Keynote: The Politics of Discovering an Emerging Disease – The *Pfiesteria* Experience

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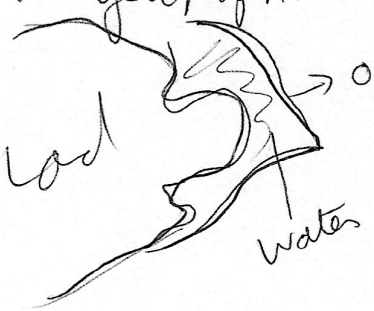
The role of toxic algae in human health historically has been considered within the remarkably narrow constraints of acute impacts, while chronic impacts from these organisms have been virtually ignored. Social/economic pressures and funding restrictions, as well as compromised ethics within contained segments of the scientific community, have fostered resistance to expanded assessment of human health impacts from harmful algal blooms. Nevertheless, accumulating evidence indicates that chronic impacts on human health from microalgal toxins can be pervasive. Documented chronic impacts on mammalian health from certain of these toxins include immune system suppression, respiratory illnesses, central nervous system dysfunction with memory impairment and learning disabilities, lingering peripheral nervous system dysfunction, and promotion of malignant tumors.

Long-term datasets strongly correlate the abundance of some harmful taxa with increasing human population growth and nutrient enrichment, enhanced by shellfish reductions from overharvesting and habitat loss. Several examples will be presented to illustrate these points, with special focus on a recently discovered harmful alga, the toxic dinoflagellate *Pfiesteria.* Within the past decade, toxic *Pfiesteria* outbreaks in the two largest estuaries on the U.S. mainland have provided a compelling illustration of strong linkages between fish kills/disease and subtle but serious impacts on human health. Two species thus far comprise the toxic *Pfiesteria* complex, and are unique from other dinofla-gellates in having (i) strong attraction to live fish; (ii) production of bioactive compounds (toxins) that cause fish distress, disease, and death; and (iii) toxicity that is triggered by the presence of fresh materials from live fish. *Pfiesteria* spp. also have a complex life cycle with multiple amoeboid and flagellated stages. They cause both focal and non-focal lesions and other disease as well as death of fish; and they have been linked to cognitive dysfunction (learning disabilities, memory loss) and other serious impacts on human health. From earliest *Pfiesteria* research, the science has been inextricably enmeshed with political pressures that have largely controlled the extent to which progress has, or has not, been possible.

Examples will be given of specific actions by representative state and federal agencies, assisted by certain academic and agency scientists, in attempts to advance vs. suppress under-standing of toxic *Pfiesteria* as a human health threat.

the geographic area is enclosed by strips of other islands.



Pfie
Discovered at Vet School & Big was called "contaminant", and it only appeared as fish kill occured. After fish dead, they disappear.
TOXIC & benign STRAINS EXIST.
Breakdown between freshwater researchers vs. salt water.

Reactivation of Lyme Disease Following Lyme OspA Vaccine

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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 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 continue 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.

Animal Models in the Study of Spirochetal Infections

James Miller, PhD
UCLA School of Medicine

Animal models are an integral part of experiments designed to elucidate virulence factors and protective immunogens of pathogenic spirochetes as well as the pathogenesis and immunology of the human diseases they produce.

There are several criteria that influence the selection of an animal model, including 1) economic feasibility, 2) ease of handling, 3) age, 4) sex, 5) dose response, 6) anatomic similarity or identity of sites of infection, 7) induction of a host response identical or closely similar to the human infection when utilizing the same portal of entry, 8) identical or similar genotypic and phenotypic properties of experimental animal isolates to human infection isolates, 9) ability to recover relatively large numbers of spirochetes from the animal host for propagation and/or biological study, 10) use of inbred animals when necessary for immunogenic studies, and 11) use of immunologically deficient animals when necessary for selected studies.

The key features of Lyme disease, relapsing fever, syphilis, and leptospirosis animal models that have contributed and continue to contribute to our understanding of the mechanisms operative during the course of human infection will be discussed.

