Lyme Center Projects

Dr. Ralph Garruto
Binghamton University
Epidemiology

Amanda Roome: Tentative Coordinator


Project 7. Garruto. Lyme Disease in Built Environments: The Neighborhood Project


Project 11. Sabounchi. Modelling the Risk of Lyme Disease in Built Environments

Project 13. Shepherd. Reproductive Physiology of Ticks


Project 19. Spathis. White-tailed Deer and Dogs as Sentinels for Pathogenic Ehrlichia Species

Diagnostics

Wes Kufel: Coordinator

Project 2. Kufel. Association of Serum Procalcitonin Concentration in Acute and Chronic Lyme Disease


Project 10. Shamoon-Pour. Optimization of A Diagnostic Protocol for Early Lyme Infection


Project 18. Garruto. Sequestration of Borrelia burgdorferi in the competent reservoir host Peromyscus leucopus and correlation with organ specific human clinical symptoms in Post-Treatment Lyme Disease Syndrome Patients.

Project 20. Darcy. Fourth Stage of Lyme Disease: diagnosis-treatment interval hypothesis

Public Health – policy, education

Sarah Lynch: Coordinator

Project 5. Roome. Lifestyle Impacts and Population Health: Impacts of Acute and Chronic Lyme Disease

Project 3. Roome.  Health Agencies and Impact on Acute and Chronic Lyme Disease


Project 17. Lynch.  Policy changes in New York State to enable prevention of Lyme disease.
Project 1:

Title: **Acarological Risk of Lyme Disease in areas of High Human Activity in the Upper Susquehanna River Basin of New York**

Amanda Roome, Department of Anthropology, Biological Anthropology

**Background:** The Centers for Disease Control and Prevention currently estimates 300,000 new cases of Lyme disease annually with 95% of cases reported from 14 states in the Northeast and Upper Midwest regions of the United States. The highest numbers of reported cases of Lyme disease come from five highly endemic counties in the upper and mid-Hudson Valley regions of New York State. Broome, Chenango, Delaware, Otsego, Tioga, and Tompkins counties, that make up the Upper Susquehanna River Basin of New York, are directly adjacent to the Hudson Valley and have recently seen a steady increase in the number of reported Lyme disease cases. It is important to determine the prevalence of infection and spatial distribution of ticks to enhance the understanding of human risk within the Upper Susquehanna River Basin, an area which directly borders the Hudson River Valley, where tick densities and infection prevalence are well documented, and are alarmingly high. These data may assist public health officials in creating and implementing public health prevention and mitigation strategies. Amanda Roome and The Binghamton University Lyme and Other Tick-Borne Diseases Research Team is well equipped to address this problem, as they have been collecting data on tick spatial distribution and infection prevalence since 2012, finding that 1 in every 3 ticks is harbors *Borrelia burgdorferi*, the causative agent of Lyme disease.

**Research Hypothesis:** We hypothesize that tick density and pathogen prevalence will be high within the six county region of the Upper Susquehanna River Basin, suggesting a high acarological risk for the transmission of Lyme disease to humans.

**Specific Aims and Methodology:**

To test this hypothesis, we will use one specific aim: **Establish the prevalence of infection with *B. burgdorferi* and spatial distribution of *I. scapularis* in the six county region of the Upper Susquehanna River Basin of New York State.** By determining the prevalence of infection and spatial distribution to ticks, we will gain a better understanding of the human risk of contracting Lyme disease within the Upper Susquehanna River Basin, an area which has been very under-studied, despite its close proximity to the Hudson River Valley, an area with the highest incidence of human Lyme cases in the country.

1. **Specific Aim 1** will operate under three sub-aims:
   a. **Aim 1A. Experiment 1: Tick Collection.** Ticks will be collected from state and county parks that encompass an assortment of ecological niches surrounded by residential, commercial and woodland areas and are areas with high human activity. *I. scapularis* ticks are collected by dragging a 1m² white corduroy cloth over low lying vegetation and leaf litter from one-meter parallel to walkways. Ticks are placed into sterile cryovials containing 70% ethanol and stored at -20°C until processing.
   b. **Aim 1B. Experiment 2: Pathogen Analysis.** Ticks collected from each site will be identified microscopically and catalogued based on life cycle stage, sex, and location. DNA will be extracted from each tick and the presence of *B. burgdorferi* will be assessed using pathogen specific primers.
c. Aim 1C. Experiment 3: Determine Acarological Risk. Density of infected ticks will be determined by calculating the number of infected ticks within the total area dragged per 1000m², revealing the acarological risk of infection in high human activity areas. We anticipate there to be a high density of infected ticks near walkways of high human use within state and county parks in the Upper Susquehanna River Basin. Data gathered from this study will aid public health officials in targeting public health prevention and mitigation strategies in the future.

References:


Project 2:

Title: Association of Serum Procalcitonin Concentration in Acute and Chronic Lyme Disease

Wesley Kufel, School of Pharmacy & Pharmaceutical Sciences, Pharmacy Practice
Sarah Lynch, School of Pharmacy & Pharmaceutical Sciences, Pharmacy Practice

Background: Lyme Disease is caused by a spirochete bacterium, *Borrelia burgdorferi*. Procalcitonin, erythrocyte sedimentation rate (ESR), and C-reactive protein (CRP) are common biomarkers used in the clinical setting to assist in the diagnosis and treatment response monitoring of various bacterial infections. These biomarkers differ considerably in their sensitivity, specificity, and time of onset in response to a bacterial infection. Of these biomarkers, procalcitonin is the most sensitive and specific for bacterial infections with a faster onset after initial insult. Sensitivity and specificity vary depending on the site and severity of infection. Currently, only one study by Lotric-Furlan and colleagues in 2002 investigated serum procalcitonin concentrations in 50 patients infected with Lyme Disease, and found that procalcitonin concentrations were normal in the majority of patients. However, this is a single-center study from over 15 years ago. Procalcitonin is more widely used in clinical practice and confirmation of a single study’s results is warranted.

Research Question: Are serum procalcitonin concentrations elevated in patients with acute and chronic Lyme Disease to aid in diagnosis and do procalcitonin concentrations decrease over time to monitor treatment response?

Specific Aims:
1. Determine serum procalcitonin concentrations in patients with acute and chronic Lyme Disease
   a. Determine reactivity in blood samples from mice
   b. Determine reactivity in blood samples from humans
2. Determine serum procalcitonin concentration after Lyme Disease treatment with doxycycline
   a. Determine treatment response in blood samples from mice
   b. Determine treatment response in blood samples from humans

Approach and Methods:
1. Determine time, effort, and money of investigators
2. Obtain ticks with *Borrelia burgdorferi*
3. Obtain mice blood samples infected with *Borrelia burgdorferi*
4. Obtain human blood samples infected with *Borrelia burgdorferi*
5. Obtain procalcitonin biomarker for research purposes
6. Laboratory space and resources with Yetrib’s lab and work in biomarkers
7. Partnership with United Health Services (UHS) and Upstate Medical University for access to potential samples, serum procalcitonin test, and microbiology expertise

Potential Investigators:
Wesley Kufel, PharmD, BUSOPPS Department of Pharmacy Practice
Sarah Lynch, PharmD, BUSOPPS Department of Pharmacy Practice
Eric Hoffman, PhD, BUSOPPS Department of Pharmaceutical Sciences
Yetrib Hathout, PhD, BUSOPPS Department of Pharmaceutical Sciences
Rajesh Dave, MD, UHS Wilson Medical Center
Scott Riddell, PhD, Upstate Medical University, Department of Microbiology
Ralph Garruto, PhD, Binghamton University, Department of Anthropology

References:
Project 3:

Title: *Impact of Health Agencies Intervention on Control of Lyme Disease*

Amanda Roome, Department of Anthropology, Biological Anthropology

**Background:** Access to proper health care and the illness burden of Lyme disease have been studied before; in a survey of over 2,000 participants, nearly half of the respondents reported seeing at least seven physicians before being diagnosed with Lyme disease. This study concluded that Lyme patients often have a delayed diagnosis, further complicating long term problems with chronic Lyme disease and simultaneously creating a very heavy disease burden which greatly impacts social and financial well-being. There have yet to be studies directly on health agencies, such as county health departments to assess trends in emerging infectious disease responses. The Upper Susquehanna River Basin is adjacent to the Hudson River Valley, which has some of the highest incidence rates of Lyme disease in the country. However, counties within the Hudson River Valley are seeing a decrease in Lyme incidence over the last 5 years, while counties within the Upper Susquehanna River Basin are seeing a drastic increase. **It is imperative to understand the public health responses and to see trends or differences between county, in relation to case counts of Lyme, to be able to determine if certain interventions were more or less effective than others.** Understanding the current public health prevention and mitigation strategies, and their efficiency are important to combating the growing problem of Lyme disease in the Northeastern United States. Amanda Roome has been in contact with county health departments over the past several years to share preliminary data gathered on tick spatial distribution and infectivity rates throughout the region.

