1994 FDA Meeting/Transcripts on LYMErix.

Ignore the comments and mark-ups. We only have the first 60 pages or so, and there is no online version.

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P R O C E E D I N G S

(8:13 a.m.)

Agenda Item: Call to Order and Administrative Remarks.

DR. LEMON: I would like to call this meeting to order. This is a meeting of the Vaccines and Related Biological Products Advisory Committee of the Food and Drug Administration.

I am Dr. Stan Lemon from the University of North Carolina, and I will be chairing the meeting today.

I would like to start by turning the microphone over to the executive secretary, Nancy Cherry.

MS. CHERRY: I would like to welcome everyone here to this meeting of the vaccines advisory committee also. And if anyone has anything they wish to tell the committee, if they would see me, I will see that the message gets to the appropriate committee member.

We are going to have open session until approximately 2:00 o'clock this afternoon, but of course, that is subject to change, depending on discussions and the open public hearing sessions.

I wanted to mention that our committee, although it has not met for a few months, has been very busy. Two of our members, or former members, have served as liaisons to other groups.

Dr. Eickhoff represented this committee on March 15th at the meeting of the National Vaccine Advisory Committee's Ad Hoc subcommittee on childhood vaccines. And Dr. Mimi Glode has accepted the position of liaison to the National Vaccine Advisory Committee's Subcommittee on Future Vaccines and, as such, she attended their first meeting last week.

I have no other administrative comments at this time, so I think Dr. Lemon wants to take over.

DR. LEMON: Thank you, Nancy. I did want to ask Jack Gertzog if he wants to make a statement.

DR. GERTZOG: Thank you, Dr. Lemon. I am Jack Gertzog. I direct the Centers for Advisory Committee Program, and I have one brief announcement.

Each year, the commissioner recognizes exceptional meritorious service on behalf of FDA, and the public whom it serves, with its personal award, known as the Commissioner's Special Citation.

The award consists of a certificate, an engraved plaque, and the Harvey W. Wiley medal.

There are a large number of nominations for this award, but only a few are selected. It is with much pleasure and gratitude on behalf of the commissioner, all of us at FDA, and the public, that I present the Commissioner's Special Citation to the immediate past chair of this

advisory committee, Richard B. Johnston, Jr., senior vice president and medical director for the March of Dimes Birth Defect Foundation.

The citation reads: For exceptional performance and accomplishments in strengthening the Food and Drug Administration's role in the approval of new biologicals and protection of the nation's health.

Dr. Johnston,

(Applause.)

DR. GERTZOG: Congratulations, sir.

DR. JOHNSTON: It has been a privilege. It is a first-rate group.

DR. LEMON: Thank you, Jack. Now we have a statement of conflicts of interest to be read by Ms. Cherry.

MS. CHERRY: The following announcement addresses the issue of conflict of interest, with regard to the open portion of this meeting, and is made a part of the record to preclude even the appearance of such at this meeting.

Based on the agenda made available, and all reported financial interests as of this date, it has been determined that all interested firms regulated by the Center for Biologics Evaluation and Research, which have been reported by the participating members and consultants, present no potential for an appearance of a conflict of interest at this meeting, with the following exceptions:

Dr. Lemon has reported that he consults with Connaught on related matters. Therefore, the agency has granted him a full waiver for the discussion on Lyme disease, and there is no other restriction of his participation after this disclosure.

Dr. Eickoff, a temporary voting member at this meeting, has disclosed that he consults with SmithKline Beecham on unrelated matters. Based on FDA's waiver criteria, Dr. Eickoff is authorized to participate after disclosure of these interests.

A copy of this waiver statement is available under the Freedom of Information Act by written request.

Pursuant to the authority granted under the VRPAK(?) charter of the director of the FDA Center for Biologics Evaluation and Research, has appointed the following individuals as voting members for the meeting of June 7th, 1994:

Dr. Claire Broome, Dr. Theodore Eickoff, Dr. Richard B. Johnston, and Dr. Patricia Ferrieri.

With regard to FDA's invited guest speakers, the agency has determined that, because the services of these guest speakers are considered potential for a thorough discussion of the issues, any reported interests of the guest speakers will be made a part of the public record for this meeting, to allow participants to objectively evaluate

their presentations:

David Dennis, M.D., an employee of the U.S. Public Health Service at CDC has disclosed that:

A, he was an invited member for the first meeting in May of an independent oversight committee of vaccinations for SmithKline Beecham Pharmaceuticals;

B, that he has accepted an invitation to meet with Connaught Laboratories to provide expert information on epidemiology and diagnosis of Lyme disease; and

C, that his employer, the Centers for Disease Control's National Center on Infectious Diseases, is participating in a cooperative research and development agreement, or CRADA, with SmithKline Beecham Animal Health.

Raymond Dattwyler, M.D., has disclosed that he is employed at the State University of New York at Stonybrook, and as such, he has worked on grants from CDC, NHD, NIAID, New York State, as well as the fact that they are currently studying strain-related variability in B. burgdorferi antigens, immune reactions to antigens, and various treatments for B. burgdorferi Infection. Some antigens under study are potential vaccine candidates. Does not disclose kits

Allen Steere, M.D., has disclosed that he has consulting arrangements with Connaught, SmithKline Beecham and Medimmune.

In the event that the discussions involve any

products or firms not already on the agenda, for which an FDA participant has a financial interest, the participants are aware of the need to exclude themselves from such involvement, and their exclusion will be noted for the record.

With respect to all participants, if any products or sponsor should be discussed, we ask that, in the interest of fairness, that they address any current or previous financial involvement with any firm whose products they may wish to comment on.

Agenda Item: Open Public Hearing.

DR. LEMON: At this time, we have scheduled an open public hearing. If there are any members of the public who wish to make a statement at this time to the committee or to the FDA, they are welcome to do so.

Such individuals have been asked in advance to make notice of this. As far as I understand, nobody has requested time, but if anyone present wants to make a statement, now is the time to do it.

If not, then I think we should proceed with the agenda today, which is a full agenda. As we have heard already, we will be discussing Lyme Disease and vaccines to prevent it today, as the primary focus.

I think we are in the exciting position of having three different vaccine manufacturers involved in the

development of vaccines for the prevention of this disease.

We will start today's discussion with an introduction of the issues by Dr. Margaret Mitrane of the FDA.

Agenda Item: Vaccines for the Prevention of Lyme Disease. Introduction.

FDA/CBER Biologics

DR. MITRANE: On behalf of the Center for Biologics, I would like to welcome everyone to the Vaccines for the Prevention of Lyme Disease session of today's vaccine advisory committee meeting.

The purpose of this section is to discuss relevant clinical issues pertaining to phase III trials with Lyme Disease vaccines. My introduction will highlight various aspects of Lyme Disease, which will be expanded upon by our guest speakers and the companies participating in this session.

Lyme Disease is a multi-system disorder caused by the spirochete Borrelia burgdorferi. It is the most common arthropod-borne infection in the United States. The arthropod vector for Lyme Disease is the Ixodes tick.

Cases of Lyme Disease have been reported in nearly all states. Most cases occurred in endemic areas. The northeast, mid-Atlantic, north central, and the Pacific coastal regions, are the endemic areas in the United States.

