TICK-BORNE DISEASES IN NORTH CAROLINA: SEROEPIDEMIOLOGY OF
SPOTTED FEVER GROUP RICKETTSIAE AND PREVENTION OF TICK BITES
AMONG OUTDOOR WORKERS

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ABSTRACT

Meagan F. Vaughn: Tick-borne Diseases in North Carolina: Seroepidemiology of Spotted Fever Group Rickettsiae and Prevention of Tick Bites Among Outdoor Workers
(Under the direction of Steven Meshnick)

Tick-borne diseases are the most common vector-borne diseases in the US. North Carolina suffers from some of the highest rates of Rocky Mountain spotted fever (RMSF), which can cause severe illness and death.

The first aim of this dissertation explored whether spotted fever group rickettsiae (SFGR) other than *Rickettsia rickettsii* are responsible for spotted fever rickettsioses in North Carolina. A retrospective seroepidemiologic study was conducted in which the reactivity of paired sera from North Carolina patients who had been tested for RMSF we evaluated against a panel of SFGR, including *R. rickettsii*, *R. amblyommii*, and *R. parkeri*. Of the 106 eligible pairs tested, 21 patients seroconverted to one or more antigens. Cross-reactivity was observed in ten patients and seroconversions to single antigens occurred in 11 patients, including one against *R. rickettsii*, four against *R. parkeri*, and six against *R. amblyommii*. These findings suggest that species other than *R. rickettsii* are associated with illness among North Carolina residents.

The second aim of this dissertation focused on tick bite prevention among North Carolina outdoor workers. A double-blind randomized controlled trial was conducted to evaluate the protective effectiveness of long-lasting permethrin impregnated (LLPI) uniforms

among workers from North Carolina State Divisions of Forestry, Parks and Recreation, and Wildlife. 159 subjects were randomized; uniforms of participants in the treatment group were factory-impregnated with long-lasting permethrin while control group uniforms received a sham treatment. Participants continued standard recommended tick-bite prevention activities and provided weekly tick bite logs during two tick seasons. 130 subjects reported 1,045 work-related tick bites over 5,251 person-weeks of follow-up. The effectiveness of LLPI uniforms for the prevention of work-related tick bites was 0.82 (95% Confidence Interval (CI): 0.66, 0.91) for the first year of follow-up and 0.34 (95% CI: -0.67, 0.74) for the second year of follow-up. These results indicate that LLPI uniforms are highly effective for at least one year against tick bites in the context of existing tick bite prevention measure usage by outdoor workers.

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LIST OF ABBREVIATIONS

BSA: Bovine serum albumin

CI: Confidence interval

CDC: Centers for Disease Control and Prevention

DEET: *N*,*N*-Diethyl-*meta*-toluamide

DNA: Deoxyribonucleic acid

ELISA: Enzyme-linked immunosorbent assay

EPA: Environmental Protection Agency

FITC: Fluorescein isothiocyanate

GEE: Generalized estimating equations

HGA: Human granulocytic anaplasmosis

HME: Human monocytic ehrlichiosis

IFA: Indirect immunofluorescence assay

IgG: Immunoglobulin G

IgM: Immunoglobulin M

IHC: Immunohistochemistry

IRR: Incidence rate ratio

LLPI: Long-lasting permethrin impregnation

NCDENR: North Carolina Division of Environment and Natural Resources

NCDFR: North Carolina Division of Forest Resources

NCDOT: North Carolina Division of Transportation

NCDPR: North Carolina Division of Parks and Recreation

NCEDSS: North Carolina Electronic Disease Surveillance System

NCSLPH: North Carolina State Laboratory of Public Health

NIOSH: National Institute for Occupational Safety and Health

PBS: Phosphate buffered saline

RMSF: Rocky Mountain spotted fever

RR: Risk ratio

SFGR: Spotted fever group rickettsiae

STARI: Southern tick-associated rash illness

Chapter 1

SPECIFIC AIMS

As the incidence of tick-borne diseases continues to rise in the US, particularly for cases of Rocky Mountain spotted fever (RMSF) in the southern and southeastern US, questions as to the reasons for these dramatic increases continue to puzzle experts. The epidemiology of RMSF appears to be changing, and there is mounting evidence that infections with other spotted fever group rickettsiae (SFGR) are at least partly responsible for the increases in incidence and the decreases in case-fatality rates of reported cases of RMSF. Among outdoor workers, it has been well documented that the risk of tick-borne diseases is greater than that of the general population. These diseases, which can cause severe illness and death, are of particular concern for workers who have frequent exposure to tick-infested habitats, such as foresters, park rangers, land surveyors and other outdoor workers. Many North Carolina state employees with outdoor occupations report multiple tick bites each year, which indicates that existing tick preventive strategies may be ineffective. A new technology which allows clothing to be impregnated with long-lasting permethrin, a chemical known for its insecticidal properties, may be a simple method to reduce tick bites and tick-borne disease.