PRESENTATIONS APRIL 22, 2001 SUNDAY

Mechanisms of Persistency of the Lyme Disease Spirochete

Charles Pavia, PhD
New York Medical College of Medicine

Evidence will be presented which reinforces the complexity of the host-parasite relationship as it exists for the North American version of Lyme disease. Infection with *Borrelia burgdorferi* evokes a somewhat complicated antibody response plus an assortment of cell-mediated immune type reactions in the untreated mammalian host. It appears that humoral immunity plays an important but limited role towards the complete elimination of Borrelia bacteria, while the cellular limb of the immune response may also be a significant host defense mechanism, along with playing a serious effector role in the development of certain pathologic manifestations associated with Lyme disease.

Many of these phenomena are reminsicent of what was discovered, many years ago, about the related syphilis spirochete, *Treponema pallidum*. It is presently unclear what percentage of untreated patients having clinically verifiable Lyme disease will actually have residual, live (persisting) spirochetes remaining in certain tissue sites, months or years later after a tick bite. It is believed that most of the dermatologic, rheumatologic and neurologic complications of Lyme disease can be attributed to local inflammatory responses elicited by the presence of relatively small but resilient numbers of *B. burgdorferi*, in the affected tissues.

Several mechanisms can be invoked to explain the delay in a fully effective immune response against borrelial infection and for the duration of disease and these include: (1) significant variation in the expression of borrelial antigens, especially the outer surface proteins (OspA, B, C, etc.); (2) production of a "capsule-like" substance on the outer surface of pathogenic Borrelia that may act as a barrier against potentially active, borreliacidal antibody; (3) this component plus other unique biologic properties of *B. burgdorferi* could enable spirochetes to escape being engulfed by macrophages and other phagocytic cells; (4) antigenic competition among different borrelial or tick-derived antigens that may lead to partial tolerance; (5) later development of so-called atypical "cyst-like" forms which do not resemble the more classic spiral-shaped Borrelia; and (6) *B. burgdorferi* infection may bring about the elaboration of immunoregulatory (suppressive) substances, of host or borrelial origin which inhibit the full expression of anti-microbial activity by host lymphocytes, macrophages, and other cell types.

view tape

Comparative Analysis of the Immune Evasion System Employed by the Lyme Disease Spirochete

Richard T. Marconi, PhD
Medical College of VA at VA Commonwealth University

The Lyme disease spirochetes are capable of chronic infection indicating that they are able to evade the immune response. The ospE and vlsE genes, both of which undergo mutation during infection, have been implicated as potential antigenic variation candidates. We have demonstrated that mutations in OspE result in the generation of antigenically distinct forms of ospE. Analyses of the closely related ospF family have demonstrated that while these genes are genetically stable during infection and do not experience mutation, they are temporally expressed. This may serve as a second mechanism by which the Lyme disease spirochetes alter the antigenic composition of the bacterial cell.

We have also conducted an analysis of the role of VlsE in immune evasion. Variants forms of VlsE that arose during infection were cloned and expressed and then used as the antigenic substrate in immunoblot analyses. Screening of the immunoblots with sera collected over the course of infection revealed that the VlsE variants that arose during infection are not antigenically distinct from one other. This observation suggests that VlsE may no play a prominent role in immune evasion. Collectively, the analyses presented provide further insight into the mechanisms utilized by the Lyme disease spirochetes in chronic infection.

VlsE infection induced variants of Bb.

- So with variants none were
- With B Flacco
- ospE - recombine during infection & antigenically distinct & help evade immune syste.
- ospF - stable
  vlsE - high mutat, point mut. limited capacity, variants ng

Regulation of Outer Surface Protein Gene Expression

Scott Samuels, PhD
University of Montana

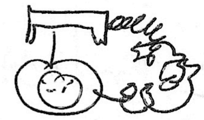
Previous studies have shown that the synthesis of the outer surface protein OspC is increased when cultures of *Borrelia burgdorferi* are shifted from 23ƒC, which models the temperature of the tick vector, to 35ƒC, which approximates the mammalian host body temperature. We demonstrate that this increase in OspC production is due to changes in DNA supercoiling. We found that DNA was more supercoiled in *B. burgdorferi* cultures grown at 23ƒC compared to cultures grown at 35ƒC. OspC synthesis increased when DNA supercoiling was artificially relaxed by treating cultures with coumermycin, a DNA gyrase inhibitor.