In 1975, Allen Steere first described Lyme Disease

as a clinical entity, manifested by oligoarticular arthritis. Seven years later, the causative bacteria was isolated by burgdorferi.

Borrelia are microaerophilic, gram negative bacteria, phylogenetically grouped with treponema and leptospira. Borrelia can be cultured in Barbour-Stenner-Kelly medium.

Slow growth in culture makes it difficult to isolate Borrelia burgdorferi from blood, cerebral spinal fluid or synovial fluid. Isolation of Borrelia from skin biopsies of erythema migrans has been more successful, with yields as high as 85 to 95 percent.

Borrelia burgdorferi have cytoplasmic and outer membranes, between which is peptidoglycan. Flagella are inserted at the ends of the spirochete.

Borrelia burgdorferi have three major outer membrane surface lipoproteins which are, 31-32 kilodalton OspA, 34-36 kilodalton OspB, and 21-22 kilodalton OspCs.

Immune response to OspA and OspB develops late in the course of infection. Early in the disease, the immune response is directed against the 41 kilodalton flagellar antigen.

Lyme Disease can be divided into three clinical stages: Stage 1 - Early localized infection; Stage 2 - Early disseminated infection which occurs in the first weeks

to months of disease; Stage 3 - Late persistent infection, which occurs in the first month to years into the disease.

A patient infected with Borrelia may manifest the infection in various ways. A patient may have isolated infection, may proceed through all stages of disease, or may present with stage 2 or 3 disease, without having had any symptomatic earlier stage disease.

An individual infected with Borrelia may also be completely asymptomatic.

Erythema migrans is the pathognomonic skin lesion that occurs at the site of the tick bite. It has a classic annular appearance with an erythematous border and central clearing.

The rash is warm to touch and half of patients experience burning or pyritis.

Erythema migrans occurs in 60 to 80 percent of patients. Some patients who do not recall the rash, may have had an asymptomatic lesion in an inconspicuous location.

Here is an example of the classic erythema migrans rash, with an erythematous outer border and central clearing. This patient also has a secondary smaller erythemal migrans lesion.

Untreated lesions usually resolve after several weeks. Treated lesions usually resolve within several days.

In stage 2 of Lyme Disease, additional cutaneous manifestations may occur. Patients may have secondary erythema migrans lesions, diffuse urticaria, malar rash, or non-specific small evanescent red lesions."

CDC

Early neurologic manifestations occur in 15 to 20 percent of untreated patients, and may manifest as meningitis, encephalitis, cranial nerve palsy -- most frequently involving the seventh cranial nerve -- and peripheral radiculoneuropathy.

Cardiac manifestations occur in four to eight percent of patients. The most common abnormality is fluctuating high grade atrioventricular block, either winky block or complete heart block.

The duration of Lyme carditis is usually brief -- from three days to six weeks. Mild, asymptomatic mild carditis, or pericarditis, may also occur.

Musculoskeletal manifestations in stage 2 are transient and migratory, and involved both articular and periarticular structures.

CDC

"Pain without swelling," of small and large joints, occurs at only one or a few sites at a time.

Neurologic manifestations in stage three include peripheral neuropathy and sub-acute encephalopathy, and become evident late in the first year, or after the first year of disease.

don't say meningitis in stage 3

The arthritis associated with Lyme Disease was found to develop, on the average, six months after disease onset and is an intermittent inflammatory, mono or oligoarticular arthritis, involving large joints, especially the knee.

Ten percent of untreated patients with joint involvement develop chronic Lyme arthritis, which is defined as joint inflammation lasting longer than one year.

Chronic Lyme arthritis has been associated with an increased frequency of the HLA DR2 or 4 allele.

Dr. Steere has identified a subset of chronic Lyme arthritis patients, who are HLA DR4 positive, have antibody reactivity to OspA or B, and are unresponsive to antibiotic therapy.

An aberrant immune response to Borrelia may play a role in the pathogenesis of arthritis in this subset of patients.

In conjunction with the clinical picture, serologic tests are used for the diagnosis of Lyme Disease. The indirect immunofluorescence Assay, enzyme linked immunosorbent assay, and western blot have been used.

The ELISA is the most widely used assay to support a diagnosis of Lyme Disease. An IgM response usually develops by two to four weeks after the onset of erythema migrans, and the IgG response is seen by four to eight

weeks.

Serodiagnosis of Lyme Disease is complicated by cross-reactivity of spirochetal antigens with other antigens, delayed development of humoral antibody response, dampening effect of early antibiotic therapy, variability of immune response in various subjects, inability to predict stages of Lyme disease, and lack of standardization.

A retrospective and prospective analysis was conducted by Dressler, to develop acceptable criteria for positive western blots.

In a retrospective analysis of 225 case and control subjects, the best discriminatory ability of test criteria was obtained by requiring 2 of 8 most common IgM bands in early Lyme disease, or 5 of 10 most frequent IgG bands after the first weeks of infection.

When these criteria were applied in a prospective study of all 237 patients seen in a Lyme Disease clinic during a one-year period, and in 74 patients with either erythema migrans or summer flu-like illnesses, IgM, by western blot, had a sensitivity of 32 percent, and a specificity of 100 percent. The specificity for IgM by ELISA, was 94 percent.

68% of pts missed by ELISA

IgG, by western blot, had a sensitivity of 83 percent, and a specificity of 95 percent. The specificity for IgG by ELISA was 72 percent.

Therefore, western blot can be used to increase the specificity of serologic testing in Lyme Disease.

Polymerase Chain Reaction has been successfully used to detect Borrelia burgdorferi DNA in cerebrospinal fluid, urine, joint fluids, skin, and serum of Lyme Disease patients.

In a study by Nocton, three separate regions of Borrelia burgdorferi genome was targeted by four sets of primers and probes.

Borrelia was detected in the synovial fluid of 75 of 88 patients with Lyme Disease, and in none of 64 control patients.

Seven of ten chronic Lyme arthritis patients, treated with multiple courses of antibiotics, had negative PCR test results.

This suggests that the arthritis in these seven individuals is not due to the persistence of spirochetes.

The ability of recombinant OspA to induce protective immunity, has been demonstrated in multiple animal models. Mice immunized with recombinant OspA were protected against challenge from Borrelia that were delivered by syringe or tick.

On the other hand, mice immunized with the 41 kilodalton flagella protein are not protected against challenge from Borrelia.

Yang developed a mouse model of Lyme Disease which allows analysis of mice with mild, moderate, and severe pathologies, after inoculation with Borrelia burgdorferi.

Infected C3H HEJ mice developed severe arthritis and severe cardiac abnormalities, while infected BALB/C mice developed mild arthritis.

Higher levels of Borrelia burgdorferi DNA were detected by PCR in the tissues of infected C3H HEJ mice, than in the tissues of BALB/C mice.

The genetic regulation of severe pathology was analyzed by infecting the offspring of a cross between C3H, HEJ and BALB/C mice.

The F1 mice developed severe arthritis and contained high levels of Borrelia DNA in the heart and ankle, similar to the C3H HEJ parent.

These findings indicate that susceptibility to severe arthritis is a dominant trait and suggest that it may correlate with high levels of persisting spirochetes.