The specific aims for this dissertation are to:

1. Evaluate the serologic reactivity of samples collected from North Carolina patients tested for RMSF to *Rickettsia rickettsii*, *R. amblyommii*, and *R. parkeri antigens.*

2. Evaluate the effectiveness of wearing permethrin impregnated uniforms for the prevention of tick bites among North Carolina outdoor workers.

Aim 1 was accomplished through a retrospective analysis of North Carolina reportable disease surveillance data and stored serum samples at the North Carolina State Laboratory of Public Health (NCSLPH). Paired sera (acute and convalescent) submitted to the NCSLPH from patients suspected to have a tick-borne illness from 2008 to 2010 were tested against a panel of SFGR antigens using an indirect immunofluorescence assay (IFA). Criteria for inclusion in the study included patients for which there were paired sera available that had been submitted to the NCSLPH for testing against *R. rickettsii* and at least one of the sera had a titer ≥1:64. Data from the surveillance report were reviewed and information regarding clinical signs and symptoms, treatment, and recovery were extracted. Disease severity and patient outcomes were compared for patients who had serologically confirmed RMSF (four-fold or greater rise in titer to *R. rickettsii* between paired specimens) and patients who had fourfold or greater rises in titers to other SFGR (*R. amblyommii* or *R. parkeri*).

Aim 2 was accomplished through a double-blind randomized intervention including employees from the North Carolina Division of Forest Resources, the North Carolina Division of Parks and Recreation, North Carolina County Parks and Recreation, and North Carolina Wildlife Resources Commission. Participants who were enrolled in the study were randomized into the placebo or treatment groups and were asked to submit all of their work uniforms to Insect Shield, LLC (Greensboro, NC). The uniforms of participants in the treatment group were treated with permethrin using a proprietary process developed by Insect Shield, which allows clothing to retain insecticidal activity for 70 machine washes. Uniforms from members of the control group did not receive permethrin treatment, and were washed and refolded. Questionnaires which collected general information on tick exposure

and tick bite prevention practices were administered to participants at enrollment and after

each tick season. All participants were asked to record any tick bites during the study

period on weekly tick bite logs. The incidence rate ratios (and 95% confidence intervals) for

tick bites comparing the treatment and control groups, stratified by year of follow-up, were

estimated using a generalized estimating equations (GEE) approach for Poisson regression.

Chapter 2

INTRODUCTION AND LITERATURE REVIEW

The most common vector-borne diseases in the US are those carried by ticks. Over the past two decades the incidence of tick-borne diseases such as Lyme disease, Rocky Mountain spotted fever (RMSF), human monocytic ehrlichiosis (HME), and human granulocytic anaplasmosis (HGA) have been increasing at an remarkable rate [1-5]. Recently the CDC estimated that there are approximately 300,000 cases of Lyme disease diagnosed in the US each year, more than ten times the number of cases that are actually reported [6]. Under ascertainment is also likely for other tick-borne diseases such as RMSF. The incidence of RMSF, the most commonly reported fatal tick-borne disease in the US, has also been rising in recent years from 2.5 cases per million in 2001 to 9.5 cases per million in 2011 [7]. These tick-borne diseases, which can cause serious illness or death if not treated early, pose a substantial public health threat in highly endemic areas. One such endemic area, commonly referred to as the "tick belt", which stretches from Oklahoma to North Carolina, is home to at least four species of ticks known to carry human pathogens [8, 9]. This region suffers from some of the highest rates of tick-borne rickettsial diseases such RMSF and HME [2] (Figure 1).
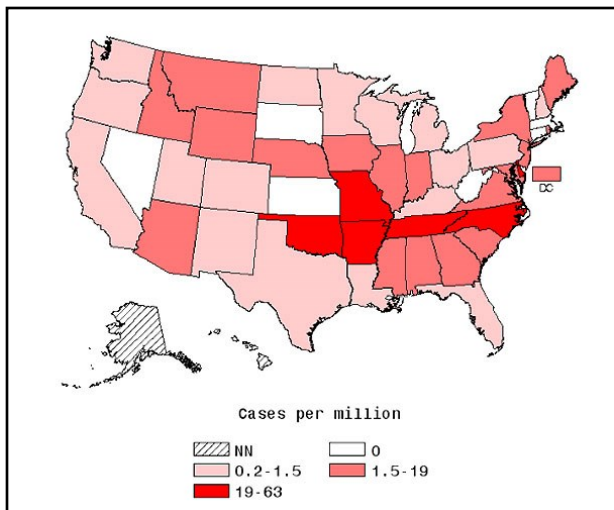
Figure 1. Average reported annual incidence of Rocky Mountain spotted fever, 2010 [10].