**Research Hypothesis:** We hypothesize that the type and intensity of prevention and mitigation strategies taken by health agencies has a large influence on Lyme disease incidence rate and health outcomes.

**Specific Aims and Methodology:**
Determine the response by regional health agencies to Lyme disease prevention and treatment. Data gathered will provide insight into trends in public health interventions and subsequent health outcomes, providing a framework to increase mitigation and prevention strategies through education, awareness and interventions that may lead to better treatment and management options.

1. **Specific Aim 1** will operate under one sub-aim:
   a. **Aim 1A.** Experiment 1: Interviews with Public Health Agencies. Data will be gathered through sit down interviews at county health departments. Interviews will be conducted with representatives from six county health departments in the Upper Susquehanna River Basin (Broome, Chenango, Delaware, Otsego, Tioga and Tompkins) and six from the Hudson River Valley (Columbia, Dutchess, Green, Orange, Putnam and Ulster). Health departments will be asked questions regarding the threat of Lyme disease within their county. They will be asked to discuss when Lyme disease became a public health threat in their county, what their initial response was, what their continued response is and how it may have changed over time, and what interventions were the most and least effective.
We anticipate that health agencies responses to the growing problem of Lyme disease will have a major influence on health outcomes. Data gathered from the interviews will provide insight into trends in public health interventions and subsequent health outcomes.

Project 4:

Title: *Improvements in Diagnostic Testing and Treatment Response Monitoring for Lyme Disease*

Wesley Kufel, School of Pharmacy & Pharmaceutical Sciences, Pharmacy Practice
Sarah Lynch, School of Pharmacy & Pharmaceutical Sciences, Pharmacy Practice

**Background:** Current Lyme Disease diagnostic testing leads to false positive results over 50% of the time. There is a critical need to simplify the testing algorithm for Lyme disease, improving sensitivity in early disease while still maintaining high specificity and providing information about the stage of infection as well as response to initial therapy. Many similarities exist between Lyme Diseases and syphilis. Both are caused by spirochete bacteria, exhibit various stages and classifications of disease state progression, exhibit ability to infect the central nervous system, and are responsive to similar antibiotic therapy with doxycycline. A comparative summary of Lyme Disease and syphilis diagnosis and response to treatment are displayed in Table 1.

<table>
<thead>
<tr>
<th><strong>Table 1. Comparison of Lyme Disease and Syphilis Diagnosis and Response to Treatment.</strong></th>
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<tr>
<td><strong>Lyme Disease</strong></td>
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| Current Diagnostic Testing (false positive results possible in certain scenarios for both Lyme Disease and Syphilis) | Two Step Approach to Diagnosis:  
- Enzyme immunoassay (EIA – IgM and IgG)  
- If positive, then confirm with Western Blot | Two Tests for Diagnosis:  
- Treponemal antibody test (TP-PA), once positive, remains positive for life  
  ○ Does not provide quantifiable results (yes/no to ever having syphilis)  
- Non-treponemal tests (Venereal Disease Research Laboratory test and rapid plasma reagin)  
  ○ Active syphilis if RPR is positive  
  ○ RPR provides titer to identify burden of disease  
  ○ RPR sensitivity: 78-86%  
  ○ RPR specificity: 85-99%  
*Generally institutions perform both of these tests in order to ensure accurate results |
| Response to Treatment Determination | ● Symptom resolution | ● Symptom resolution  
● Four-fold reduction or two-fold dilution in RPR titer for treatment response at 6-12 months |

**Research Question:** What is the utility in using RPR, or a similar diagnostic test specifically for Lyme Disease, for diagnosis and determination of response to treatment for Lyme Disease?

**Specific Aims:**
1. Determine the impact of non-treponemal RPR diagnostic test on Lyme Disease infected mice samples
   a. Determine reactivity and titer response for RPR diagnostic test on blood samples
   b. Determine reactivity and titer response for RPR diagnostic test on tissue samples
   c. Determine if the time period from time of infection with *Borrelia burgdorferi* impacts RPR reactivity and titer

2. Determine the impact of non-treponemal RPR diagnostic test on Lyme Disease infected human samples
   a. Determine reactivity and titer response for RPR diagnostic test on blood samples
   b. Determine reactivity and titer response for RPR diagnostic test on tissue samples
   c. Determine if the time period from time of infection with *Borrelia burgdorferi* impacts RPR reactivity and titer

3. Develop a Lyme Disease specific RPR diagnostic test for use on blood samples
   a. Determine sensitivity and specificity of diagnostic test
   b. Determine quantitative titer response to acute Lyme Disease
   c. Determine quantitative titer response to chronic Lyme Disease
   d. Determine quantitative titer response following treatment with doxycycline to monitor response to Lyme Disease treatment

**Approach and Methods:**
1. Determine time, effort, and money of investigators
2. Obtain ticks with *Borrelia burgdorferi*
3. Obtain mice tissue and blood samples
   a. Samples infected with *Borrelia burgdorferi*
   b. Samples not infected yet - infect using ticks
4. Obtain human tissue and blood samples infected with *Borrelia burgdorferi*
5. Obtain nontreponemal RPR syphilis diagnostic test for research purposes (already exists - would need access to one)
6. Laboratory space and resources with Raju's lab and work in immunology
7. Partnership with United Health Services (UHS) and Upstate Medical University for access to potential samples, RPR diagnostic test, and microbiology expertise

**Potential Investigators:**
Wesley Kufel, PharmD, BUSOPPS Department of Pharmacy Practice
Sarah Lynch, PharmD, BUSOPPS Department of Pharmacy Practice
Eric Hoffman, PhD, BUSOPPS Department of Pharmaceutical Sciences
Kanneboyina Nagaraju, PhD, BUSOPPS Department of Pharmaceutical Sciences
Rajesh Dave, MD, UHS Wilson Medical Center
Scott Riddell, PhD, Upstate Medical University, Department of Microbiology
Ralph Garruto, PhD, Binghamton University, Department of Anthropology

**References:**
Project 5:

Title:  
Lifestyle Impacts and Population Health: Impacts of Acute and Chronic Lyme Disease

Amanda Roome, Department of Anthropology, Biological Anthropology

Background: Lyme disease is commensurate with other findings of other infectious disease, where human behaviors in combination with biological and ecological variables all mediate the transmission and propagation of disease. Many individuals infected with Lyme disease are unaware of tick bites, with 90% of cases diagnosed during the summer when ticks are in their small nymphal stage, no larger than the size of a poppy seed. The CDC reports that 10-20% of acute Lyme patients who are treated continue on to develop Post-Treatment Lyme Disease Syndrome (PTLDS) where chronic symptoms develop and continue to persist. As the incidence of Lyme disease is rapidly increasing, so are PTLDS cases. By gaining an understanding of the long term impact on patient’s lifestyle (physical and social activity, dietary, emotional and sleep patterns, and overall health), public health and health care professionals can assist in developing better treatment, prevention and mitigation strategies in the future.

Amanda Roome is well equipped to address this problem, as she has been working closely with Lyme patients for the past 3 years within the highly endemic Northeast region of the U.S. and has given multiple scientific research presentations regionally and nationally regarding her previous research throughout the region.

Research Hypothesis: We hypothesize lifestyle changes will be more drastic in individuals with Post-Treatment Lyme (PTLDS) than acute Lyme disease, and that the degree of change will be positively correlated with symptom duration.

Specific Aims and Methodology:
To test this hypothesis, we will use one specific aim: Determine the impact of Lyme disease on the health and lifestyle of patients in the Northeastern United States. Data gathered from this survey will provide insight into how people adapt or adjust to the emergence of Lyme disease and what lifestyle changes occur with duration of their symptoms. It is anticipated that this research will lead medical professionals to better understand the impact of long duration disease symptoms on quality of life.

