff, and this is just to show you that these filters behave according to what you expect, according to their exclusion size.

Now, this cartoon here. I'd like to see some possible scenarios for the traditional TSE agents. So if the TSE agent is the prion protein, or even an aggregated form of the prion protein, like for 5 or 10 molecules altogether, we would expect this protein to go through all the filters and to have infectivity recovered in the 15 nanometer filtrate. On the other hand, if the TSE agent we are filtering is in the fibril form, then we know that during sonication and preparation fibrils, we are breaking down the fibrils, and I want you to know that the size, the unit size, of this broke down fibril is not more than 15 nanometers.

So, what we would expect is that it's going to be some large fibrils that will be blocked by the 75 nanometer filter; some will be blocked by the 35; and obviously if it's more than 35, it would be blocked by the 15 nanometer filter and we

find no infectivity on the 15 nanometer filtrate.

On the other hand, as a hypothesis and as a model, we can also think of an additional component, a TSE agent that is not fibrils but is associated with fibrils, indicated here by the red dot. Now, how would -- if this component exists, how would that be filtered? Well, it depends on what kind of interaction it has with fibrils.

If the interaction is strong, then it would behave just like fibrils in this filtration experiment. On the other hand, if interaction is weak, or if we somehow can break this interaction, then this component, this TSE agent, then will be filtered according to its size. So it could be potentially also be smaller than 50 nanometers and be found on the 15 nanometer filtrate.

So, the question now that we ask in this experiment is: What is the smallest

unit size of infectivity? If we cannot get to this question, then the next important question is what is, then, the operational ---- size of filtration? Do the filter-removed TSE infectivity stochastically and selectively? That's the spike composition ---- infilterability.

There has been some early work on trying to determine the size of the TSE agent. There was some work done using ---- filters, and they determined the particle size is between 30 and 50 nanometers. Also some other filtration studies in which they use 100,000 ---- filters, and what they found is that only 1 percent of infectivity passed the filter and 99 percent was retained in retintate (phonetic).

There were also two studies done by Tateishi's group in which they used the Planova filters for the removal of TSE agent. This is the first study they used a CJD mouse brain homogenate and the

infectivity titers measured by incubation time.

This is their results. I'm not going to show you everything because I don't have time. The studies here basically demonstrate that the TSE agent has a size between 60 nanometers and 75 nanometers. This is not what we found in our studies but that's what is showed here.

The panel that I'd like you to focus your attention on is this experiment here. When they challenged the 40 nanometer filter with a 50 ---- unit of TSE infectivity, they found only 23 that passed the filter. However, the same filter changed with the same spike but in the presence of sarkosyl.

They have complete -- the lost the removal and they have complete passages through filter of the TSE infectivity, clearly showing that sarkosyl has a solublizing effect. Then it appears to

reduce the apparent size of the TSE agent.

The same group also investigated farther the effect of sarkosyl. So, this is the second experiment that they did. They used the --- filter 35 nanometer, 15 nanometer and 9 nanometer. The vehicle was human with or without .5 percent sarkosyl. This is the spike, the TSE spike, and, again, to measure the titers by incubation time.

The results are shown on this slide. So, when they looked at a 35 nanometer filter, this is when they challenged it. This is the reduction factor that they found, almost 5 logs of reduction without sarkosyl.

The same identical experiment, in the presence of .5 percent sarkosyl reduced the reduction factor of 1.6. So, basically, they had demonstrated again what I just showed you on the 40 nanometer filter, that sarkosyl has a solublizing effect. It

reduces the size of infectivity.

They also looked at 15 nanometer system. That was a very interesting experiment. They challenged the same way without sarkosyl and with sarkosyl. Unfortunately, these data -- I'm not -- well, what they found basically, they reached the detection limit of their assay. So all they can say is that this is more than 5.86 and this is a reduction factor more than 4.2. We cannot tell whether there is a difference in these two numbers or not.

So, we really don't know where there is a difference in the presence and without sarkosyl for the 15 nanometer filtrate. The same problem is for the 9 nanometer filtrate.

When we designed our experiment, the objective that we had in mind was what is the operational filtration size of TSE infectivity, do 15 nanometer filters retain infectivity. These are what seems to be the