I would like to conclude my introduction with FDA's questions to the advisory committee. We ask that the committee consider these questions while they listen to the presentations this morning.

Number one. Is the CDC case definition for Lyme disease appropriate for a pivotal efficacy trial. Please comment on laboratory assays to support the diagnosis of the

disease -- that is, culture, western blot and polymerase chain reaction.

Two. Lyme disease has a wide range of clinical manifestations which occur in the acute and chronic phases of infection by Borrelia burgdorferi. Please comment on appropriate primary and secondary end points that provide specificity in diagnosis of the disease for a pivotal efficacy trial with an OspA vaccine.

Three. How should the safety of OspA vaccines be evaluated, especially as it relates to individuals with HLA DR2 or 4 haplotype.

Four. How long should immunized individuals be followed to attain adequate safety and efficacy data.

Five. How could the safety and efficacy in children be assessed.

Six. What other studies could be performed to answer additional safety and efficacy questions with the OspA vaccine. For example, how should the use of the vaccine be evaluated in seropositive individuals and in those with a history of Lyme Disease. Thank you.

DR. LEMON: : Thank you, Dr. Mitrane. As always, the FDA has given us a set of challenging questions to address here. Are there any questions for Dr. Mitrane from members of the committee before we go on.

If not, then maybe we should proceed with the next

speaker. I would like to thank Dr. Mitrane for sticking within her allotted time. I encourage people to do the same. We are, of course, ahead of schedule, but I would like to remain so, so that we have more time for discussion when the time comes.

We are luck to have a number of experts in Lyme Disease to help us address these difficult questions posed by the FDA. And I would like to ask Dr. David Dennis to begin these presentations with a discussion of the epidemiology of Lyme Disease.

Agenda Item: Epidemiology of Lyme Disease.

DR. DENNIS: Thank you and good morning. I am going to address some broad epidemiological issues and try to relate them to the vaccine studies that are in place.

As I represent the Centers for Disease Control, which is the nation's prevention agency, it is quite obvious that our roles are in national surveillance, epidemiologic studies, and research leading to prevention and control strategies.

This bar diagram shows the reported numbers of cases to the Centers for Disease Control by states over time. This describes the curve of increasing reports showing that Lyme Disease is a rapidly emerging disease in the United States. There have been more than 55 thousand cases now reported totally, from the nation, about 9,000

cases a year in the last three or four years when there has been a uniform national surveillance system in place, about a 20-fold increase from the less than 500 cases reported by 11 states in 1982.

The distribution of the reports of cases by state in the nation -- this is for 1993 where there were about 9,000 cases reported, you can see that 45 states reported cases, but the vast majority of cases -- in fact, over 90 percent of cases -- occurred in the localized areas of the northeast, the upper north central, and the Pacific coast.

And those states that are in green are states in which we have identified enzootic cycles of the parasite, Borrelia burgdorferi, in situations where humans are exposed to infection.

Lyme Disease is a tick-borne zoonosis, Borrelia burgdorferi, in sensu latu -- latu is the agent. The vector ticks are of the Ixodes risctanis complex. This is a picture of the ixodes scapularis, the principal vector in the northeast, in the north central United States, and similar to the vector on the Pacific coast, Ixodes Pacificus. It is a three-host tick, involving a two-year life cycle.

Rodents -- in particular, mice -- this is the peramiscus lucopis, the white-footed mouse -- served as the reservoir host of the Borrelia burgdorferi in nature. Other

wild rodents also served as reservoir hosts.

The deer, however, does not serve as a reservoir host of the parasite, but it does serve as a maintenance host of the ticks, because it is a principal site of mating and feeding of the adult ticks that need to take a blood meal in order to lay eggs.

It is the deer, the introduction of the deer, to new geographic areas that allows the introduction and the establishment and maintenance of populations of the ticks that transmit Lyme Disease in the United States.

This is a typical environment in which intense enzootic cycles occur in the United States, exposing most people to risk. This is a suburban area in which homes have been placed into deciduous woodlots. These are succession forests that have ample saplings for feeding of the deer. They have a deciduous leaf litter that is a favorable environment for the ticks.

You can see stone walls, other places, that the rodent reservoir can use as nesting sites.

In addition to a pararesidential exposure, of course, there are high risk occupational and recreational exposures throughout the areas in which Lyme Disease is endemic.

The force of infection showed by these magenta arrows in nature, is from rodents to the immature stages --

larvae and nymphs. There is transtadial transmission, but not transovarial transmission. And the nymph is the primary source of infection for humans. The nymphs also transstadially transmit their infection to adult ticks, which less frequently, are a source of infection for humans.

If we look at the graphs of the distribution by dates of onset, or months of onset, of cases with erythema migrans, you can see in the lower chart there, that there is a very marked seasonal incidence in May, June, July, August time period.

That is the time period in which the nymphal stage of the tick is most active, supporting the observations of the sufficiency of nymphal ticks transmitting infection to humans.

Lyme Disease is a disease of equal effect upon males and females. There is, however, a very pronounced bimodal distribution with high risk, and high rates of infection in young children and older adults.

The distribution of Lyme Disease throughout the world is driven by the distribution of Ixodes ticks of the ricinus complex. And you can see here their distribution is limited to the northern hemispheres.

The disease is endemic across Europe -- Russian -- to Korean peninsula, to Japan, and to northeastern China. In the United States, it is a limited foci in Canada and

widely distributed in the northeast and, to a lesser degree, in the north central and the western coastal states.

We mapped the distribution of the principal vectors of Lyme Disease in the United States several years ago. Ixodes damini now has been renamed -- reverted to its original name, Ixodes scapularis.

These are the distributions of the known populations of Ixodes scapularis in the northeast and the north central area, and Ixodes Pacificus on the west coast. Red is where there are established populations. Almost always there is also enzootic cycling of Borrelia burgdorferi identified in those counties. Yellow is where the tick has been reported, but not yet have there been established cycles of the parasite, Borrelia burgdorferi.

If we look how this distribution relates to the frequency of occurrence by rates of disease in states, it follows quite well that the seven states with the highest rates -- and we are only talking about rates of about 80 to 50 per 100,000 -- are clustered in those areas where we saw that the tick population occurs.

The point of this slide is that these seven states account for more than 80 percent of all cases and yet, you can see the rates are really not very high. It is not a disease of very high frequency of occurrence.

It is also a disease that is very focally

distributed, even within states. These are the counties that, in 1992, had rates greater than 30 per 100,000. And you can see that there are only 13 counties -- some of them with very limited populations and rather unstable rates -- that had rates per 100,000 or greater.

And there are just a few states as well that have rates exceeding 30 per 100,000. And even in these counties, the disease is highly focalized.

This summarizes some of the studies that have been done in the past, looking at the most highly endemic communities, to try to get some understanding of the prevalence and incidence rates that do occur in these almost outbreak situations, in some instances.

And you can see that using serologic and clinical -- and these are both on standardized serologic and standardized case definitions, that the prevalence was found to be in the range of eight to fifteen percent, in an incidence in the range of two-and-a-half to three-and-a-half percent, in the most highly endemic foci communities that have been studied.