In addition to rising incidence rates, the public health threat posed by tick-borne diseases has been compounded by the emergence of several "new" tick-borne diseases. The lone star tick (*Amblyomma americanum*) is one of the most abundant species of ticks in the southeastern US and is known for it's aggressive feeding on large mammals, including deer and humans [11]. Formerly considered a "nuisance" species, the lone star tick is now known to be an important vector. Since the 1980s, increasing reports of an illness with clinical manifestations similar to Lyme disease has been described among people bitten by lone star ticks [12-14]. The etiology of this disease, named southern tick-associated rash illness or STARI, is still unknown. There is also evidence that other species of spotted fever group rickettsiae such as *Rickettsia amblyommii* and *Rickettsia parkeri*, previously thought to be non-pathogenic in humans, are capable of causing disease [15-19].

A. Seroepidemiology of spotted fever group rickettsiae

Spotted fever group rickettsiae (SFGR) are obligate intracellular gram negative bacteria that are transmitted to mammals through the bite of an infected tick, flea, or mite. Up until the 1980s, the only SFGR known to cause disease in humans in the United States was *Rickettsia rickettsii*, the agent of RMSF [20]. In the US, the primary tick vectors for *R.*

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rickettsii are the American dog tick (*Dermacentor variabilis*), the Rocky Mountain wood tick (*Dermacentor andersoni*), and more recently the brown dog tick (*Rhipicephaulus sanguineus*). Contrary to its name, the highest rates of RMSF are in central and eastern parts of the US, with North Carolina, Tennessee, Arkansas, and Oklahoma representing the states with the highest incidence of reported cases [2, 20]. *D. variabilis* is the established vector for *R. rickettsii* in this region, although *R. rickettsii* has been detected in less than 1% of these ticks in recent tick surveys [21-25].

1. Clinical presentation of RMSF

The typical constellation of symptoms for a case of RMSF includes fever, headache, and myalgia, followed by the development of a maculopapular rash which may progress to a petechial rash. Other symptoms reported include malaise, abdominal pain, nausea, vomiting, anorexia, or altered mental status. The onset of symptoms usually occurs within 14 days of the tick bite, although many patients do not recall being bitten. The rash is ultimately observed in approximately 80% of cases, although less than 50% have it at the time of first presentation to a medical provider [2]. The majority of cases reported have an onset of illness between April and September. RMSF is often a severe illness, and historical estimates of case-fatality rates in the early 20th century were well above 20%, while more recent estimates range between 2% and 6% [26]. Even with appropriate antibiotic treatment many patients are hospitalized; of the cases reported to the CDC between 1997 and 2002, 35% were hospitalized with a 1.4% case-fatality rate [3].

2. Clinical and laboratory diagnosis of RMSF

Diagnosis of RMSF can be difficult due to the nonspecific symptoms in the early stages of the illness. If a tick-borne rickettsial disease is suspected, doxycycline is the antibiotic of choice and treatment should not be delayed for a laboratory confirmation since sensitive diagnostic tests are not available in the early phase of disease. Several tests can be used to confirm infection with *R. rickettsii*, including serologic tests on acute and convalescent

sera, detection of rickettsial DNA in blood or skin biopsy samples, immunohistochemical staining of skin biopsy tissue, and culture. Serologic testing is the primary method of laboratory diagnosis for RMSF; the most common methods used being the indirect immunofluorescent antibody assay (IFA) and enzyme-linked immunosorbent assay (ELISA). The IFA is considered the gold standard since it is quantitative (unlike the ELISA) and has a high sensitivity and specificity (85-97% sensitivity and 100% specificity for a single convalescent sample) [27]. A four-fold or greater change in IgG titer (seroconversion) against *R. rickettsii* antigen between appropriately timed acute and convalescent serum samples is sufficient for laboratory confirmation. Elevated IgG and IgM titers are usually not observed until the second or third week of illness, and detectable antibodies are often not found in acute samples collected in the first seven days of illness, therefore a second sample collected 2-4 weeks after onset of illness is required to demonstrate seroconversion. For surveillance purposes, a single elevated IgG or IgM titer against *R. rickettsii* antigen can be used as supportive laboratory evidence for infection, but is not sufficient for confirmation as it may only represent the presence of antibodies from a prior infection. It is unknown how long antibodies to SFGR persist, but one study demonstrated that patients infected with *R. rickettsii* still had detectable antibodies after 12 months [28].