In addition, OspC synthesis was higher in DNA gyrase mutants than wild type cells. These results, taken together, suggest that *B. burgdorferi* senses environmental changes in temperature by altering the level of DNA supercoiling, which then affects the program of gene expression. This implies that DNA supercoiling acts as a signal transducer for environmental regulation of outer surface protein synthesis.

How Genes switched - Osp A & C

DNA - Double Helix -

relaxed coils vs supercoiled



smaller
migrate slower

only way to conver - take off hook, uncoil & put back on - & enzyme DNA gyrase does this

In tick - ~23°C - Osp A & B - predominant

mamml - ~35°C - OspC - predominant

other factors infl. ph -

How *Borrelia Burgdorferi* Responds to Oxidative Stress

John Skare, PhD
Texas A&M University System Health Science Center

We have isolated a mutation in the BB0647 gene in non-infectious *Borrelia burgdorferi* strain B31, a locus originally assigned as a homologue of the ferric uptake regulator (fur) by The Institute for Genomic Research (TIGR). Our goal was to initiate studies directed at identifying and characterizing genes regulated by BB0647 since fur genes from other pathogens have been associated with host adaptation and pathogenesis.

A recent database search indicated that *B. burgdorferi* fur (BB0647) is homologous to the peroxide regulatory protein PerR, a metal-containing DNA binding protein that regulates expression of catalase or peroxidases required to breakdown hydrogen peroxide upon exposure to oxidative stress. Consistent with this, the resulting perR mutant was approximately 3000-fold more resistant to 1 mM hydrogen peroxide relative to the isogenic parent strain.

Additionally, the perR mutant demonstrated detectable peroxidase activity when the indicator substrate o-phenylenediamine was used whereas no activity was observed in the parental strain. Taken together, these data suggest that the absence of PerR results in the constitutive expression of putative peroxidase gene(s) required to combat oxidative stress. Comparison of total protein profiles indicated only subtle differences between the perR mutant and the parental strain.

However, Western immunoblot analyses indicated that the decorin binding proteins A and B (DbpA and DbpB), known *B. burgdorferi* adhesins, were approximately 4-fold derepressed in the perR mutant suggesting that the PerR homologue regulates additional genes unrelated to the peroxide-resistant response. As such, this putative PerR regulon may be required not only for adaptation to oxidative stress during the tick blood meal and within the mammalian host but may also, by analogy to dbpBA, regulate genes involved in colonization of *B. burgdorferi* during infection.

Serology in Early Lyme Disease

Maria E. Aguero-Rosenfeld, MD
Westchester Medical Center; New York Medical Center

During the early phase with EM the diagnosis of Lyme disease is mostly clinical. Nevertheless, the clinical diagnosis of EM may be difficult at times due to lack of the characteristic features and perhaps misdiagnosis occurs. After the EM phase the symptoms are protean and may mimic several other infectious and non-infectious diseases. The most frequent laboratory tests used to support the clinical diagnosis are those detecting antibodies to *B. burgdorferi*. Several methods have been used to detect antibodies to *B. burgdorferi* including enzyme-linked immunosorbent assays (ELISA); indirect immunofluorescent antibody assays (IFA) and western immunoblots.

Tremendous knowledge on the serology of Lyme borreliosis has been gathered since the original development of these methods. Early studies used organisms highly passed in culture that were devoid of significant immunodominant antigens. Later it was found that there is differential antigen expression of *B. burgdorferi* between the tick and host environments that explain the antibody reactivity observed in early Lyme disease. In our institution we have characterized the antibody response of patients with EM defined clinically and as well as those culture-confirmed. Majority of patients develops antibodies within 2 – 4 weeks from onset of illness and most fulfill the criteria established by the ASTPHLD/CDC.

The early antibody response is mainly IgM directed to the 23-kDa (OspC) and the 41-kDa. The presence of antibodies during the acute phase correlates with the duration of disease and/or evidence of disseminated disease. IgG antibodies are produced in early disease but usually do not meet the ASTPHLD/CDC criteria. Individuals receiving a recombinant OspA vaccine preparation develop mostly IgG antibodies that are detected by the first step assays such as the currently used ELISAs. Antibodies are of higher intensity after the third vaccine dose with high Lyme ELISA values. Immunoblots of these sera show strong IgG reactivity to the 31-kDa and to other bands below OspC but the IgM IB shows mostly moderate reactivity to the 31-kDa. Although Lyme vaccine reactivity is characteristic and distinct from natural antibodies to *B. burgdorferi*, it could present some confusion to the inexperienced laboratorian. In our experience since most of the reactivity is IgG it does not pose a problem in reading or interpreting the IgM blot of individuals who failed vaccine and present with clinical evidence of early disease.

