## Vector-borne disease and bioterrorism preparedness: Tularemia, Q-Fever, Venezuelan equine encephalitis, plague, and relapsing fever

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## West Nile update, eastern equine encephalitis

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West Nile virus was introduced from abroad into the New York City area sometime prior to August 1999. It has spread rapidly and has been found in southern counties of northern New England states, throughout the state of New York, as far south as southern Florida, and westward into Louisiana and an eastern county in Iowa. Birds are the likely means by which this virus is transported long distances. The virus has been isolated from or detected in more than 20 species of mosquitoes. The virus has been associated with at least 75 species of birds.

WN virus has now been found in the greater New York City area for three consecutive years and is likely established. Humans and horses have been infected from New England to Florida. A phylogenetic analysis of a 921 nucleotide (nt) sequence of 82 different isolates from Connecticut showed differences at 30 positions when compared to the WN virus isolated from a flamingo in New York City in 1999. Thirty-four of the isolates had sequences identical to the flamingo isolate, 37 had one nucleotide change, 8 had two changes, and three had three changes.

This virus is likely to remain a health threat in temperate climates and become an even more serious problem in southern states where the mosquito season is longer, and where there is a greater variety of species of mosquitoes.

# PRESENTATIONS APRIL 6, 2002 SATURDAY

***Discussions and Questions***

## Genetic Systems and antibiotic resistance markers

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(Scott Samuels, Kristi Frank, Michele Kresge, Nathan Dague, Kendal Galbraith, and Sharyl Fyffe, Division of Biological Sciences, The University of Montana, Missoula MT)

Ten years ago, we isolated mutants of *Borrelia burgdorferi* that were resistant to the clinically irrelevant antibiotic coumermycin. The gyrB antibiotic resistance marker was used to develop a rudimentary genetic system. Recently, the field has matured remarkably. A couple new selectable markers are available and several genes have been disrupted, allowing for a molecular dissection of pathogenesis. In addition, shuttle vectors have been developed to introduce new genetic material into *B. burgdorferi*. Of the three extant antibiotic resistance markers, there are caveats for using two of them. Regardless, an efficient genetic system requires several markers for complementation studies as well as disruption of multiple genes.

Therefore, we fused a strong constitutive *B. burgdorferi* promoter to a spectinomycin and streptomycin resistance gene open reading frame in a similar fashion as previously described for constructing the kanamycin resistance marker. The resulting hybrid antibiotic resistance marker has been cloned into the shuttle vector pBSV2 and confers resistance to spectinomycin and streptomycin. Selection with spectinomycin has not been feasible due to a high frequency of background mutants.

However, streptomycin functions well as a selective agent. Therefore, another useful antibiotic resistance marker is available for genetic studies. Now, the only molecular tool missing from our genetic arsenal is a counter-selectable marker. This allows researchers to select for the loss of genetic material from the experimental organism. A counter-selectable marker has recently been developed for Borrelia's spirochete cousin Leptospira.

One strategy for counter-selection involves using a merodiploid with a recessive antibiotic resistance allele on the chromosome and a dominant antibiotic sensitive allele on a plasmid. The merodiploid is susceptible to the antibiotic (unless the plasmid is lost). Therefore, plasmid loss is selected with the antibiotic. We have isolated mutants of *B. burgdorferi* that are resistant to fluoroquinolone antibiotics. We are developing the fluoroquinolone antibiotic resistance marker, which is recessive, into a counter-selectable marker.