1. Specific Aim 1 will operate under one sub-aim:
   a. Aim 1A. Experiment 1: Survey Data Collection. Data will be gathered through a public survey. This survey will involve three groups of participants; individuals that have never been diagnosed with Lyme disease, individuals that have been diagnosed with acute Lyme disease, and individuals that have been diagnosed with Post-Treatment Lyme Disease Syndrome (PTLDS) by a physician. All participants will fill out a self-administered online survey questionnaire which will assess demographics and lifestyle patterns (physical and social activity, dietary, emotional and sleep patterns, and overall health, and prevention measures for tick bites). Individuals that have had confirmed Lyme disease previously (acute or PTLDS) will continue on to a second portion of the survey which will address their Lyme diagnosis, treatment, symptoms, duration, lifestyle patterns (physical...
and social activity, dietary, emotional and sleep patterns, overall health, and measures used to prevent tick bites) both before and after their Lyme disease diagnosis.

It is expected that Post Treatment and Chronic Lyme patients will have a significantly lower quality of life and more mental and physical health problems than acute Lyme patients. Data gathered from this survey can provide crucial insight into how people suffer from and adapt to Lyme disease symptoms and how their lives are changed, informing future medical intervention and public health prevention and mitigation strategies, as well as prompting new studies on the early diagnosis and treatment of the disease.

References:

Project 6:

Title: Providing evidence for efficacy of acute doxycycline prophylaxis

Sarah Lynch, School of Pharmacy & Pharmaceutical Sciences, Pharmacy Practice

Background: Lyme Disease is widespread across the United States. Current guidelines from the Infectious Disease Society of American recommend prophylactic treatment with 200mg Doxycycline at the sign of a tick bite in populations who meet the following criteria:¹

- Adults and children ≥8 years' old
- Adult tick with likely attachment for >36 hours
- Ability to start antibiotic within 72 hours of tick removal
- Bite occurring in a region with ≥20% of ticks infected with B. burgdorferi
- Doxycycline treatment is not contraindicated

Doxycycline is a prescription only tetracycline antibiotic. Barriers to utilizing this prophylactic treatment can include making an appointment with a provider and filling a prescription within the 72-hour window after tick removal. Rhode Island pharmacies have been establishing the first pharmacist-prescribed antibiotic prophylaxis available to eligible patients.² A collaborative practice agreement between a pharmacist and local infectious disease physician establishes the treatment protocol which pharmacists follow in order to prescribe and dispense the antibiotic.

Additionally, although this prophylaxis is guideline based, there is very little evidence suggesting it is effective. The guidelines are based primarily on a 2001 NEJM study.³ This placebo controlled trial enrolled 506 individuals between 1987 and 1996 and administered Doxycycline or placebo to those who presented with a bite from an Ixodes Scapularis tick and had kept the tick to be sent in for analysis. The primary critique of this study is that the end point was development of an erythema migrains rash within 6 weeks after treatment with Doxycycline or placebo; the erythema migrains rash has been reported to occur in anywhere from 20-80% of B. burgdorferi infections, making this sign a poor predictor of incidence.⁴ In addition, although ticks were analyzed, they were not tested for B. burgdorferi, making it unclear what the actual incidence of possible transmission could have been in this study.

Research Idea: Development of community pharmacist prescribed Doxycycline for Lyme Disease prophylaxis in New York State.

Specific Aims:

1. AIM 1: Develop and assess a community pharmacy practice protocol to prescribe Doxycycline for Lyme Disease prophylaxis.
   a. Gather statistics on service utilization
   b. Administer surveys to local pharmacists, physicians and patients to gather opinions on their support for this practice
   c. Use the successful results to support need for greater role of the community pharmacist in prescribing???
2. **AIM 2: Determine efficacy of community pharmacy based doxycycline prescribing in preventing infection with Lyme Disease**
   a. Submit ticks for testing for *B. burgdorferi* to determine possible infection rates
   b. Follow up with individuals for up to one-year post-treatment and administer survey to gather information about s/sx of chronic Lyme Disease
   c. Perform serologic testing to treated individuals at various points following treatment to track development of Lyme Disease

**References:**
Project 7:

**Title:** *Lyme Disease in Built Environments: The Neighborhood Project*

*Ralph Garruto, Department of Anthropology, Biomedical Anthropology*
*Chelsea Clark, Department of Biological Sciences, Biology*
*Matt Goldman, Department of Biological Sciences, Biology*
*Bryan Levine, Department of Biological Sciences, Integrative Neuroscience*
*Andrew Wagner, Department of Biological Sciences, Biochemistry*
*Amanda Roome, Department of Anthropology, Biomedical Anthropology*

**Background:** Lyme disease is the most prevalent tick-borne disease in the United States, affecting an estimated 300,000 people each year. It is spread to humans through the bite of an *I. scapularis* tick infected with *B. burgdorferi*, the causative agent of Lyme, and can significantly impair the quality of life of those infected. This study seeks to determine the density and infectivity of *Ixodes scapularis* ticks, along with risk factors associated with Lyme in urban and peri-urban residential areas in the Southern Tier region of New York, now endemic for Lyme disease and compare residential risk to non-residential areas such as State Parks in rural areas of upstate New York.

**Research Hypothesis and Aims:** This Project’s overall aim is to assess the risk of Lyme and other tick-borne diseases in fragmented ecosystems, specifically suburban and urban neighborhood households, for comparison with surrounding more rural areas, by collecting the black legged tick and analyzing them for the infectious agent for Lyme and other tick-borne diseases. *We hypothesize that the risk of Lyme and other tick-borne disease in residential areas to be equal to or higher in comparison to more rural environments with less human activity in the 6 county region of the Upper Susquehanna River Basin.* We expect that the risk of Lyme disease is a direct threat to human health in areas people spend most of their time living, eating, recreating and working in and around the home.

1. **Specific Aim 1:** Collect and test ticks for *B. burgdorferi*, the infectious agent for Lyme disease. Houses are randomly chosen from volunteer residents living in Broome County, New York. The collection method includes dragging and measuring the perimeter of the property, house, vegetation, and 5 meter cross sections of the lawn with a 1-meter square corduroy cloth, which the ticks attach to. Ecological characteristics of the property are also noted, including vegetation types, whether neighborhood property backs onto wooded areas, etc. Identification of the pathogen is through standard isolation and purification of pathogen DNA and Real Time PCR.

2. **Specific Aim 2:** Test all ticks for co-infectious agents for babesia and human anaplasma using the methods in Aim 1 above.

**Future aims** of the study include specific vegetation analysis of each household and its influence on Lyme disease risk, analysis of tick feeding and molting times on prevalence of ticks on neighborhood household properties, collection of the competent reservoir host *P. leucopus* to determine density and infectivity levels by household as well as expansion of the project to the all 6 counties that make up the Upper Susquehanna River Basin in southcentral New York.
Title: Development of a Diagnostic Protocol for Tick-Borne Co-infections

Michel Shamoon-Pour, Department of Anthropology, Molecular and Biomedical Anthropology
Rita Spathis, Department of Anthropology, Anthropology

Background:
Lyme Disease is one of the fastest growing infectious diseases in the United States, with incidence rates of up to 80 per 100,000 residents in the Northeastern states. The tick bite in an estimated 10% of these individuals also leads to transmission of one or several other tick-borne diseases (Knapp and Rice, 2015). This might be a conservative estimate, given that online surveys put the co-infection rate at over 30% (Johnson et al., 2017). Nevertheless, the rate of these infections in both humans and black-legged ticks is growing, and some of these infectious agents are feared to eventually occupy the same distribution of Borrelia burgdorferi, the Lyme Disease bacteria.

While co-infection with many of these agents is left underdiagnosed in patients, tick studies reveal the presence of more than a dozen infectious agents in ticks collected in the United States (Nelder et al., 2016). The human pathogens most commonly coinciding with B. burgdorferi are: Babesia microti, Batronella spp., Anaplasma Phagocytophilum, Ehrlichia muris-like species, and Powassan virus (POWV). While these pathogens cause well-described diseases in human (babesiosis, human granulocytic anaplasmosis, etc…) their coinfection with Lyme also leads to an increase in intensity and frequency of Lyme Disease symptoms.

Research Idea:
This proposal aims to address the lack of an effective testing protocol for tick-borne co-infections. When co-infected with Lyme bacteria, other tick-borne infections often go unnoticed, and consequently untreated. The sharp increase of these infections in the US in the past two decades call for better diagnostic protocols for detection of Lyme and other tick-borne human diseases.