3. Surveillance case definitions

In 2010 the case classification for RMSF was changed by the Council of State and Territorial Epidemiologists to reflect the likelihood that some cases being reported as RMSF under the prior classification may be due to other SFGR, and is now designated as "Spotted Fever Group Rickettsiosis (including Rocky Mountain spotted fever)". The current case definitions used for surveillance include [29]:

- Suspected: A case with laboratory evidence of past or present infection but no clinical information available (e.g. a laboratory report).

- Probable: A clinically compatible case (meets clinical evidence criteria) that has supportive laboratory results.
- Confirmed: A clinically compatible case (meets clinical evidence criteria) that is laboratory confirmed.

A clinically compatible case must have reported fever and one or more of the following symptoms or clinical findings: compatible rash, eschar, headache, myalgia, anemia, thrombocytopenia, or any hepatic transaminase elevation [29]. Clinical findings of anemia, thrombocytopenia, or hepatic transaminase elevation are defined as exceeding the laboratory defined upper or lower bounds for normal values. Laboratory confirmation can be achieved by any one of the following methods:

- Serological evidence of a fourfold change in immunoglobulin G (IgG)-specific antibody titer reactive with *Rickettsia rickettsii* or other spotted fever group antigen by indirect immunofluorescence assay (IFA) between paired serum specimens (one taken in the first week of illness and a second 2-4 weeks later).
- Detection of *R. rickettsii* or other spotted fever group DNA in a clinical specimen via amplification of a specific target by PCR assay.
- Demonstration of spotted fever group antigen in a biopsy or autopsy specimen by immunohistochemistry (IHC).
- Isolation of *R. rickettsii* or other spotted fever group rickettsia from a clinical specimen in cell culture.

For supportive laboratory results, the patient must have serologic evidence of elevated IgG or IgM antibody reactive with *R. rickettsii* or other spotted fever group antigen by IFA, enzyme-linked immunosorbent assay (ELISA), dot-ELISA, or latex agglutination [29].

4.   Trends in incidence and mortality

Since national surveillance for RMSF began in 1920, incidence in the US has gone through large fluctuations (Figure 2). Experts have suggested several possible reasons for some of the major periods of change, such as changes in agencies responsible for collecting surveillance data, the death of prominent RMSF researcher Ralph Parker in 1949, changes in case definitions, the availability of improved diagnostic tests, improved education and disease reporting, and recognition of RMSF in the eastern US [26, 30]. Other factors that may have played a role in the changes in incidence observed in the US include variation in the virulence of *R. rickettsii*, changes in use of pesticides (DDT), competition with other species of *Rickettsia* in tick vectors, and increased human contact with tick vectors due to increases in population, recreation, and suburbanization [26].

A general decreasing trend was observed through the 1980s and 1990s, reaching its lowest point at 1.4 cases per million in 1998. Since 1998 there has been unprecedented and dramatic rise in the incidence of RMSF, with more than a 5-fold increase in the span of a decade to an all-time high of 8.4 cases per million in 2008 [10]. This time period also marked a rapid decline in the reported case fatality rate for RMSF, which also reached an all-time low of 0.5% in 2008. Since treatment options and clinical management of cases of RMSF has not changed for many years, this decrease in mortality is difficult to explain. Several events related to RMSF surveillance occurred in the past decade that may account for some of the increase in incidence, including the introduction and promotion of a new case reporting form in 2001, changes in the case definition in 2004, publication of national guidelines for diagnosis, treatment, and management of tick-borne rickettsial diseases in 2006, and increases in the amount of funding for public health preparedness available to state health departments [30].

A decreasing trend in the percent of RMSF cases that are confirmed and an increase in the number of probable cases accompany the increases in incidence and decreases in

case-fatality from 1998 to 2008. The case-fatality rate among confirmed cases was 10 times that of probable cases between 2000 and 2007, and suggests that probable cases may represent previous exposure to a SFGR rather than an incident infection [30]. This theory is supported by several studies showing that seroprevalence to SFGR is between 6% and 12% based on serosurveys of adults and children in the US [31, 32]. In addition, a recent geospatial analysis of the relative severity of RMSF cases in the US by county found two clusters of less severe disease in central and south-central North Carolina [33]. This finding may indicate that there is better detection, diagnosis and treatment of RMSF in North Carolina, or that milder strains or less pathogenic species of rickettsiae are circulating in this region. Since subclinical infection or mild infections with *R. rickettsii* are considered by experts to be rare or even non-existent [18], exposure to non-pathogenic or less pathogenic species of SFGR is likely to account for the relatively high seroprevalence to *R. rickettsii* and may also account for some of the increased incidence and decreased case-fatality observed in recent years.