# PRESENTATIONS APRIL 7, 2002 SUNDAY

## Treatment panel - Case presentations

Ami Katz, MD, Leslie Fein, MD Kenneth Liegner, MD, Edwin Masters, MD, Sam Donta, MD
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## The Southern and Eastern Tick Triads: Lyme disease, Master's disease, ehrlichiosis and babesiosis

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Examples of clinical erythema migrans in Missouri are presented along with other clinical presentations and examples of sequelae. Etiological and epidemiological theories are presented and discussed. This includes the hypothesis that just as there exists a clinical triad of *Ixodes scapularis*-vectored borreliosis (Lyme disease), ehrlichiosis (HGE) and babesiosis in the Northeast, there may also exist in the South, including Missouri, a similar clinical triad vectored by lone star (*Ambylomma americanum*) ticks. Patients with signs and symptoms explained only by a borreliosis following lone star tick bites are presented. This southern lone star-vectored erythema migrans has also been called Master's disease. *Babesia* MO 1 has been identified in a Missouri patient and although vector studies have not been done, other *Babesia* are known to be carried by *Amblyomma* ticks.

Ehrlichiosis (HME) and Ehrlichia ewingii are known to be carried by lone star ticks in the South. Parallel evolutionary paths in these two tick lines might explain what clinicians around the United States are seeing. Each of the three illnesses might have northern variants associated with *Ixodes scapularis* ticks and southern variants associated with lone star ticks. The clinical disease variants appear clinically similar, but have testing, microbiological, and culturing differences. Ehrlichiosis represents the prototype for this theory. Evidence of tick to tick (even different species) transmission *of Borrelia burgdorferi* while feeding on hosts and the isolation of *B. burgdorferi sensu lato* from a lone star tick feeding on a rabbit at one of my Missouri erythema migrans patient's farm are both consistent with this theory. The role of Borrelia lonestari in southern EM's is still uncertain.

Given this history of the evolving geographic association of Borreliosis, Babesiosis and Ehrlichiosis (although different species or strains may be involved), when there is evidence for the presence of two of these ticks-vectored illnesses, a high index of suspicion for the third is warranted, along with the possibility of co-infection. More research is needed.

## Human Ehrlichiosis: an update on the pathogens, the diseases, and changes in their nomenclature

William Nicholson, PhD
*Centers for Disease Control and Prevention*

Ehrlichiosis was first described as sennetsu fever in humans in 1954. Since then, three additional diseases with ehrlichial etiology have been described (in 1986, 1994, and 1999). The species making up what was known as the genus *Ehrlichia* have been shown to exhibit distinctive biological, serological, and genetic differences. The species that clustered together have been referred to as genogroups. A recent taxonomic paper has reclassified these agents based on 16S rRNA and other gene sequencing to reflect these genogroups. Thus, the genus *Ehrlichia* remains the home of the human pathogens, *Ehrlichia chaffeensis* and *E. ewingii*. The agent of human granulocytic ehrlichiosis is assigned to the species, *Anaplasma phagocytophila*. The human pathogen causing sennetsu fever is now named *Neorickettsia sennetsu*. While these taxonomic changes may appear disruptive, no revision of the genera will be needed for awhile. This presentation will review these taxonomic changes, and provide updated information on the biology and epidemiology of each organism, the clinical features of the diseases, and treatment of these infections.

## *Bartonellae* and *Bartonella*-associated disease: A brief introduction

Russ Rugnery, PhD
*Centers for Disease Control and Prevention*

Recognized member of the genus *Bartonella* are typically rarely cultivated in clinical diagnostic bacteriology laboratories due to their fastidious nature and slow growth characteristics. So perhaps it is not altogether surprising that until recently, relatively little was known about members of the genus *Bartonella* and the infections they can cause. The application of modern methods of DNA analysis has made it possible to not only appropriately group the few recognized members of the genus together, but also to begin to recognize the incredible spectrum of *Bartonella* organisms not previously appreciated.