Specific Aims
1. Identifying incidence of Lyme, anaplasmosis and babesiosis in Southern Tier region.
   1.1 Identifying the incidence of tick-borne infections in Southern Tier region: This goal will be achieved by testing blood samples from tick-bitten individuals using the existing quantitative PCR protocols for the three infecting agents.
   1.2 Identifying the prevalence of co-infection in tick populations of Southern Tier region: This goal will be achieved by testing tick samples using the existing quantitative PCR protocols for the three infecting agents that includes a) ticks brought by the bitten individuals and b) from existing ticks (collected by our laboratories).
2. Developing effective multiplex genetic testing for Lyme, anaplasmosis and babesiosis. Suboptimal sensitivity and/or specificity of available genetic tests calls for development of more effective tests. A multiplex quantitative PCR protocol will be optimal for fast and cost-effective evaluation of samples for medical purposes. Our lab
has adapted several genetic approaches to testing for Lyme infection and has the capability to develop tests with higher sensitivity and specificity. Furthermore, our archive of tick and white footed mouse specimens (the reservoir host) provides an excellent source of samples that can be utilized for development and optimization of genetic tests.

2.1 Development of a highly sensitive and specific quantitative PCR test
2.2 Evaluation of the newly-developed tests: Sensitivity and specificity of our protocol will be evaluated in comparison with the existing protocols. This goal will be achieved by applying the newly-developed protocol to test the same set of samples described under aim#1.

References:
Project 9:

Title: Systems Dynamics, Modelling and GIS Mapping of Lyme Disease

Nasser Sharareh, Department of Systems Science and Industrial Engineering, Systems Science and Industrial Engineering

Background: Lyme disease was first discovered in Long Island on New York State and since that time it has spread throughout the Northeastern region of the U.S. However, public awareness towards Lyme disease and the deer tick or black-legged tick, the vector of Lyme disease bacterium (*Borrelia burgdorferi*) is insufficient and people show risky behaviors in tick-infested areas. In addition, healthcare organizations and health departments do not implement suitable interventions to impede the increasing prevalence of Lyme disease. This issue is complex because of several factors, including demographic, spatial, and behavioral factors that are interacting with each other. Therefore, there is a need for a systems thinking approach to study this problem in order to design appropriate mitigation and prevention strategies to help ameliorate the Lyme disease epidemic. Hence, as an engineer, who is experienced with simulation modeling, data analysis, and GIS mapping, I would like to develop simulation models that propose the best interventions that can control this epidemic. Below is our initial research publication, findings and our first modelling attempt:

Research Hypothesis: Based on the research we have completed over the past two years, our hypothesis is that the number of Lyme disease cases will continue to grow due to the forest fragmentation in built environments with high human activity in the Northeast U.S. leading to an increase in small rodents, the primary reservoir host for Lyme disease, and in the deer tick population (the vector for Lyme), combined with a low level of public awareness and situational awareness.

Specific Aims:  
1. System Dynamics Simulation Modeling
   The model would present the interaction between demographic, environmental, behavioral, and spatial factors that are creating the final behavior of the system.  
2. Integration of the Simulation Model with GIS Mapping
   This approach would be of benefit by providing a dynamic GIS map which would illustrate the influence of spatial factors on Lyme disease epidemic.

Future Aims: I plan to expand our current simulation model to broader geographical areas of the U.S. and validate the model for other states. In addition, I am interested in investigating the influence or impact of spatial factors, including the location of providers and healthcare centers, on the incidence of Lyme disease in the U.S.

References:  
Project 10:

Title: Optimization of A Diagnostic Protocol for Early Lyme Infection

Michel Shamoon-Pour, Department of Anthropology, Molecular and Biomedical Anthropology
Rita Spathis, Department of Anthropology, Anthropology

Background:
Lyme Disease is one of the fastest growing infectious diseases in the United States, with its incidence rate ranging from 13 to 80 per 100,000 residents in the Northeastern states. Lyme infection can lead to chronic Lyme Disease with prolonged symptoms and risk of disability and death. Despite the high health impact of Lyme Disease, current diagnosis laboratory tests lack in specificity and sensitivity, and there is no effective test available for early diagnosis of infection. Currently, CDC recommends only one laboratory test for Lyme Disease (CDC, 2015). A two-tiered serology protocol, this test suffers from low sensitivity (46%) in diagnosis of early Lyme Disease (Waddel et al., 2016). This approach is especially lacking in sensitivity (14%) during the first week following the onset of erythema migrans (bulls eye rash) (Marques, 2016).

Research Hypothesis:
Given the slow spread of infection from the bite site, the highest sensitivity in diagnosis of borreliosis during the first 2-4 weeks will be achieved by genetic testing of skin biopsy.

Specific Aims:
This proposal aims to address the difficulty of early diagnosis of Lyme infection, particularly during the first 4 weeks on infection. Overall, while a skin biopsy might prove to be most effective, the invasiveness of this approach dictates an attempt to evaluate efficacy of genetic and serological testing of skin, blood and urine samples.

1. Evaluating serological and genetic testing of skin, blood and urine during early stage Lyme.
   Due to invasive nature of skin biopsy, less invasive methods should be evaluated and compared with skin testing. This goal will be achieved by:
   1.1 Collecting skin, blood, and urine samples from individuals bitten with ticks. Blood and urine samples should be collected weekly for the first 8 weeks following first visitation. Skin samples will be collected during the first 3 or 4 weeks. Skin will be tested if individual can identify the location of the tick bite, even if a rash is not present.
   1.2 Testing samples with 2 or 3 most effective serology and genetic protocols.
   1.3 Developing a protocol by identifying the most effective solution(s) during each week (since the bite/potential infection)

2. Specific aim#2: Developing effective Lyme biomarker and genetic diagnostic testing.
   Suboptimal sensitivity and/or specificity of available tests calls for development of better tests. Our lab has adapted several genetic approaches to testing for Lyme infection and has the capability to develop tests with higher sensitivity and specificity. Furthermore,
our archive of tick and mice samples provides an excellent source of samples that can be utilized for development and optimization of biomarkers and genetic tests.

2.1 Developing a highly sensitive and specific quantitative PCR test
2.2 Developing Biomarkers (I leave this to our friends)
2.3 Comparing sensitivity and specificity of the newly-developed tests with the most effective conventional protocols. This will be achieved by applying newly-developed test to the skin, blood and urine samples to be collected in this study.

References:
Title: *Modelling the Risk of Lyme Disease in Built Environments*

Nasim Sabounchi, Department of System Science and Industrial Engineering, Systems Science and Industrial Engineering  
Ralph Garruto, Department of Anthropology, Biomedical Anthropology

**Background:** It is estimated that 300,000 Americans are diagnosed with Lyme disease (LD) each year and is now the fifth most common National Notifiable Disease in the U.S.\(^1\). The disease incidence is increasing and is now endemic in more than 15 states\(^2\) with about 96% of reported cases occurring in the Northeast and upper Midwest\(^3\). This is concerning as LD treatment entails a huge cost, currently $712 million to $1.3 billion per year for the health care system in the United States\(^4\). Studying the impact of human behavior on disease risk, and the barriers to adopting protective measures is key to improve LD prevention\(^5\).

**Research Hypothesis:** The main research objective of this proposal is to test the hypothesis that demographic, social and behavioral factors and increasing awareness and public attention in conjunction with ecological variables will assist in effectively decreasing LD incidence in the US. Our central hypotheses regarding the increased incidence of LD is that: 1) Contrary to previous studies describing higher risk for tick bites in more remote areas with smaller, more dispersed populations\(^6\), our preliminary data show that risk is higher in ecologically fragmented built environments that have high human foot traffic and high human activity\(^7\), 2) low awareness with regards to tick prevalence and its spatial distribution in peri-urban built environments, leads to an increase in LD incidence and 3) As a prevention strategy, raising awareness so that risky behavior is modified leads to decreased contact with infected ticks and a decline in LD incidence. We will address these hypotheses through the following three specific aims:

**Specific Aims:**

1. **Specific Aim #1:** Test the hypothesis that risk for tick bites is high in ecologically fragmented peri-urban built environments with high human foot traffic and human activity. **Experiment 1:** Collect field data and information on vegetation, the LD reservoir (*Peromyscus leucopus*) and the vector, the blacklegged or deer tick (*I. scapularis*). This data will include rodent and tick density and infectivity with *B. burgdorferi*, the causative agent of LD in the Upper Susquehanna River Basin in peri-urban built environments for two consecutive years (a total of 4 years) to account for any seasonal or yearly variation in vector and reservoir populations. Furthermore, we will collect two additional years of observational data on behavioral factors related to pedestrians behaving and perambulating on various walkways at each of the 16 sites. In order to provide support for our hypotheses, field data from the 16 sites within the Upper Susquehanna River Basin should match our 2 years of preliminary findings.