Now, the Center for Disease Control, working with others, have developed diagnostic tools for surveillance of immunologic studies of Lyme Disease. Most important is the case definition based primarily on clinical findings.

The simplest description of this case definition

is a physician diagnosed erythema migrans rash, or at least one objective manifestation of a major later stage illness in the musculoskeletal, cardiovascular, or neurologic systems, with so-called laboratory confirmation.

I will just show you how we have qualified the clinical aspects, just using erythema migrans as an example. It can't just be a rash that occurs after a tick bite. It is a rash that has particular characteristics -- a solitary lesion for surveillance purposes must reach five centimeters or greater in diameter, it must be diagnosed by a physician -- it cannot be something that arises quickly and disappears quickly. It usually occurs three to thirty days after the tick bite.

In addition to that, the serodiagnostic tests, CDC, working together with clinical researchers, manufacturers, with the state territorial public health laboratory directors, the FDA, NIH, and others, had a primary research priority to standardize and improve serodiagnostic tests.

We have been working with a flagellant ELISA and have standardized this, and have used it in conjunction with the Western blot.

We now, with a broad range of highly characterized serum specimens, many coming from patients from whom Borrelia burgdorferi has been isolated, achieve a

sensitivity of about 85 percent and a specificity of 98 percent or greater when this combination of flagella and ELISA and Western blot are used, and a high degree of precision with cases, non-cases, and serum specimen from patients with the disease thought to be cross reactive.

Also, we have had a chance to look at the performance of this testing schema in patients with early disease which would be most important to following patients immunized in a vaccine trial.

And you can see that, even in early stages of disease, we have a fairly high sensitivity. In patients seen in the period day zero through thirty -- and I will say day zero through seven is underrepresented in this sampling -- we have nearly 80 percent sensitivity.

These are patients that all erythema migrans. Most of them had Borrelia burgdorferi isolated from their lesions. They were seen early and treated.

Similarly, we got about an 80 percent sensitivity in persons in the period of 30 to 100 days, and it fell off after 100 days, and we had follow ups up to more than a year.

So, using this two test approach, we have a fairly sensitive test now, even for early stage disease.

There has been a big movement, not only to standardize the ELISA but also to standardize the Western

Dave Dennis, CDC officer, June 1994 FDA meeting:

blot. We have made progress. We think that in the coming months we will be able to have a standardized western blot.

A working group of people most active in the United States in the clinical and research development of serodiagnostic tests, met at CDC and have come up with the interim recommendations on serodiagnosis, in which they are recommending a two test approach with an ELISA, with taking care for setting the negative cut-off using certain criteria, and then testing all persons who have a positive or equivocal test obtained by the ELISA with the immunoblot, using a low passage strain of Borrelia burgdorferi as antigen, and with the immunoblot using the criteria of Dressler, et al, discussed by Dr. Mitrane.

It appears that these immunoblot criteria will be simplified, and it appears that three bands along in the IgM -- p45, p31 and the OpaC -- will probably be sufficient for use in the IgM criteria, when two of those three, and including the OpaC, are used as the criteria.

So, we expect that this will be standardized and simplified in the fairly near future.

Just some thoughts on trial design issues. Lyme Disease and the testing of vaccines, obviously you can't have experimental challenge of subjects. But there are animal models -- particularly the primate and the canine models -- that are very good models of human disease, not

only for the clinical aspects but also the immunologic aspects in the primate, mimicking the response that we see in humans, very closely.

Population sampling, I think it is very important that we use a sampling that tries to achieve a representative sampling of the population that we think would be targeted for vaccine, because of their risk.

Obviously, in thinking about statistical power and alpha and beta errors, we not only have to think about efficacy, but also safety issues. And some of the safety issues are ones that theoretically may be of very low frequency of occurrence and, because of that, there will be a need for setting into place epidemiologically placed surveillance systems for monitoring not only of the effect over time, but also some of the safety issues over time.

The targeting of the use of the vaccines, I think we have to work very closely with state and local health departments and research groups that are working to really define the risk of population groups.

Obviously, epidemiologic studies can be useful in developing cost benefit analyses of the vaccine, and certainly for helping develop strategies of distribution of the vaccine, if and when it becomes available.

Thank you very much.

DR. LEMON: Thank you, Dr. Dennis. Perhaps I

could open the discussion with a couple of questions that came to mind listening to you.

One of your first slides is very impressive, in that it showed an increase in reported cases over the past decade or more. And one question is, how much does this reflect a true increase in the incidence of the infections, the disease, versus an increase in physician recognition and the availability of diagnostic tests.

DR. DENNIS: I should have mentioned, there are considerable caveats in that bar graph. There is a big problem -- but a decreasing problem -- of misclassification of cases. And I think that there has been a big effort by state health departments over the past three or four years in particular, in order to validate the cases that are reported to them, as cases that truly meet the surveillance case definition.

There still is a big problem with misdiagnosis, not only because the clinical signs and symptoms can be protein and not as specific as we would like, but also because we haven't had the best serodiagnostic tools.

On the other hand, there is a very big problem with underreporting. And i think this is most important in those states that have the most endemic disease. And it would not surprise us if, in states like Connecticut, New York, New Jersey and Pennsylvania that there may be 70, 75

percent underreporting. So, this graph represents a combination of things.

DR. LEMON: And the second question, you may have already answered for me, and that deals with the role of the OspA antigen in the immunoblot. The data you showed utilizing a combination of the flagella and ELISA immunoblot, with a high degree of sensitivity and specificity, if you were to eliminate the OspA band from the immunoblot in that analysis -- because that may become irrelevant in a vaccinated population -- how does that affect the overall sensitivity and specificity of the combination of the serologic procedure.

DR. DENNIS: It won't, because OspA is not an important band for diagnostic criteria. And as I mentioned, it looks, at least for the IgM, that the three bands -- p39, p41, p23 -- will probably be as sensitive and specific as what is in place now.

DR. LEMON: Will it be different for the IgG, do you think.

DR. DENNIS: Perhaps others can best address that. Dr. Steere may be able to later.

DR. LEMON: Are there other questions about this to the community.

DR. EICKOFF: Could you go back to the underreporting issue for just a second. Is this, in your

view -- well, Lyme Disease is almost a little too upper socioeconomic strata disease, I would infer. It is not a disease in the urban ghetto.

Is the underreporting a physician failure to diagnose, a patient failure to seek medical attention, or simply physician failure to report, having made the diagnosis, or all of the above, or can't you tell.

DR. DENNIS: I think it is all of the above, but I think it is probably mostly a failure of physicians to report.

The Connecticut Department of Health did a study a year ago. They searched their reportable diseases records, and they searched their records of primary care physicians in the state, and they found that all cases of Lyme Disease reported to them, had been reported by only seven percent of primary care physicians.

They then went and did a survey of primary care physicians, and they got a very good sampling and a good rate of response. And 65 percent of the physicians -- primary care physicians -- sampled, said that they had seen and diagnosed and treated at least one case in that same year.

So, there is an obvious significant underreporting by physicians of cases that they do see.

There is a problem with asymptomatic infection,

sub-clinical infection and misdiagnosis of cases of true Lyme Disease. But we don't know how important this is, as it relates to our surveillance data.