The recent revolution in the understanding of the roles of *Bartonella* as infectious agents began in the early 1990's with the isolation and characterization of an organism (*Bartonella henselae*) found in the blood of an febrile HIV-infected patient. Concurrently, DNA sequences that matched the same organism were also found in lesions of HIV infected persons with a syndrome described as bacillary angiomatosus. In the process of developing a simple serologic diagnostic test to evaluate risk factors for acquiring bacillary angiomatosus an even more important discovery was made. It was found that persons diagnosed with cat-scratch disease (CSD), the etiology of which had evaded recognition for over 50 years, had high-titered antibodies to *B. henselae*. As the name implies, there is a strong association of human cat-scratch disease is strongly associated with a traumatic cat-scratch, typically by a kitten. It was rapidly shown that *B. henselae* could be isolated from infected tissues of CSD patients, thus helping to clinch the etiology of CSD. It was also rapidly demonstrated that *B. henselae* could be isolated from the blood of cats. This lead to the recognition that *B. henselae* infections are quite common in domestic cats in most areas of the world. Cats don't appear to show any overt symptoms of infection. Unlike Lyme disease, there appear to be few geographic restrictions on the distribution of *B. henselae* infections of cats and humans. The distribution of *B. henselae* infections in the U.S. roughly parallels the distribution of the cat flea, suggesting that the flea is an important vector of *B. henselae* between cats; the arid areas of the country that don't support cat flea populations don't appear to have evidence of feline (or human) *B. henselae* infections. Experimental transmission of *B. henselae* between cats by cat fleas has been demonstrated. The complete role of fleas, and possibly other vectors including ticks, in the transmission of *B. henselae* between cats or to humans remains under investigation. Cat-scratch disease is not commonly reported to states of to CDC so solid estimates of the number of human cases are illusive; the best estimate for the number of CSD in the U.S. is 22,000 per year.

The rapid progress that characterized the discovery of the agent and reservoir of CSD suggested that similar progress could be made in understanding the reservoir for *Bartonella quintana*, the agent of trench fever, another related infectious agent associated with the bacillary angiomatosus syndrome reported in persons with immune impairment, as well as a disease that was commonly recognized among troops during WWI. *Bartonella* culture was attempted from a wide range of non-human animals (in addition to cats). Curiously, no non-human vertebrate reservoir for *B. quintana* has ever been reported, suggesting perhaps that humans *are* the reservoir and that all that is required is the appropriate invertebrate vector, the human body louse, which thankfully is not a common feature of most of our lives.

Despite the observation that the initial search for a non-human reservoir(s) for *B. quintana* was unsuccessful in finding a non-human reservoir for trench fever, it was wildly successful at discovering an unexpected array of novel *Bartonella* species, recovered from a wide variety of mamma-

lian species sampled. And the frequency of bacteremia in the species tested is often remarkably high; quite unlike many other microbial agents which are typically episodic. It is now recognized that most species of rodents that have ever been sampled have at least one genotype or species of *Bartonella*. Similarly, when larger mammals (e.g., rabbits, elk, deer, cattle) are sampled they too are frequently found to host novel *Bartonella* species.

Clearly, *Bartonella* species are an extremely common, perhaps ubiquitous, part of the systemic microbial flora of many mammalian species. The most common *Bartonella* species found in cats is a good example of classic zoonotic disease. In environments where body lice are common, trench fever appears to have the potential for epidemic disease, sans alternate vertebrate host(s). And while the potential for many of the other *Bartonella* agents that are associated with non-domestic species to cause human disease is still being evaluated, it is clear that several of what are considered rodent *bartonellae* have now been associated with occasional zoonotic human disease.

Although still in its infancy, the study of *Bartonella* species and the diseases they may cause is now an international field of medical research. However, many many questions remain to be answered. These include elucidating the details of interaction between hosts and pathogens, the roles of various arthropod vector species, and the potential interaction between *bartonellae* and other infectious agents and their host species.

## New data on tick-borne diseases: Bartonella, mycoplasma

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300 patients were tested for both Lyme and Bartonella over the past 12 months. Data presented will include correlation between PCR's and antibodies, frequency of the coexistence of both Lyme and Bartonella, and a discussion about the response to therapy.

## Neuropathy and Leukoencephalopathy Associated With LymeRix Vaccination

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We've recently seen several patients who developed demyelinating neuropathy, multifocal motor neuropathy, or leukoencephalopathy, after receiving the Lymerix vaccine. These syndromes are similar to those described in patients with active Borrelia burgdorferi infection, or chronic Lyme disease.