2. **Specific Aim #2:** Test the hypothesis that low awareness with regard to tick prevalence in peri-urban built environments, leads to an increase in LD incidence. **Experiment 2:** Identify and collect social, behavioral and demographic data on human hosts and LD incidence and develop the causal structure and feedback mechanisms that describes how these factors relate to LD incidence. For this purpose, we will collect data during group model building (GMB) sessions to be used in developing a system dynamics
(SD) model, to identify the causal mechanisms that lead to an increase in LD incidence and identify both effective and non-effective prevention strategies. We will have separate GMB sessions with current and former adult LD patients who have undergone treatment and were cured compared to those that have continuing chronic symptoms and also with health officials from the 6 county health departments in the Upper Susquehanna River Basin and physicians and providers from each of the 6 counties. We will implement online anonymous surveys for the general public to collect de-identified data for awareness level about LD.

3. **Specific Aim #3: Test the hypothesis that raising situational awareness as a prevention strategy to prevent risky behavior leads to decreased contact with infected ticks and a decline in LD incidence.** Experiment 3: Identify and compare different prevention strategies for LD using SD simulation modeling and determine the most cost-effective prevention strategy for LD within the 6 county region with a goal towards determining risk of disease in the Upper Susquehanna River Basin region of New York that can be used for other geographic regions in New York, the Northeastern U.S. and potentially the Upper Midwest. For this purpose, we will **refine, simulate and validate** the qualitative model developed in Aim 2 using the data and information collected on the human host, the tick vector, the competent reservoir host (i.e. the white-footed mouse), and the LD crude incidence rates in Aim 1. Using the validated and predictive SD simulation model, we will run “what-if” scenarios to test various strategies with the goal of decreasing the overall prevalence of LD and develop strategies for prevention, accounting for probabilities and rates of disease outcomes according to each prevention strategy. Incorporating the calculated and simulated results, a cost-effectiveness analysis will be conducted.
Project 12:

**Title**: *Shared Resource for Proteomics and Biomarker Studies of Lyme Disease.*

Yetrib Hathout, School of Pharmacy & Pharmaceutical Sciences, Pharmaceutical Sciences

**Specific Aims.** The need for reliable molecular biomarkers for Lyme disease is becoming critical for several reasons: (i) current diagnostic biomarkers are neither sensitive nor specific, especially in detecting early infections (see project, Dr. Kufel and Dr. Lynch), (ii) Biomarkers to assess disease stages/complications and treatment outcomes are lacking, (iii) Characterization of post-Lyme disease biomarkers are also highly important to understand why some patients develop chronic illness even after the infectious agent has been cleared (See project, Dr. Nagaraju), (iv) finally proteome signature of infected versus non-infected ticks and rodent host (white footed mouse) are also important to bring insight into the molecular mechanism of *Borrelia burgdorferi* life cycle and infectivity (See project, Dr. Garruto). We hypothesize that large scale proteome and metabolome profiling on these available samples will lead to the identification of reliable biomarkers for accurate diagnosis as well as biomarkers to assess disease stage and response to treatments and eventually gain insights into novel molecular mechanism of *Borrelia burgdorferi* infectivity and develop innovative treatment strategies in the future. Our proposed aims are as follow:

1. **Aim-1:** Define novel and reliable biomarkers for Lyme disease using high throughput proteome and metabolome profiling. Through collaboration with Dr. Scott Ridell (Director of Microbiology Lab at Upstate University Hospital, Syracuse) we have access to sera samples from infected and non-infected patients. A discovery phase will be performed using cross-sectional sera samples from clinically confirmed cases (n = 10) and age matched non-infected controls (n = 10). Identified biomarkers will be then validated on the entire cohort (n = 136) and tested on sera samples of newly infected patients as they become available. For discovery of serum protein biomarkers, we will use a combination of highly multiplexing TMT technology and LC-MS/MS. Metabolome profiling will be performed on the same set of samples. For both assays we will use our ultimate 3000 nano-LC connected to the high sensitivity and high accuracy Thermo QExactive HF instrument. Identified biomarkers will be validated in all samples and newly collected samples using highly sensitive Meso Scale Discovery Electrochemiluminescence ELISA assay (MSD ELISA).

2. **Aim-2:** Define the Proteome or peptide signature of Borrelia infected ticks and infected host rodent. Through collaboration with the Biological Anthropology Department (Dr. Ralph Garruto, Professor/Director Biospecimen Archive Facility at Binghamton University) we have access to lysates from infected and non-infected ticks as well as sera and tissues samples from infected and non-infected white footed mice. We propose to perform high throughput proteome profiling on these samples using TMT technology and LC-MS/MS to gain insight into the molecular mechanisms involved in *Borrelia burgdorferi* life cycle and infectivity. These peptide signatures might be also targeted in
samples collected from infected patients to develop additional specific diagnostic markers.

3. **Aim-3: Provide specialized support to internal investigators on Lyme disease.** Our facility is committed to help investigators in Lyme disease developing and implementing specific assays for their projects. Dr. Kufel and Dr. Lynch are aiming to evaluate the clinical utility of procalcitonin as a biomarker for treatment response by *Borrelia burgdorferi* infection using serum samples collected from both human and host rodents. We are committed to helping them develop highly sensitive and highly specific MSD ELISA assay. Dr. Nagaraju is aiming to define the molecular mechanisms that lead to chronic inflammation in post-Lyme disease patients. We are committed to help him implement proteomics approach to define these targets using a combination of gel electrophoresis, antibody-antigen reaction and mass spectrometry. And Dr. Garruto project is to ..........

**Experimental Approach and Methods:**

We have all the equipment and expertise in place to achieve the proposed aims. Dr. Hathout is an expert in proteomics method development and biomarker studies. Samples will be collected by his laboratory personnel and stored in workable aliquots at – 80 °C until analysis.

**Biomarker discovery and validation:**

Biomarker discovery will be performed on sera samples from infected human and rodents. Sera samples will be depleted from highly abundant albumin then processed for high throughput multiplexing analysis using Tandem Mass Tag (TMT) technology. This technique was first developed as Isobaric tag for relative and absolute quantitation (iTRAQ) method that could analyze four samples simultaneously and more recently refined by Thermo Fisher as Tandem Mass Tag (TMT) technology to provide highly robust multiplexing analysis able to quantify up to 1,500 proteins in 10 sera samples simultaneously (O'Brien & Timms, 2014). This technique will be highly suitable for analysis of longitudinal samples collected from both patients and rodents.

Validation of newly discovered biomarkers and targeted biomarkers such as procalcitonin will be performed using our MSD platform. MSD technology is a relatively novel electrochemiluminescent multiplex immunoassay platform that uses a carbon electrode surface that has 10 times greater binding capacity than classical polystyrene wells with less background interference when dealing with multiplex assay (Bastarache et al. 2014). Antibodies that show good specificity and less background will be selected to transition to custom made MSD plates. MSD has additional rigorous incoming materials and a QC program to ensure materials are well characterized before being put into kits. These include antibody purity, activity, stability, and specificity. Antibodies will be prioritized depending on their clinical utility in Lyme disease.

**Statistical analyses:** Tools to be used are already established among our biostatistical collaborators Drs. Dang, Xu and Cosler. The biostatistical team has comprehensive and complimentary expertise in all these areas, from both statistical methodological research and its health outcome applications.
Project 13:

Title: Reproductive Physiology of Ticks

Julian Shepherd, Department of Biological Sciences, Biological Sciences

Background: My research interests are in the field of physiology (especially reproduction) and ecology of arthropods, working with moths, ticks and mosquitoes. I am especially interested in sperm maturation, activation, and motility, and the function and fate of sperm in the female reproductive tract. My current research with ticks is now focused on the final maturation of tick sperm in the female reproductive tract, and the mechanism by which they fertilize eggs. Despite 100 years of research, the latter is still a matter of conjecture. I have been working with ticks for over 40 years, have a laboratory colony of 2 species of soft ticks, and the subjects of my research are applicable to all species of ticks.