- OppA → C,
OspC =

Relevant to understand - Vaccine
2-step test - 1. Screen ELISA + IFA
2. separate IgG / IgM,
Problem is lack of screening of 1st step

Western Blot Testing for Diagnosing Lyme Borreliosis: Potentials, Problems, Performance

Paul T. Fawcett, PhD
Alfred I. duPont Hospital for Children

Western blot (WB) tests have been used routinely as an aid for diagnosing Lyme borreliosis for over 10 years. In 1994 criteria for interpreting WB were established at the "Second National Conference on Serologic Diagnosis of Lyme Disease" held in Dearborn, Michigan and in 1996 the Food and Drug Administration approved the first commercial WB for in vitro diagnostic use with suspected cases of Lyme disease. Widespread interest in, and acceptance of, WB as a test method for detecting antibodies to *B. burgdorferi* resulted from several factors, not the least of which was the relative novelty of WB as an effective immunochemical detection method, during the time when *B. burgdorferi* was first isolated and identified as the causative agent of Lyme disease in 1982.

Although electrophoretic separation of proteins using polyacrylamide gels had been in use for some time, as had various immunoblotting methods for immunochemical detection, it was not until 1979 that the two methods were combined into the system now called Western blot. Western blotting allows one to employ the highly sensitive and specific detection power of immunochemistry with the resolving power of gel electrophoresis. In contrast with other assay methods, WB allows simultaneous detection of antibodies of any class to essentially any and all of the individual proteins of *B. burgdorferi*, which are identified as bands on the WB membrane. In essence, WB allows one to take a fingerprint or snapshot of an individual's humoral immune response to *B. burgdorferi* at the time the serum sample was obtained.

Despite the potential power of this method there are many variables that can cause slight to large variations in the electrophoretic migration of a given protein in a polyacrylamide gel. These include degree of polymerization, uniformity of polymerization, buffers, impurities, time, temperature, power, and the preparation of the material being electrophoresed. Irregularities in separation cause variability in the location of given proteins from one gel to another, and following transfer can result in detection of WB "bands" of a particular protein in different locations.

Results from our laboratory, which has been manufacturing its own WB for 10 years, has shown that despite extensive QC one can expect variations of several millimeters in the location of given proteins between different lots of strips over time. Analysis of WB kits approved for in vitro diagnostic use indicates that commercial manufacturers experience similar, perhaps even greater variation, in protein band location between lots. This particular problem is exacerbated if one attempts to strictly adhere to current recommendations for WB interpretation, which requires specific band identification. Our own criteria, unlike that recommended by CDC, requires reactivity in two areas of a WB (41 kDa and a 60-70 kDa range band) in addition to any other 2 bands for IgG WB testing. In essence, we rely on pattern matching when reading strips. An example of the usefulness of our criteria, blot scoring and the power of WB, is provided by our use of WB to successfully diagnose reinfection with *Borrelia burgdorferi*.

We have also evaluated the effects of different manufacture on WB reactivity resulting from immunization with the OspA vaccine using two commercial WBs and our in-house WB. Results showed dramatic differences in reactivity. One of the commercial blots had vaccine recipient

sera actually scoring positive by recommended criteria, the other developed several bands and a dark graying of the high molecular mass region. Our blot showed 3-4 lower molecular mass bands with no graying. These findings indicate that it is not feasible to compare immunoreactivity between different tests when testing sera from vaccine recipients.

In conclusion, WB is capable of providing excellent diagnostic utility for Lyme disease. However, manufacture and interpretation requires significant levels of expertise and experience to fully realize the potential of this test method.

WBOB have migration of bands on same gel of about 10 kdas.

Unable to have exact location.

- Dearborn neg results are impossible for commercial labs to be precise on bands -

- One individual specimen in high level lab. can be done.