The observation suggests that these syndromes might be caused by molecular mimicry, or immune reactivity to antigenic determinants in human central and peripheral nerves that are shared by the Borrelia burgdorferi outer surface protein A (OspA). This immune reactivity could be induced by active infection, or by the Lymerix vaccine which contains OspA.

When comparing the OspA DNA sequence to sequences in the GenBank database, we found 2 stretches of identity, or 100% homology, over 19 base pairs, with 2 cDNA clones derived from human brain. One encoded a 5 amino acid sequence, VSEKI, corresponding to the cDNA sequence of a clone derived from human primitive neuroectoderm (GenBank gi: 11987184), and the second encoded a 6 amino acid sequence, FTKENT, corresponding to the cDNA sequence of a clone derived from an anaplastic oligodendroglioma (GenBark gi: 5528532). The functions or distribution of the corresponding gene products, or proteins, are unknown.

Humoral or cellular immune reactivity to these antigens in human nerve or brain might be responsible for the neuropathy or leukoencephalopathy observed in patients with Lyme disease or following Lymerix vaccination. Continued reactivity following antibiotic therapy might be responsible for some of the manifestations of chronic Lyme disease. Assays for antibody or T-cell reactivity to these proteins might be useful in the diagnosis and evaluation of affected patients. Deletion of these sequences from the OspA vaccine might prevent the neurological complications.

## Immunoreactivity related to Lyme disease vaccination

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Immunization with the recombinant OspA vaccine for preventing infection with Borrelia burgdorferi remains a controversial topic despite the recently announced removal of the vaccine from commercial distribution. Previous studies have reported that the vaccine may induce production of antibodies which bind to several different components of the spirochete in addition to native OspA. Other studies which examined the potential of the vaccine to elicit adverse reactions have yielded conflicting conclusions.

For this study, serum specimens from over 150 recipients of the "LYMErix" vaccine were tested by Western blot using a home brew and the 2 FDA approved products. All of the individuals tested for this study claimed to have developed adverse reactions as a result of receiving the vaccine. Individuals included in the study had received 1 to 3 doses of the vaccine with an elapsed time from the last dose to sampling ranging from several months to over three years. Adverse reactions claimed to be related to the vaccine ran the gamut from memory defects to active joint disease.

Results of testing indicated that less than one percent had serologic reactivity beyond that which could be expected to result from the vaccine alone. A significant percentage of individuals did not have antibodies to OspA by at least 2 Western blot assays indicating that they had either failed to seroconvert as a result of vaccination or that their response to the vaccine had fallen below detection levels. The majority of individuals tested were found to have antibodies which bound several components of B. burgdorferi in addition to OspA. The extent of this reactivity made interpretation of the W.B. strips difficult as several CDC/Dearborne criteria bands were routinely scored as positive, although only a few individuals met that criteria for positivity. The difference between reactivity detected on the different W.B. assays indicates that blot manufacture can be a critical variable for interpreting serologic results.

The results obtained from this study indicate that with few exceptions these vaccine recipients, claiming adverse reactions to the vaccine, do not have evidence of infection with B. burgdorferi which could account for their symptoms. It remains to be determined whether some other cause/ causes or the vaccine itself is responsible for their symptoms.

## Lyme vaccine reactivation of latent Lyme disease

Sam Donta, MD
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*Background*: Lyme Disease may result in a multisymptom disorder characterized by chronic fatigue, musculoskeletal dysfunction, and neurocognitive dysfunction. The underlying basis for these symptoms remains to be delineated, but accumulating evidence supports the existence of chronic infection. A vaccine based on the OspA protein of the causative organism, *Borrelia burgdorferi*, was developed to try and prevent Lyme Disease and its various manifestations. Although there were a few reports of adverse reactions during the testing of the vaccine, the numbers of reactions since the vaccine was approved and marketed have been increasingly being recognized.