DR. JOHNSTON: The chronic arthritis is worrisome, of course. It has received a lot of play in the lay press. In trying to understand what is going on there, I have a couple of questions.

Are there enough data in children to detect any difference in the likelihood of getting a persistent arthritis. Number one.

And number two, is immune response -- has it been studied enough -- let's say in toddlers, even -- to know whether there is any difference in the immune response in children.

DR. DENNIS: I am probably not the best person to answer those questions. But I will say from surveillance data -- hematologic studies that we have done -- that the spectrum of illness in children now seems to be about the same as what we are seeing in adults, I think as physicians and the public become more sensitized and aware and understanding of the disease.

As far as the questions of immunologic response, I think Dr. Allen Steere and Dr. Dattwyler and others would be better placed to answer that.

DR. GLOBE: Back to the issue for a second, of

sub-clinical disease. If you look at your seroprevalence studies that I think you showed us from endemic areas, were those done with any questionnaires to know what percent of those, I think it was 8 to 15 percent seropositive had had disease that might have been compatible with Lyme Disease, just to try to get a handle on how much asymptomatic self limited infection there is.

DR. DENNIS: Yes. Actually, I should have been more clear in the title of the slide, but that represents -- there were seroepidemiologic studies and that represented input both from serodiagnostic testing as well as questionnaires on past history of disease.

And each of those investigators asked it in a different way, and with a different degree of sensitivity and specificity, I would think. But that was a combination.

If you look at asymptomatic seropositivity, if you look at sero conversion over a transmission season, the ratio of symptomatic to asymptomatic is about one to one, or a little bit greater than one to one.

If you look at those who are seropositive at a community prevalence bleed at one time, more than half of them in the studies that have been reported do give a history of having had disease compatible with Lyme Disease.

DR. LEMON: Let's have this be the last question before we move on.

DR. O'BRIEN: I am not sure I am asking the right person. In the background material that we got for description of Lyme Disease, there is a comment that not all strains of Borrelia make OspA, OspB, or OspC. How does that -- it is a concern when it comes to making a vaccine made of OspA, but also in serodiagnosis, when you are relying on OspC in your western blot, how is that -- is that taken into consideration and is that really a major problem in diagnosis.

DR. DENNIS: We don't know how large a problem it is. We are just now trying to do the comparisons with different geographic strains from throughout the United States. But we do know that the strains that have been identified in the areas that are highly endemic in the northeast and in the north central part of the country, have a considerable homogeneity and would be expected to have both OspA and OspC.

There are greater differences in organisms isolated in the Pacific coast and from some enzootic cycles that we do not think cause a public health risk of any significant amount in the south and in the Rocky Mountain states.

DR. LEMON: I think we had better move on. Thank yo, Dr. Dennis. I am sure we will come back and revisit some of these issues in later discussions.

pages 32-33 are missing

can occur.

→ The hallmark of dissemination, I think, is erythema migrans. And studies have demonstrated an incidence of that from anywhere from 10 to 50 percent. Generally, in our experience, it runs somewhere around 15 percent.

An enteric hepatitis can occur quite commonly, and this is just a transemanitis, by and large. Acute arthritis has been reported in this phase of the infection but, as has already been pointed out, arthritis is usually a later manifestation.

Cardiac involvement, in our experience at Stonybrook, occurs now less than one percent of the time.

There are differences in the clinical presentation of disease, perhaps some region. In Europe there seems to be a link, perhaps, between certain strains of this Borrelia and the clinical manifestations. But this has not been demonstrated in the United States, although it remains a possible explanation.

The chronic phase of the infection occurs months to years after the onset of infection. Arthritis has certainly been well described. It is generally a large joint arthritis.

Individuals begin usually with a more vague type of symptom complex of myalgias and arthralgias and, only

after some period of having this sort of prodromal syndrome begin to develop, a good arthritis.

The knee is overwhelmingly the most commonly infected joint. And quite interestingly, it is usually associated with very large effusions.

The small joints are very uncommonly involved, and symmetrical arthritis is also uncommon. So, by and large, it is a mono or oligoarticular arthritis of the large joints.

Acrodermatitis chronic A. trophicans is seen in Europe. It is only rarely reported in the United States, but it is a manifestation that one has to pay attention to.

Peripheral neuropathies tend to be axonopathies, and they tend to be diffuse in nature, not involving any one specific nerve.

The neurologic involvement in late disease can be encephalitis, chronic meningeal encephalitis, or a vaguer symptom complex and then encephalopathy.

Again, this has not been terribly well studied. And I don't know of any very long term population based study that has delineated the full repertoire of disease.

We have, for example -- these are some things on erythema migrans -- some problems in how we define it. There is even, in something as classic as erythema migrans, there is a considerable amount of variability of the

presentation.

The classic description is this target-like lesion where the tick bite occurred centrally. The tick bite occurred centrally here, and one sees erythema, clearing, erythema and then clearing skin. That is the classic description. We can get other types of things where this a more homogeneous erythematous area, still others where it is almost like a patch, and others where it is raised as opposed to being flat.

One can see it is almost like a bruising central area in this particular lesion, and still others where there is some vesiculation which occurs centrally.

One of the problems that we have observed is that many physicians are not terribly familiar with erythema migrans, and have failed to understand the fully range of this particular skin lesion, which is associated with this disease, and it is easy to make mistakes.

And even people who are somewhat experienced with Lyme Disease frequently have difficulty with erythema migrans.

Anybody that is familiar with dermatologic manifestations realizes that, although the classic target lesion is easy to recognize, some of these others may be confused with such things as fixed drug eruptions, and a detailed history is important.

What about, can we get some hints about disease based on natural history studies. Unfortunately, there are few natural history studies of this disease. Dr. Steere reported one in the annals of internal medicine a number of years ago, in which he took 55 patients who had presented to the clinic at Yale with erythema migrans who remained untreated.

They remained untreated because this was in the era when people didn't realize that this was caused by this spirochete.

These individuals were followed from anywhere from three to eight years, and there is a fair age range and fairly equal distribution of males to females.

The interesting thing in this is that the highest incidence -- greatest incidence of problem in this area was intermittent arthritis with arthralgias occurring in a significant number of individuals. Only six of the fifty-five developed chronic arthritis.

The intermittent arthritis in these individuals tended to be self limited and just resolved with time, even untreated. The arthralgias in this group didn't progress.

So, we seem to see a spectrum of rheumatological manifestations in this disease, even in its untreated state. Of course, treatment would change those numbers.

→ As far as the non-rheumatological manifestations

in this population, one saw fatigue, fever, headache or stiff neck, myalgias and recurrent erythema migrans.

Certainly, of these, the first four are fairly non-specific and, I think, would be difficult to categorize from the point of view of a study population when one talks about vaccine trials.

Another natural history of Lyme Disease studied also came from Dr. Steere's group, which was of 46 children who had been selected for arthritis. These were individuals who presented to Dr. Steere's group with arthritis. None had been treated for four years after the diagnosis, and there was a 10 to 13 year follow up in 39 of them.

Erythema migrans was the most common initial manifestation of the disease, with a viral-like illness alone in 15 percent. And I think that highlights one of the difficulties with this disease, in that the best marker of early infection is not a universal marker.