Research Hypotheses:
1. Movement and final maturation of sperm in the female genital tract are dependent on factors in the female generated by a blood meal. This was the implication of studies reported in Resler et al. 2009 (see below) and needs to be ascertained by isolation of such substances from the female tract.
2. Fertilization takes place in the ovaries of the female. Proposals have been advanced that it occurs in the oviducts, but research by others and in my laboratory have made this seem unlikely, without documenting the actual mechanism of fertilization.

Specific Aims: to investigate the above using anatomical, histological, and biochemical analyses.

Relevant publications:
Project 14:

**Title:** Vegetation Analysis and Lyme Disease Transmission

**Julian Shepherd, Department of Biological Sciences, Biological Sciences**

**Background:** I have been working with Ralph Garruto to enhance his model of Lyme Disease transmission by analyzing dominant vegetation in all of the 6-county sites where his group have collected ticks and mice, analyzed them for disease microorganisms, and monitored human behavior. As a part of a long-term inventory of natural areas in Broome County, I have learned the local flora and habitat analysis, and so feel well-qualified for this project.

**Research Hypothesis:** Habitat will significantly affect tick distribution and tick density, and so vegetation analysis is an essential part of the model.

**Past work:** With the help of several undergraduates, vegetation data on all sites within the upper Susquehanna river basin to date (several hundred) have been collected in the field and entered into a database that is now ready for analysis and available for modelers.

**Current work:** sites for analysis continue to be identified, particularly in some types of habitat that are underrepresented in the initial surveys. Surveys also will continue with the Lyme neighborhood project which just started in summer 2017.

**Anticipated results:** a comprehensive, detailed model of potential Lyme Disease risk and transmission will be developed in a 6-county area of the Southern Tier of New York.
Project 15:

Title: *Molecular mimicry in chronic lyme*

Kanneboyina Nagaraju, School of Pharmacy & Pharmaceutical Sciences, Pharmaceutical Sciences

**Background:** Antibiotic therapy resolves clinical symptoms of Lyme disease in most cases, however, about 5-15% of patients will develop persistent and debilitating residual symptoms such as fatigue, musculoskeletal pain, and cognitive complaints that persists long after antibiotic treatment. This is generally referred as post treatment Lyme disease syndrome (PTLDS). The pathogenesis of PTLDS is currently unclear and requires systematic investigation. PTLDS patients neither respond to retreatment with antibiotic therapy nor have ongoing *Borrelia burgdorferi* infection suggesting other host mechanism such as infection triggered autoimmune response may be responsible for some of the persistent symptoms (Chandra et al., 2010). It has been postulated that some of the PTLDS symptoms could be due to antibacterial (e.g., *Bacterial* outer-surface protein A (OspA)) immune response that cross-react with host self-proteins (autoantigens), commonly called as molecular mimicry. In fact, previous studies have found evidence for several candidate autoantigens such as human lymphocyte function–associated antigen-1, endothelial cell growth factor, apolipoprotein B-100, annexin A2, MMP10, Cytokeratin10, Cardiolipin and Gangliosides in treatment resistant Lyme disease patients and in mouse models (García Moncó et al., 1993; Gross et al., 1998; Drouin et al., 2013; Crowley et al., 2015; Pianta et al., 2015; Crowley et al., 2016). Previous studies have used tandem mass spectrometry, database searches, and manual spectral interpretation to identify MHC class II (HLA-DR)-presented peptides from the synovia of Lyme arthritis patients. While this approach was useful to identify some autoantigens in Lyme arthritis patients however the pathological consequences of immune response (T cells and autoantibodies) remained unclear because of their low affinity interaction with target autoantigens. Further, the scope of this approach is very limited because epitope selection is more dependent on HLA-DR genotype of the individual patient and tissue specific autoantigens are presented in different tissues (e.g., Brain, skeletal muscle, heart). Therefore, we would like to use human serum samples from well PTLDS patients (Dr. Scott Riddell’s lab at Upstate University Hospital Syracuse, NY) using ImmuneProfiler™ human proteome microarray containing >20,000 unique and individually purified proteins covering 81% of the human proteome (16,152 genes). This approach has potential to identify disease specific autoantigens in an unbiased manner.

**Research Hypothesis:** Debilitating symptoms associated with PTLDS are due to immune response to host self-antigens.

**Specific Aims:**

1. **Specific Aim 1.** Identify autoantigens in well-defined cohorts treatment responsive (N=10) and post treatment Lyme disease syndrome (N=10), Arthritis (n=10), Myositis (N=10) and Lupus patients (n=10) using a novel and comprehensive ImmuneProfiler™ HuProt proteome microarray platform.
Rationale. Fatigue, pain, or joint and muscle aches associated with PTLDS are also common features of systemic autoimmune rheumatic diseases such as arthritis, myositis and lupus. Autoantibody titers in rheumatoid arthritis (anti- anti-citrullinated protein antibodies (ACPA)), myositis (anti-Jo-1, anti-SRP) and Lupus (anti-ds DNA) are associated with clinical severity and prognosis of the disease in these patients.

Research Hypothesis: Tissue specific autoimmune response is mediates symptoms associated with organs in PTLDS.

2. Specific Aim 2. Determine PTLDS specific autoantibodies recognize tissue specific autoantigens using protein lysates from various human tissues

Rationale. Symptoms associated with synovial tissue (e.g., arthritis) skeletal muscle (e.g., fatigue), brain (e.g., cognitive complaints and Pain) and heart (e.g., carditis) are commonly present in PTLDS patients. We will screen serum samples using Western blots containing protein lysates from synovial tissue, skeletal muscle, and different regions of brain and heart tissue.

Research Hypothesis: Tissue specific autoantibodies cause organ specific symptoms in mouse models


Rationale. If tissue specific autoantibodies are pathogenic they should be able to induce pathology and clinical symptoms associated with PTLDS

Impact: Identification of PTLDS specific autoantibodies would be helpful not only in the diagnosis of this conditions but also prognosis and treatment.
Title: New York Community pharmacists’ knowledge of Lyme Disease and current treatment regimens

Sara Spencer, School of Pharmacy & Pharmaceutical Sciences, Pharmacy Practice

Background: New York ranks 3rd for having the highest confirmed Lyme Disease cases in the country. Pharmacists have an obligation to remain abreast of the most current evidence-based recommendations. Conflicting guidelines exist for the treatment of Lyme disease and there is no current system for reconciling conflicting guidelines. The International Lyme and Associated Diseases Society (ILADS) recommends the long-term use of antibiotics due to the controversial view that the bacteria can persist silently in the body. The Infectious Disease Society of America (IDSA) medical association discourages the use of long-term antibiotics and recommends to treat the continuing aftereffect symptoms of the infection. Below is a comparison table of the IDSA and ILAD guidelines.

<table>
<thead>
<tr>
<th>ILADS</th>
<th>IDSA</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Management of Ixodes species bites</strong></td>
<td></td>
</tr>
<tr>
<td>Doxycycline 100 – 200 mg twice daily for a minimum of 20 days for all Ixodes tick bites in which there is evidence of tick feeding, regardless of the degree of tick engorgement or the infection rate in the local tick population</td>
<td>Single 100 mg dose of oral doxycycline for Ixodes scapularis if the following criteria are met: a. Tick attached for minimum of 36 hours b. Tick infection rate &gt; 20% in local where bite occurred c. Treatment can begin within 72 hours of tick removal</td>
</tr>
<tr>
<td><strong>Management of Erythema Migrans</strong></td>
<td></td>
</tr>
<tr>
<td>Amoxicillin, cefuroxime, or doxycycline for a minimum of 4-6 weeks OR azithromycin for a minimum of 3 weeks</td>
<td>Doxycycline for 10-21 days OR amoxicillin or cefuroxime for 14-21 days</td>
</tr>
<tr>
<td><strong>Management of Patients with Persistent Post-treatment Manifestations</strong></td>
<td></td>
</tr>
<tr>
<td>4-6 weeks of retreatment with antibiotics based on individualized risk-benefit assessments</td>
<td>Recommends against antibiotic retreatment for patients with persistent post-treatment manifestation of Lyme disease</td>
</tr>
</tbody>
</table>

Research Question: What is the knowledge base of community pharmacist for Lyme Disease and treatment regimens?