- Freq of bands - **

10 most common bands - Pediatrics
NOT dearborn band

Cross Reactive - EBA
- JRA
- Pre Surgical Vol. adequate

Clinical Evaluation of the Borreliacidal Antibody Test

Steve Callister, PhD
Gunderson Lutheran Medical Center

Serodiagnosis is often the only viable option for confirming Lyme disease. The CDC currently recommends a two-tiered serodiagnostic approach consisting of a nonspecific immunofluorescence assay (IFA) or enzyme-linked immunosorbent assay (EIA) screening test followed by confirmation of equivocal of positive results by Western blotting (WB). This approach can be accurate when testing patients with a high pretest probability of Lyme disease. Because of a lack of specificity, however, WB may be inaccurate, especially when testing patients with atypical clinical symptoms. Borreliacidal (lethal) antibodies are a highly specific immune response induced by infection with *B. burgdorferi*. We determined the ability of a flow cytometric borreliacidal antibody test (BAT) to confirm Lyme disease in patients being evaluated during 1998 in a primary-care practice and compared the results with those obtained using WB.

Both procedures correlated exactly ($k = 1.00$) when confirming *B. burgdorferi* infection in patients with Bell's palsy or Lyme arthritis. However, the BAT was significantly more sensitive (79% vs. 65%, $p = .090$) during early Lyme disease, especially when patients had a single erythema migrans lesion and other symptoms (93% vs. 67%, $p = .021$). The potential misdiagnosis that could result from the decreased sensitivity and lack of specificity of the WB were apparent when sera from patients with atypical symptoms were evaluated. Eleven BAT positive early Lyme disease patients would have been missed since an antibody response sufficient to yield positive WB results had not developed.

In addition, 14 patients would have been erroneously diagnosed with Lyme disease since the positive WB results could not be confirmed by the BAT. Increased use of the BAT, especially when testing patients with less obvious symptoms, would solve many of the problems that have confounded serodiagnostic testing for Lyme disease.

Serodiagnosis of Lyme Disease With the C6 Peptide

Mario Philipp, PhD
Tulane University Medical Center
Tulane Regional Primate Research Center, Department of Parasitology

Lyme disease is caused by genetically divergent spirochetes including three pathogenic genospecies: *Borrelia burgdorferi*, *sensu stricto*, *Borrelia garinii* and *Borrelia afzelli*. *B. burgdorferi* sensu stricto is the pathogenic species in the US. In Europe all three species are found, a fact that complicates serodiagnosis. A synthetic peptide (C6) based on the invariable region IR6 of VlsE, the "antigenic variation" antigen of *B. burgdorferi*, was used as an ELISA antigen to assess its diagnostic potential. With serum samples collected from US patients, the sensitivities for acute, convalescent and late disease-phase specimens were 74% (29/39) 85-90% (34/40-35/39) and 100% 59/59), respectively.

Specificity was assessed with 77 serum samples from patients with other spirochetal or chronic infections, autoimmune or neurologic diseases, and 99 serum specimens from hospitalized patients of a nonendemic area for Lyme disease. Only two potential false positives from the hospitalized patients were found and the overall specificity was 99% (174/176). Sera collected from mice experimentally infected with the three *Bb* genospecies and from European patients with Lyme disease also were assessed. Regardless of the infecting genospecies, mice produced a strong antibody response to C6, indicating that IR6 is antigenically conserved among the pathogenic genospecies. 20 of 23 patients with culture-confirmed erythema migrans had detectable antibody response to C6. A sensitivity of 95.2% was achieved with sera collected from patients with well-defined acrodematitis chronica atrophicans. 14 of 20 patients with symptoms of late Lyme disease also had a positive anti-C6 ELISA. Antibodies elicitied by the OspA vaccine did not cross-react with C6. Thus, it may be possible that C6 may be used to serodiagnose Lyme disease worldwide, regardless of patients' OspA vaccination status.

Immune Complex Serodiagnosis

Steve Schutzer, MD
University of Medicine and Dentistry of New Jersey

Host Targeted Acaricidal Treatments on Rodents to Control Densities of *Ixodes Scapularis* Subadult Ticks

Edward Bosler, PhD
SUNY at Stony Brook School of Medicine

Historically, tick abatement has consisted of a variety of chemical and non-chemical methodologies. Methods to manage *Ixodes scapularis* have largely relied on either habitat modification (e.g., controlled burns) or applications of acaricides.