Neurologic involvement, as was expected, was seen in facial palsy in seven percent and meningitis in fifteen percent.

The interesting thing, these were individuals all with arthritis, but there were latent neurologic complications -- encephalopathy in 2 of the 39, a seizure disorder in 1 of the 39, and a demyelinating disease in 1 of the 39.

It is unclear whether the latter two manifestations were associated with Borrelia burgdorferi infection, although if one looks at these types of numbers and compares it to what we know about untreated T. pallidal infection, these numbers are not terribly different than that.

The other manifestations of disease that were not associated with frank arthritis were continuing arthralgia, marked fatigue and keratitis, in 2 out of the 39 individuals.

So, what we see from this, I think, is something that had not initially been described in the classic literature -- i.e., the keratitis -- which again, I think, points out that we perhaps don't know the full repertoire of this disease.

Well, what can we learn from other studies. It is very difficult to do a non-treatment trial. I just pulled this out of a recent trial which we were involved in where amoxicillin was compared to azithromycin. And what we saw in this was that the azithromycin protocol had a higher failure rate.

But it is interesting what the manifestations were. Certainly, arthritis was very common. But muscle tenderness was also quite common in this patient population. And these were -- all these individuals had erythema

migrans. So, this is, by definition, Lyme Disease and they were photographed and reviewed, so that we have a fair confidence.

But pain on flexion of the neck was also quite common, paresthesia was seen in one and meningitis was seen in one. So, I think that non-classical manifestations can be a part of someone who developed Borrelia burgdorferi infection.

Again, going on with this group, even to make it more difficult, fatigue, joint pain, headache, muscle pain, stiff neck, numbness and tingling, were also quite common manifestations of failure in this group of patients who had been treated for erythema migrans.

Now, to change tacks and talk to you a bit about what laboratory has to contribute to the clinical evaluation of individuals.

Certainly, one in any infectious disease, would like to have microbiological proof of infection. Unfortunately, this has proved difficult in many instances.

The best results in culture come from individuals with erythema migrans in which, now, with modern culture techniques, 90 percent-plus in individuals can have the organism isolated from their skin.

Unfortunately, this is the area where it is least necessary to provide culture results because, in the right

hands, the diagnosis of erythema migrans is straightforward.

ACA is the next most common place where one can culture the organism. Again, unfortunately, ACA is usually not seen in the United States -- that is acrodermatitis chronical antrophicans.

Where I think it becomes more interesting, especially from the view of a trial like this, would be we would like to isolate it from the central nervous system or the joints.

And in these instances, even in untreated individuals, the ability to get cultures under these circumstances is low.

Now, this is older data and perhaps with more modern techniques and better culture media, that we can get more isolates from CSF -- we certainly hope so. But still, I think it points out a significant problem, that certainly culture doesn't seem to be the answer in defining this. I think that is fair.

Well, what about PCR. I didn't put my PCR slide in there. (I think PCR offers a real opportunity, and certainly, one can do PCR on cerebral spinal fluid or synovial fluid, and get fairly high yields, as has been demonstrated in the literature.) And this could be something that is, I think, a very important adjunct in the microbiological definition of this disease.

Dattwyler - See Primers.

PCR, though, is not an easy technique and it has to have very vigorous controls. As anyone who has done it realizes, if you have amplicons contaminating your laboratory, that you can turn everything positive.

The other thing that you must take great care in handling samples and preparing them for PCR, in that it is very easy to contaminate it. So, you have to have no Borrelia in the area where you are alloquatting your samples, or you can easily contaminate it.

And it is my understanding that some results of PCR, when analyzed further, it turns out that the organism was really high passage, laboratory strain bacteria which, somehow, accidentally contaminated the sample.

So, PCR, in well controlled circumstances, can be quite good.

Obviously, the question of serologies comes up and that is probably one of the most useful tools that we have. This is a study of 217 individuals with erythema migrans, and they are serial serographies -- and this is combining both IgM and IgG responses.

And what we see in these individuals is that, by day 20, everyone who was going to seroconvert, seroconverted. So, we see that we can utilize early serologic testing to define things. We don't have to, as sometimes, wait four to six weeks. So, I think the feeling

that there is a marked delay in the serological responses in this disease are not supported by out studies -- our most recent studies.

And the way this is done, day zero would be presenting time with erythema migrans and the duration after presentation with erythema migrans.

What also should be said under these circumstances is that if we recognize erythema migrans as an infection, that every one of these individuals was put on antibiotic therapy at time zero.

So, whether that had some influence in the subsequent seroconversion and whether, if we didn't put them on antibiotics we would have seen 100 percent seroconversion, we don't know, because I don't think anybody could ever do that study today.

Now, what serologic assays could one use in a study such as a vaccine trial. Would a single ELISA be adequate. Would a single Western Blot be adequate. Or, should one do serial ELISAs and serial Western Blots.

It is my opinion that the best way to assess most infectious diseases is to get an acute and a convalescent serology. If one thought that the person was acutely infected, I think that that is a classic way of assessing.

We will know that, if you immunize someone with a vaccine and get an appropriate immune response, that they

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should have some antibody and perhaps be positive in a single ELISA. So, a single ELISA under those circumstances, I don't think, would be terribly useful.

A single Western Blot, since we are immunizing -- at least in this discussion -- with OspA, would that be useful. The answer is, I think, yes, and I will get back to that in a minute.

A serial ELISA certainly could be helpful if one did it in an acute and convalescent. A rising serologic response would suggest an infection. And the same, I think, would be true about serial Western blots, where one would see an increase repertoire of immune response against various antigens to the bacteria.

Now, when one looks at some of the difficulty with serology in this disease, one has to look at the major antigens. And the problem, I think, becomes very apparent. The 41 kilodalton flagellant antigen induces an early immune response. But it has been fully sequenced, and there is a high degree of homology with other flagellant antigens from other spirochetes or other organisms -- things like trepanimadenticolon borrelia bucalus, which can cause gum infection and induce an immune response. There, flagellant antigens are highly cross reactive, with the flagellant of Borrelia burgdorferi.

The other thing is that Borrelia burgdorferi

expresses a number of common bacterial antigens. The best characterized ones are at 60 to 66 range, and another at the 73 kilodalton range. These belong to the heat shock family 70 and 60 family members, respectively.

Antibodies directed against these are non-specific and we have been able to demonstrate that in individuals with subacute endocrinities causes by strep varigans, that one can see the productions of antibodies against these common bacterial antigens, which are highly cross reactive with Borrelia burgdorferi.

Some of the more specific antigens of this bacteria tend to be the outer surface protein antigens. The OspC is particularly interesting in that it produces an early immune response.

The difficulty with OspC, though, is that it is a plasmid encoated antigens. And most strains of this bacteria that express OspC, as you passage them repetitively over time in tissue and in culture, they will lose the plasmid that encodes for this, and that is a problem.

And there are numbers of commercial laboratories right now that have organisms which are simply not expressing this any longer.

The 93 is an antigen which is of import. And we recently went over, at the meeting that Dr. Dennis alluded to, and the committee at that CDC meeting really specified a

number of bands which we felt were important in developing Western Blot criteria. And these included 18, 22, the 23 which we call OspC -- because OspC is a fairly variable molecule, and some papers have put its molecular weight anywhere from 20 to 25; we arbitrarily called it 23 -- a 28 kilodalton antigen, a 30 kilodalton antigen -- which is not in our surface protein antigen -- 39, 41, 60, 66, and 93.