Specific Aims:
1. To assess New York Community pharmacists’ knowledge regarding current evidence for the initial and maintenance treatment of Lyme Disease
Approach and Methods:
1. Invite pharmacists to complete a knowledge-based questionnaire relating to Lyme Disease treatment, specifically ILADS and IDSA regimens

Potential Investigators:
Sara A. Spencer, PharmD, BUSOPPS, Department of Pharmacy Practice

References:
Background: Lyme disease may present with many chronic debilitating symptoms, such as cardiac and neurological problems that can significantly impact the quality of life of affected individuals. During chronic infection, *Borrelia burgdorferi* survives in certain tissues(s), although the exact tissue tropism of this bacteria remains unknown. Considering that the Lyme latency also can lead to Post Treatment Lyme Disease Syndrome (PTLDS), identifying the Lyme bacteria tissue tropism can help optimizing treatment and reducing the risk of PTLDS in infected individuals. *Peromyscus leucopus*, the white-footed mouse, is the competent reservoir host for *B. burgdorferi*. Since this pathogen is able to sequester within various tissues of *P. leucopus*, an understanding of this process has the potential to explain long duration symptoms in Post-Treatment and Chronic Lyme disease in humans.

Research Hypothesis: We hypothesize that the latent Lyme infection in human involves the same tissues where it is sequestered in the white-footed mouse. Furthermore, we hypothesize there is a significant relationship between the latent and long-duration clinical symptoms exhibited by humans with PTLDS and the type of tissues/organs where it is sequestered in the white-footed mouse.

Specific Aims and Methodology:
Determining *B. burgdorferi* tissue tropism in mice. This study has the potential to inform diagnosis and treatment of PTLDS, and will be conducted through the following sub-aims:
1. **Collecting naturally-infected Mice:** We have and will continue to live trap the competent reservoir host for Lyme, *P. leucopus*, adjacent to walkways with high human foot traffic within state and county parks in the 6 county region of the Upper Susquehanna River Basin and sacrifice captured rodents according to an approved IACUC protocol (#746-15).
2. **Genetic testing of 9 tissues:** DNA is extracted from harvested bladder, kidney, spleen, heart, liver, gut, lungs, skin, brain, as well as blood and saliva, and the presence of *B. burgdorferi* determined by quantitative PCR.
3. **Compare naturally infected white footed mice with tissue tropism in lab mice (previous studies)**
4. **Compare tissue tropism in white footed mice with PTLDS symptoms in humans:** We will study the correlation between the presence and level of infection in the mouse tissues and tissues/organs involved in PTDLS, based on the clinical symptoms (from a cohort of over 200 patients, John Darcy Clinical Study).

Preliminary data: Tissues from 30 white footed mice (the natural reservoir for the Lyme pathogen) suggests that skin, heart, and bladder display the highest pathogen sequestration with rates of 37%, 33%, and 31%, respectively, while blood has the lowest at 9%. Brain, gut and
saliva have not been tested as yet. We plan to test approximately 100 white footed mice to determine if a significant relationship exists in the rates of infectivity in the 9 mouse tissues listed above to determine organ specific sequestration and the level of infectivity. These results will be compared with previous studies investigating tissue dissemination in experimentally infected laboratory mice (Barthold et al. 1993). Future aims using qPCR include testing the white footed mouse reservoir host for sequestration of Lyme co-infectious agents, *anaplasma* and *babesia*.

References:

Project 17:

Title: Policy changes in New York State to enable prevention of Lyme disease.

Sarah Lynch, School of Pharmacy & Pharmaceutical Sciences, Pharmacy Practice

Background: Prescribing by community pharmacists is currently not possible in New York State. However, there are three potential ways this could be achieved with the help of legislators.

Specific Aim: We envision three possible models for rapid provision of prophylactic antibiotics for suspected or potential Lyme disease by pharmacists. The short term goal is to carry out a feasibility assessment to prioritize one of these approaches, and then engage the legislative liaisons at Binghamton University, DC lobbying groups, Schumer’s office, and local legislators to develop the legislative language and process for policy change.

1. Inclusion of community pharmacists in legislation allowing clinical pharmacists to enter collaborative practice agreements (CPA) with physicians in NYS. This would allow individual pharmacists to work with individual practitioners to establish their own protocol for pharmacist prescribing (including eligibility criteria, required documentation and reporting to collaborating physician, and treatment protocol). Currently in NYS, only pharmacists who practice within a clinic or hospital with a physician may prescribe for patients per CPA. In other states (such as RI) any pharmacist is eligible to enter a CPA as long as they can find a willing physician.

2. Allowance of a standing order for Doxycycline prescribing by pharmacists. A standing order is a prewritten medication order with specific criteria and instructions from a prescriber. This is how immunizations are provided in NYS: pharmacists must get a signed standing order by a physician in their county and all immunizations are processed electronically with the standing order physician as the prescriber. The standing order physician does not get patient specific information about the individuals vaccinated under this standing order.

3. Development of a state-wide protocol for pharmacist-prescribed doxycycline. This would basically establish that either all pharmacists or certain pharmacists who have completed a specified training would be eligible to prescribe doxycycline as long as they stuck to the protocol developed. The state board of pharmacy generally develops the protocol and pharmacists follow the protocol and prescribe under their own license. This is an example of how pharmacists in Oregon are prescribing contraceptives – the state has developed a specific protocol that must be followed. Pharmacists must complete a 5-hour training program in order to become “certified” to prescriber the contraceptives, but then they are eligible to prescribe with no reporting requirements to a collaborating physician.
Project 18:

Title: *Sequestration of Borrelia burgdorferi in the competent reservoir host Peromyscus leucopus and correlation with organ specific human clinical symptoms in Post-Treatment Lyme Disease Syndrome Patients.*

Ralph Garruto, Department of Anthropology, Biomedical Anthropology  
Rita Spathis, Department of Anthropology, Anthropology  
Michel Shamoon-Pour, Department of Anthropology, Molecular and Biomedical Anthropology  
John M. Darcy, II, PhD, MS, MA  
Julia Townsend, Department of Anthropology, Biochemistry

Background: Lyme disease may present with many chronic debilitating symptoms, such as cardiac and neurological problems that can significantly impact the quality of life of affected individuals. During chronic infection, *Borrelia burgdorferi* survives in certain tissues(s), although the exact tissue tropism of this bacteria remains unknown. Considering that the Lyme latency also can lead to Post Treatment Lyme Disease Syndrome (PTLDS), identifying the Lyme bacteria tissue tropism can help optimizing treatment and reducing the risk of PTLDS in infected individuals.  

*Peromyscus leucopus*, the white-footed mouse, is the competent reservoir host for *B. burgdorferi*. Since this pathogen is able to sequester within various tissues of *P. leucopus*, an understanding of this process has the potential to explain long duration symptoms in Post-Treatment and Chronic Lyme disease in humans.

Research Hypothesis: We hypothesize that the latent Lyme infection in human involves the same tissues where it is sequestered in the white-footed mouse. Furthermore, we hypothesize there is a significant relationship between the latent and long-duration clinical symptoms exhibited by humans with PTLDS and the type of tissues/organs where it is sequestered in the white-footed mouse.

Specific Aims and Methodology:

**Determining *B. burgdorferi* tissue tropism in mice.** This study has the potential to inform diagnosis and treatment of PTLDS, and will be conducted through the following sub-aims:

5. **Collecting naturally-infected Mice:** We have and will continue to live trap the competent reservoir host for Lyme, *P. leucopus*, adjacent to walkways with high human foot traffic within state and county parks in the 6 county region of the Upper Susquehanna River Basin and sacrifice captured rodents according to an approved IACUC protocol (#746-15).

6. **Genetic testing of 9 tissues:** DNA is extracted from harvested bladder, kidney, spleen, heart, liver, gut, lungs, skin, brain, as well as blood and saliva, and the presence of *B. burgdorferi* determined by quantitative PCR.

7. **Compare naturally infected white footed mice with tissue tropism in lab mice (previous studies)**

8. **Compare tissue tropism in white footed mice with PTLDS symptoms in humans:** We will study the correlation between the presence and level of infection in the mouse tissues and tissues/organs involved in PTDLS, based on the clinical symptoms (from a cohort of over 200 patients, John Darcy Clinical Study).

Preliminary data: Tissues from 30 white footed mice (the natural reservoir for the Lyme pathogen) suggests that skin, heart, and bladder display the highest pathogen sequestration with rates of 37%, 33%, and 31%, respectively, while blood has the lowest at 9%. Brain, gut and
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infected laboratory mice (Barthold et al. 1993). Future aims using qPCR include testing the
white footed mouse reservoir host for sequestration of Lyme co-infectious agents, anaplasma
and babesia.