*Methods and Results*: Patients who had received one or more injections of the Lyme OspA vaccine and who had systemic reactions to the vaccine lasting more than a few weeks were seen and evaluated at our Lyme Disease Clinic at Boston University Medical Center. The results of the first 15 patients are reported here.

Six males and nine females ranging in age from 17 to 56 were studied. Three knew they had prior Lyme Disease, five had symptoms compatible with chronic Lyme Disease, and eleven reported prior exposure to Ixodes ticks. Five patients had received one immunization, three patients two immunizations, and the remaining seven three immunizations. In those receiving a single immunization, the onset of reactions began within 3-5 days in all but one patient, whose reactions began two months later. Two patients receiving two vaccines had reactions within 1-3 days, while the third patient's reactions began 2 months later. The remaining patients who received all three immunizations had the onset of their reactions generally within 24hrs of the 3rd injection, although a few reported their reactions 1-2 months later; some of these patients had milder reactions following their first and/or second immunization. All but one patient had the combination of symptoms including fatigue, musculoskeletal and neurocognitive dysfunction. Patients with prior symptoms related that their symptoms post-vaccination were very similar to those pre-vaccination. HLA-DR4 studies were positive in one patient, negative in two others. Lyme Western Blots revealed the presence of reactions against specific proteins of *B. burgdorferi* in addition to reactions against the OspA 31kd protein. IgM reactivities against the OspA and other proteins were noted in 11/15 patients. While patients continued to be studied, 10/15 patients are responding to antibiotic treatment with intracellular-type antibiotics.

*Conclusions*: The Lyme OspA vaccine appears to be reactivating symptoms characteristic of chronic Lyme Disease. Individuals without known prior infection with *B. burgdorferi* who had vaccine-associated reactions had evidence of prior infection by Western Blot analyses. As the numbers of reactions amongst vaccine recipients appears to be increasing, and the magnitude of this problem is yet to be delineated, it would seem appropriate to withhold the vaccine from patients with a prior history of Lyme Disease and/or have patients tested with a sensitive Western Blot prior to receiving the vaccine.

## Comparison of Lyme disease vaccine adverse event reports and comparison to other vaccine results

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*Genetic Centers of America*

We have analyzed arthritic adverse reactions following recombinant Lyme vaccination in the adult population in the United States. A certified copy of the Vaccine Adverse Events Reporting System (VAERS) database was obtained from the Centers for Disease Control and Prevention (CDC) and analyzed from December 1998 through October 2000 using Microsoft Access. The arthritic reactions analyzed were: arthritis, arthrosis, arthralgia and joint disease. We found that arthritic reactions were reported for patients in the age group 35-62 year-olds within four to six days after Lyme vaccination. Arthritic reactions were fairly evenly reported for men and women. Because of the molecular design of the recombinant form of the Lyme vaccine, it was assumed that this vaccine would be well-tolerated and result in few serious adverse reactions. This prediction was not borne out by our analysis of the VAERS database. Rather, our analysis showed a statistical increase in arthritic reactions over those reported following an adult Td vaccine control group.

Additionally, we determined that the incidence rate of acute and chronic arthritis was statistically increased following Lyme vaccination in comparison to adult rubella vaccination. This is particularly remarkable considering the Institute of Medicine of the U.S. National Academy of Sciences has determined that there is a casual relationship between adult rubella vaccination and acute and chronic arthritis. Our results indicate a less reactogenic Lyme vaccine is needed. Lathrop and colleagues, from the CDC, in recently analyzing the same VAERS database, remarkably concluded there were no unexpected or unusual patterns of reported adverse events following Lyme vaccination. Obviously the vaccine manufacturer, GlaxoSmithKline Beecham Pharmaceuticals, agreed with our assessment of the Lyme vaccine, since they withdrew the vaccine from the market in early 2002. This action seems well justified based upon the results of our study and the Lyme vaccine should probably not be used until processes have been developed to produce a safer vaccine.