The proposed criteria, which are going to be studied, which is going to be five out of these ten bands on an IgG blot, will be considered to be positive.

The proposed criteria for an IgM blot is that IgM blots are only important in the first month of infection. And one must have two out of three antigens. And the antigens of import for IgM are 41, 39, and the OspC. And one must have two out of the three to be considered positive under those circumstances.

Those are preliminary proposals. I hope I didn't step on the CDC by telling people about that, but I think that that is important.

With regards to a question that I heard from the panel, the OspA band is not included in this, because the immune response to OspA usually only occurs late, and it occurs in only a minority of individuals. So, it would not felt to be a critical band in the development of IgG Western blot criteria. I think that is somewhat important.

Now, there are other problems with serologic samples. Because of the lack of reproducibility, samples run at different times cannot be compared. If one takes a serum sample and divides it, say, into 10 and runs it at 10 different times, the amount of variability in that serum sample is usually quite high, particularly when you use a commercial lab.

So that, if one were to compare two samples, I think that a prerequisite would almost be that it has to be run on the same ELISA plate, one right next to the other.

Another problem which we delineated is, we have been unable to find a correlation between serologic response and clinical response. This is both in early and late disease.

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And in fact, in early disease, there seems to be somewhat of a negative correlation, in that individuals who failed to mount a vigorous immune response tend to have a higher risk of subsequent failure.

And as already has been pointed out, rarely patients may fail to mount a measurable immune response. These are generally individuals who have been given antibiotics early in the course of infection and the course has been inadequate. I should emphasize the word, rarely, in here, since seronegative disease are rare for that.

DR. DENNIS: Do those three comments apply to

immunoblot as well as ELISA.

DR. DATTWYLER: Yes.

DR. DENNIS: The inter-assay variability.

DR. DATTWYLER: I think there is less variability in the Western Blot. But again, I think it is important, if one were to do these assays, especially the ELISA, one would do them at the same time.

Western Blots, I don't think that is important, if appropriate controls are used in the assays. I think the key with Western Blotting is to do appropriate controls. And also very critical is what antigen substrate one uses.

One should use a low passage Borrelia burgdorferi for these assays. And if one carefully monitors that, and makes sure that that organism is expressing a full range of its protein antigens -- if has lost, say, the OspC, then it should not be utilized. And I think that that is a key thing.

So, in summary, I think that what we have is that we have, perhaps, an incomplete view of the full range of clinical manifestations. We are now just evolving and standardizing serological tests to the point that they can be utilized. But that is still work in progress that I think the CDC is doing a great job, but we are still not totally there yet.

I would think that we should do epidemiological

studies on large populations of heavily-at-risk people, both following serology and also applying clinical responses, to fully delineate the full range of this disease. I am not sure that I know the answers yet, or anybody does at this point. Why don't I stop there.

DR. DENNIS: Thank you, Dr. Dattwyler. Are there questions from members of the panel.

DR. O'BRIEN: I was concerned about your last slide where you said there was a poor correlation between serologic response and clinical disease. And as I heard you to say, some people who mount better responses get worse disease. Did I hear you say that.

DR. DATTWYLER: No, no, I said the reverse. The better responses tended to have a better response. And I should clarify where this is from. This is from antibiotic trials. These are treatment trials of erythema migrans, in which individuals given an antibiotic regimen which was not optimal -- we didn't know that it was not optimal at the time -- the ones that failed to mount a vigorous immune response tended to do worse, clinically. So, there was an inverse correlation between the degree of serologic response and the outcome.

So, individuals with a poor immune response tend to have worse disease.

DR. O'BRIEN: One other question, back to your

natural history that you presented. There were some patients who were not treated for four years because they were not recognized to have the disease. And this had to do with the development of understanding Lyme disease.

DR. DATTWYLER: Yes.

DR. O'BRIEN: What happened to patients that were treated after four years. How did they, people that had been ill for a long time and then were treated.

DR. DATTWYLER: Actually, the best person to answer that, because I was quoting Dr. Steere's data, is Dr. Steere. I think he can answer that question much better than I can.

DR. STEERE: It is the usual response to antibiotics whenever you treat it, although it may be more difficult later. You may have to treat longer. And neurologic disease requires intravenous antibiotic therapy.

DR. O'BRIEN: Sort of like syphilis.

DR. STEERE: It is like syphilis. But if a person already has deficit -- and particularly neurological deficit -- it usually improves, but it may not become totally normal.

DR. O'BRIEN: Thank you.

DR. DATTWYLER: I must comment, that is our experience as well, in people that present with late disease, that the antibiotic response is usually quite good.

DR. ROOS: There was a question before, about how valid serologic tests are with different types of genotypes. (Portion of question off microphone.) Or making it more broad with respect to serology, how about PCR. There are other kinds of Borrelia which may be not wanted --

DR. DATTWYLER: I don't think we know a full answer to that. We know, from studies in Europe, that there are three genotypes -- genospecies -- that have been defined.

Studies in the United States would suggest there is only one genospecies, but it has really not been studied fully at this particular point in time, and we know that, from tick isolates, that there appear to be some variability in Borrelia from ticks.

Whether these additional isolates can cause human disease, I don't think we know at this particular point in time.

As far as serologic import, there is enough cross reactivity between the various Borrelia at this particular point in time, that serologic assays should pick them up, because the flagellant and the common bacterial antigens are well served across spirochetal species.

With regard to other tick-borne infectious diseases, these ticks certainly carry a number of other things. I guess the best characterized thing would be

lovizia(?), and that can present with a viral like illness after a tick bite.

And it does not produce erythema migrans lesions. There could be some confusion, however, in an individual who presents after a tick bite with a viral-type illness. What is it. And that would create some difficulty in the differential diagnosis. And one would have to back off and look at, perhaps, serologic responses in those circumstances.

DR. LEMON: I wonder if you could speculate a little bit about the pathogenesis in patients with multiple EM lesions. Is this really hematogenous dissemination from a primary site with a single tick bite, or is it possible that these are actually representative of multiple tick exposures.

DR. DATTWYLER: Multiple tick exposures would be, frankly, uncommon. I live in a very highly endemic area, in Suffolk County, Long Island, and multiple tick bites would be, frankly, uncommon.

So, I think, by and large, these represent hematogenous dissemination of the organism.

The other thing in support of that is that individuals with other symptoms and other abnormalities would suggest a systemic disease.

DR. LEMON: There is no evidence that multiple EM

lesions are less common in low prevalence areas, for example.

DR. DATTWYLER: I am not aware of any data that would suggest that.

DR. LEMON: Does that men that the parasitemia is very low level, if you are seeing such a few number of lesions.

DR. DATTWYLER: No. I think that it speaks to the variability of the disease. I should also say that there may be regional differences, because in Connecticut, in Dr. Steere's work, they had a much higher incidence of multiple erythema migrans lesions, than we had observed.

Also, from different regions of the country, we seem to see different incidence of other manifestations, say, carditis.