References:
2. Barthold SW, de Souza MS, Janotka JL, Smith AL, and Persing DH. Chronic Lyme
Borreliosis in the Laboratory Mouse. Am. J. Pathol. 1993; 193(3):959-973
Project 19:

Title: *White-tailed Deer and Dogs as Sentinels for Pathogenic Ehrlichia Species*

Rita Spathis, Department of Anthropology, Anthropology
Michel Shamoon-Pour, Department of Anthropology, Molecular and Biomedical Anthropology

**Background:** Ehrlichiosis is a human bacterial disease caused by several *Ehrlichia* species including *E. ewingii*, *E. chaffeensis* and *E. muris*-like (EML) agent. Symptoms of ehrlichiosis, which typically appear 1-2 weeks after the bite of an infected tick, include fever, headache, fatigue and muscle aches. According to the CDC, ehrlichiosis is a serious illness that can be fatal if left untreated. In 2016, 156 cases of ehrlichiosis were reported state wide to the New York State Department of Health (NYSDOH) with greater than 50% reported from Suffolk County on Long Island. The primary vector responsible for transmitting these bacteria, collectively referred to as Ehrlichia, is the lone star tick (*Amblyomma americanum*). Lone star ticks also transmit tularemia and southern-tick associated rash illness (STARI), in addition to two Borrelia genospecies *Borrelia andersonii* and *Borrelia americana* which have also been associated with Lyme-like illness in the Southern US. *A. americanum*, which are much more aggressive than the black-legged deer tick, are distributed geographically in the southeastern and Eastern United States. Although these ticks have been widely reported on Eastern Long Island, there is, as yet no documented or anecdotal evidence for their existence in the Upper Susquehanna River Basin.

The white-tailed deer (*Odocoileus virginianus*) serves both as the primary mobile vertebrate host for the lone star tick as well as the primary reservoir for both *E. chaffeensis* and *E. ewingii*. In a 2003 study, Yablsey et al. performed immunological and PCR analysis on blood samples collected from 2,101 white-tailed deer from 18 states in the southeastern and eastern US. The authors demonstrated that that white-tailed deer could be used as natural sentinels for the emergence of *E. chaffeensis* and describe a “prototypical framework” for such a surveillance system.

*A. americanum* also transmits *E. ewingii* and *E. chaffeensis* to dogs along with a third *Ehrlichia* species, *E. canis*. Both *E. ewingii* and *E. canis* can cause serious disease in dogs displaying symptoms such as fever, anorexia, hemorrhage, polyarthritis and central nervous system abnormalities. An extensive study funded by IDEXX Laboratories (Beall et al. 2012) performed serological surveys for antibodies to *E. ewingii*, *E. chaffeensis* and *E. canis* from 8,662 dog blood samples collected from veterinary clinics and diagnostic labs in 41 states. Their results document the widespread distribution of both *E. ewingii* and *E. chaffeensis* and also demonstrate the feasibility of using pet dogs as sentinels to monitor regional emergence of *Ehrlichia spp.* and the potential risk of transmission to humans.

**Research Goal:** The goal of this project is to use deer and pet dogs as sentinels to monitor the emergence of the human pathogens *Ehrlichia ewingii* and *Ehrlichia chaffeensis* and their primary tick vector *Amblyomma americanum* in the Upper Susquehanna River Basin of New York.

**Specific Aims:**
1. **Develop a real-time multiplex PCR for the detection of E. ewingii and E. chaffeensis** This novel qPCR assay will be designed and standardized through the use of commercially available control pathogen DNA.

2. **Collect and test blood obtained from deer and dogs in the 6 County region of the Upper Susquehanna River basin.** Local veterinarians will be recruited to participate by donating blood samples collected from symptomatic dogs. Deer blood will be collected from butcher processing centers on the opening day of deer hunting season. From previous experience we know that the deer brought to these processing centers are typically infested with ticks, so in addition to collecting blood we will also be checking the deer for the appearance of lone star ticks. DNA extracted from dog and deer blood will tested for the above mentioned pathogens.

**References:**

3. [https://www.cdc.gov/ticks/geographic_distribution.html](https://www.cdc.gov/ticks/geographic_distribution.html)
   https://doi.org/10.1089/153036603322662183
Project 20:

Title: *Fourth Stage of Lyme Disease: diagnosis-treatment interval hypothesis proof of concept proposal*

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**Background:** Lyme disease infects more than 300,000 Americans annually according to the United States CDC. Approximately 10-20% of these infections develop into a post-treatment Lyme disease syndrome and may progress into “chronic Lyme disease”, which is recalcitrant to treatment, constitutes a significant patient burden and is medically and socially controversial. In 2013-2014, data was collected on a cohort of 250 post-treatment Lyme disease patients as part of a Binghamton University PhD dissertation (JM Darcy II 2014, *Quaternary Lyme Disease: Symptomatology, Epidemiology and Anthropology of an Emerging Disease*). Data and symptoms were analyzed for trends. Despite many receiving antibiotic treatment, a significant number of patients reported symptoms consistent with continued post-treatment Lyme disease and chronic Lyme disease. The dissertation proposed that a fourth chronic stage of Lyme disease persists in patients beyond the currently acknowledged three acute (non-chronic) stages of the disease even with medical attention. One outstanding question in Lyme disease treatment is how does delay or long intervals between infection and treatment affect outcomes?

**Research Hypothesis:** The interval between time of first Lyme disease infection signal (first appearance of symptoms or tick attachment with erythema migrans) and medical diagnosis and antimicrobial treatment (known as Symptom-Treatment Interval) impacts longitudinal disease severity, duration and symptomatology that underwrites a chronic fourth stage of Lyme disease.

**Specific Aims:**

1. **Symptom-treatment interval and symptom quantification.**
   From the data previously collected in the JM Darcy II 2014 dissertation, the symptom-treatment interval will be calculated and correlated with the number of symptoms each patient reported in the study. This can be interpreted as a proxy for disease severity relative to the symptom treatment interval.
   a. Specific Aim 1a - Access current Lyme blinded patient database to identify and collect appropriate subjects.
   b. Specific Aim 1b - Tabulate and run correlations in SPSS version 22 (or higher if available) and analyze for trends.

**Anticipated Results Aim 1:** Detection or recognition of a positive trend or statistically significant correlation between length of time and number of symptoms reported with respect to length of time.

2. **Symptom-treatment interval and symptomatological trends.**
   From the subject data selected in Specific Aim 1, the symptom treatment interval will be correlated with groupings of symptoms based on the prespecified categories from the JM Darcy 2014 dissertation that includes categories such as neurological, musculoskeletal and pain. This analysis will look for trends that may identify specific pathological associations related to time intervals that will be important in identifying and characterizing the fourth stage of Lyme disease and its relationship to the symptom-treatment interval.
   a. Specific Aim 2a - Analyze subject data from Specific Aim 1 for suitability of detailed symptom correlations.
b. Specific Aim 2b - Tabulate and run correlations in SPSS version 22 for trends to specific symptom groupings.

Anticipated Results Aim 2: Detectable difference or correlation between neurological symptoms and disease-treatment interval.

3. Determination and design of a study for testing the symptom-treatment interval hypothesis.
   From the results of Specific Aims 1 and 2, analyze signals from the data and determine the feasibility of a study on the symptom-treatment interval hypothesis with human subjects. While post-hoc studies are useful, additional prospective or registry based studies with primary end points around the symptom-treatment interval are required to advance the hypothesis.
   a. Specific Aim 3a - Identify the key data required to test the symptom-treatment interval hypothesis. Such data required may include specific diagnostic criteria, specific antibiotic treatment and uniformly accepted symptomatological criteria for patients.
   b. Specific Aim 3b: Calculate (using SPSS or epi calculator) the minimum number of subjects needed to determine statistically significant associations (P value equal to or greater than 0.05) between symptom-treatment interval and quantitative measure of disease severity (developing a scale).

Anticipated Results Aim 3: Production of a research study design that tests the symptom-interval hypothesis in patients identified with a fourth stage of Lyme disease. The study design would be expected to be ready for human subjects research review committee and formal proposal submissions to federal or state funding agencies.

Impact: The testing of the symptom-treatment interval hypothesis could have a significant impact on how patients identified in medical settings are treated for Lyme disease. If the hypothesis is confirmed, then the benefit of quick and aggressive treatment of Lyme disease with antimicrobials would far outweigh the risk and cost associated with current conservative approaches in medical practice.