Several studies demonstrate the efficacy of habitat spraying of commercial pesticides to reduce densities of *I. scapularis*. While effective, these types of applications may kill non-target organisms and pose environmental hazards. Host-targeted abatement procedures reduce both the risk of pesticide exposure to non-target species and usually require less chemical for efficacious results than area wide spraying. Initial host-targeted attempts to kill immature *I. scapularis* ticks relied on the nesting behavior of *Peromyscus leucopus* through the use of acaricide (Permethrin) impregnated cotton balls (Damminix).

Results from several studies have met with variable and conflicting success. In 1988 we began testing the utility of Damminix over a 6-year period. Use of the product resulted in an immediate and dramatic decline in subadult ticks on mice and mice collected from treated areas remained tick free following annual treatments. Free ranging nymphal ticks were collected annually on all study sites to determine product efficacy. Questing nymphs in treated areas were consistently lower than in non-treated areas. However, during most years *Borrelia burgdorferi* nymphal infection rates were higher in treated areas indicating other mammals function in maintaining both the tick and spirochete cycles in nature.

A common approach to treating wildlife with pesticides has been to attract them to bait stations containing the chemicals. In 1995 we modified Tomahawk traps of varying sizes to serve as bait stations wherein permethrin was mechanically dispensed to topically treat mammals other than mice. Our preliminary data strongly suggested that treatment of other mammals in addition to mice will produce a significant reduction in tick densities. Due to the cost of the mechanical sprayers we have attempted to develop non-mechanical (passive) bait stations to treat mice and medium sized mammals. To date passive bait stations have not proven to be as efficacious as the mechanical system. An historical overview of all our host-targeted studies and data will be presented.

Community Prevention Programs: New Technologies in Tick Control

Kirby Stafford, PhD
Connecticut Agricultural Experiment Station

Objective: Lyme disease continues to increase with a record number of cases in Connecticut in 2000. Prevention of tick-associated diseases has focused on education, tick-bite prevention, and residential tick control. The CDC, through cooperative agreements, has shifted the emphasis on basic research of tick control methods to supporting community-based programs and integrated tick management to try to reduce the incidence of Lyme disease. Host-targeted methods are being evaluated in community-based programs with CDC and USDA support

Methods: Target Lyme disease is a pilot educational-research program aimed at teaching residents about ways to reduce ticks and protect them selves from Lyme disease. The project is funded through a cooperative agreement with the CDC and the Connecticut Department of Public Health, the Connecticut Agricultural Experiment Station, and the Westport Weston Health District. Host-targeted approaches offer the potential to reduce tick populations on a community scale. With a grant from the USDA, the control of *Ixodes scapularis* on white-tailed deer using a USDA-patented passive topical treatment device known as a 4-poster is being evaluated in 5 states in the Northeast. A fipronil-based bait box system for the treatment of white-footed mice and other rodents was developed by the CDC and was tested initially in Colorado and then for two years in a Connecticut community.

Results & Conclusions: Target Lyme disease has provided a beginning model for community-based Lyme disease prevention programs. Project activities include Lyme disease and tick surveillance, a demonstration site to illustrate landscape management techniques for tick control and a consumer education campaign on proper pesticide use for tick control, training on tick ecology and control strategies for licensed pesticide applicators and landscape professionals, and outreach to the community and involvement of community groups on Lyme disease prevention. Host-targeted approaches can be effective in reducing ticks feeding on deer and mice and may provide tick control on a neighborhood or community-wide scale.

Although there was low usage of the 4-posters by deer for a year or two at some sites because of heavy acorn production, utilization of the devices has been generally high (> 90%) during 3 years of the 5-year project. Following some adjustments to the delivery of the amitraz pesticide, tick control on the deer has been good and, in Connecticut, nymphal tick populations have declined by > 60% in the treated core area relative to the control community. The topical treatment of rodents with fipronil can provide virtually 100% tick control for over 7 weeks and has been found to protect mice from infection with *Borrelia burgdorferi*. The system has been patented by Aventis and current trials with the fipronil bait box will be expanded in Connecticut in 2001.