On Long Island, one sees carditis occurring less than one percent of the time in individuals with erythema migrans. And I have talked to physicians in Connecticut and they report a higher incidence of that.

From treatment trials, which are national based treatment trials of erythema migrans, there appears to perhaps be some regional differences in response to antibiotic therapy.

Now, there is not enough statistical power in those studies to prove that. That is just an off-hand

observation. So, I really don't think we know at this point.

DR. FERRIERI: Could you comment on the universality of the antigenic profiles relative to the European strains and whether one can expect the immune responses to be similar, so that the criteria for WB would be similar.

DR. DATTWYLER: Yes. I think that, fortunately, there is enough shared proteins between various genospecies that the Western Blot criteria should hold up fairly well.

There are some strains in Europe which don't express OspA or OspB, but that is not an important criteria.

There seems to be a universality to the flagella and the common bacterial antigens -- 93, some of the others. So, I think that from that perspective on Western Blots, we are fairly safe.

DR. EICKHOFF: With other tick-borne infections, the tick-borne recipsiosis(?), in particular, a history of tick exposure is highly variable. What proportion of patients, who fit the CDC case definition of Lyme, actually give a history of tick exposure.

DR. DATTWYLER: A little less than half.

DR. EICKHOFF: Just like other tick-borne diseases.

DR. DATTWYLER: And the difficulty in certain

regions, though -- say, Suffolk County -- if you ask the population of a very heavily endemic areas in Suffolk County, have you ever had a tick bite, the answer is yes. So that, it fails to be much of historic import, because tick bites are so common in certain populations.

And I have been bitten by ticks, my children have been bitten by ticks, my wife has been bitten by ticks. We don't have Lyme Disease. So, it is a fairly universal thing.

In our region, one of the scientists at Stonybrook tried to look at dogs to find some negative dogs to study. And he couldn't find any seronegative dogs. All the dogs had been exposed to Borrelia burgdorferi in our area.

DR. ROOS: Just one further point regarding the serological differences. There are some articles in the literature about similarities in the central nervous system, syndromes in European Lyme and American Lyme, but differences regarding at least some aspects of the serological response in the spinal fluid so that aligoclonal bands, I guess, are commonly seen in Europe and rare here, suggesting that there are certain important qualitative differences.

Now, I don't know how much confirmation there is at present in those studies, and whether those qualitative differences also have some quantitative differences and

aspects, and whether you have done Western Blot studies on those European spinal fluids, et cetera.

DR. DATTWYLER: The last question, no, we haven't done studies on the European cerebral spinal fluids. I can tell you, though, that there appears to be a real difference between European serologic responses in the central nervous system and North American responses in the central nervous system.

It is almost universal in Europe to have a serologic response in the CSF and it is uncommon in the United States. And we have individuals who we have PCRed the DNA out of the cerebral spinal fluid, who failed to mount a serologic response, and clinically had Lyme meningitis -- had erythemal migrans and meningitis.

So, I think that the serologic response in the CSF in North American patients is of less value than it is in Europe. It is a less reliable marker of disease. And there, the PCR might be more useful.

DR. BROOME: Two questions. One is just following up on the point of the CSF antibody. Is that using the same serologic tests.

DR. DATTWYLER: Yes.

DR. BROOME: And there is no explanation.

DR. DATTWYLER: There is just no explanation, using the same type of serologic methodologies.

DR. BROOME: I wanted to ask about the variant erythema migrans lesions you showed, and the feasibility of using those as part of a case definition for a vaccine trial. I guess part of it is just the specificity of such variant lesions on clinical grounds.

And the second part is whether people have done biopsies and cultures of such lesions, and is that a reasonable approach for the less typical clinical.

DR. DATTWYLER: The answer is yes, people have done biopsies and cultures of those types of lesions. And yes, they have isolated Borrelia from that type of lesion.

I think, in experienced hands, it is easy to recognize those lesions as erythema migrans. I think the difficulty becomes, in inexperienced hands, if one were to show those slides, blinded, to an experienced dermatologist, I think there would be no trouble in identifying that as erythema migrans. So, I don't see that that is a problem.

I think that most individuals who become used to seeing all the varieties of this would have no difficulty under those circumstances.

DR. LEMON: Perhaps we should move on. Thank you very much. I would like to see if we could have Dr. Steere give his presentation prior to our break. And then, after the break, we can proceed with the manufacturers presentations in open session.

infection. And one of the sites that, at least in the United States, and at least in New England, there is common hematogenous spread, is to joints.

Usually, that event is rather vague, in terms of joint symptoms, at that time. They seem to be dampened. And it is only months later -- usually within the context of an expanded immune response to the spirochete -- that one gets a picture like this.

This is a child who, six months into the illness, had the sudden onset of marked knee swelling. And one gets huge infusions in this disease.

Typically, they don't last very long, at least in initial attacks. This will often go away within several weeks to at least several months, but it may recur. And it is only at the far end of the spectrum that this lasts for a longer period of time.

This is a study that Ray showed, that we did with patients back when we -- well, it was started in 1976, when we suspected that erythema migrans was a manifestation of this disease, and in which one also got arthritis.

And we simply identified patients with these skin lesions and followed them prospectively to see what would happen. We did not know about antibiotic therapy for the disease or Borrelia burgdorferi at that time.

In about 20 percent of the patients, nothing else

Well, now maybe the number of spirochetes that got to joints were different, maybe there are differences in virulence of the spirochete that we don't know anything about that.

We have investigated the issues of, are host responses different in these people, and in particular, rheumatologists are interested in immunogenetic markers because so many of the types of arthritis that we deal with have associations with Class I or Class II MHC molecules.

So, we looked at, well, both class I and class II MHC molecules in 80 patients who had Lyme arthritis. And they had a spectrum of disease, including arthritis of short duration which was defined of less than five months, moderate was six to twelve months, and chronic was one to four years, not forever.

There was an increased frequency of HLA DR4 in the patients who were at the severe end of the spectrum, compared to the mild end of the spectrum. And this is the only significant difference, in this group of 80 patients.

There was a suggestion of an increased frequency of HLA DR2, also, in the group with chronic or moderate arthritis. But this was not a significant difference.

If these things are increased in frequency, what was less. And the answer to that was HLA DR5 and maybe DRW6, although the number of patients with that specificity

patient with severe involvement, disease onset -- which also included neurologic involvement, but it went away. Remember, this is all untreated disease.

Then, during the second year of the illness was the period of arthritis, which there was some fluctuation but he had continuous involvement for about a one-year period, and then it went away.

During the sixth year of the illness and through the eighth year of the illness, he had a chronic encephalopathy, and he was treated with antibiotic therapy at this point in the illness.

First, I would like for you to focus on the IgM and the IgG response to these non-outer-surface proteins -- p39, p41, and p93. These are probably the immunodominant proteins that are non outer surface proteins.

One does see an IgM response to these proteins and then, quickly, as one would expect, an IgG response, which just remains high throughout these years of the illness.

The responses to the outer surface proteins are somewhat different -- this is OspA, OspB, OspC. And incidentally, there are other outer surface proteins. We have not been able to find an antibody response yet in anyone to outer surface protein D, but outer surface proteins E and F have now been described, and there may be more as well.