Figure 2. Incidence and case-fatality rates for Rocky Mountain spotted fever, 1920-2008. Source: http://www.cdc.gov/rmsf/stats/

5. Risk factors for RMSF

RMSF occurs more frequently in males, of the cases reported between 2000 and 2007, 57% of cases were male [30]. While whites make up the majority of the reported cases (87% of cases reported from 2000-2007), American Indians suffer from disproportionately high rates of RMSF (16.8 cases per million compared to 4.4 cases per million for whites) [30]. Historically, the highest incidence of RMSF was seen in children under age 10 [2, 4], but in recent years the incidence of RMSF in older adults has surpassed that of children (Figure 3).

RMSF Incidence by Age Group, 2000-2008
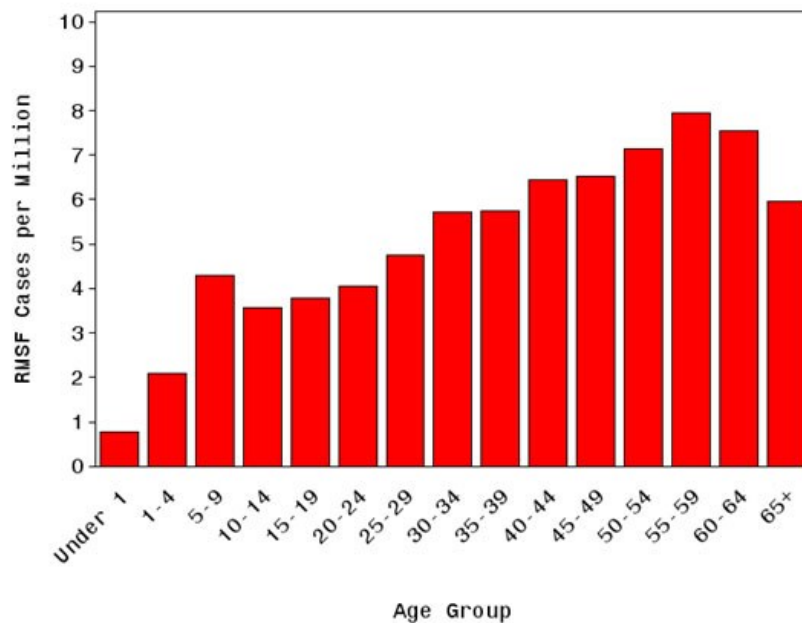
Figure 3. Age-specific incidence of Rocky Mountain spotted fever cases reported between 2000 and 2008. Source: http://www.cdc.gov/rmsf/stats/

6. Emergent and possible emerging SFGR in the US

Worldwide there are a large number of SFGR that are known to cause disease in humans, including *Rickettsia africae* (African Tick Bite Fever), *Rickettsia conorii* (Mediterranean Spotted Fever), *Rickettsia sibirica* (Siberian Tick Typhus), and many others.

In addition to *R. rickettsii*, several other species of SFGR have been isolated from ticks in the US, but most have not been implicated in causing human illness.

a. *Rickettsia parkeri*

Rickettsia parkeri was first isolated in 1939 from Gulf Coast ticks (*Amblyomma maculatum*) and was considered to be nonpathogenic for many years until 2002, when it was isolated from a patient in Virginia [19, 34]. Additional cases from Virginia, Mississippi, Kentucky, South Carolina, and Maryland were described in subsequent years [24, 35, 36]. *R. parkeri* appears to cause a milder illness than classic RMSF, and eschars at the site of inoculation are common (which are rarely described in cases of RMSF). A review of case reports submitted to the CDC between 1998 and 2007 for which an eschar or vesicular rash was reported found 12 confirmed and probable cases of *R. parkeri* rickettsiosis based on laboratory results using culture, PCR, IHC, or group-specific IFA against several species of SFGR [36]. *R. parkeri* rickettsiosis cases were less severe overall compared to RMSF cases; with lower percentages of hospitalization, death, nausea/vomiting, diarrhea, and coma/delirium/seizures. Eschars were present in nearly all cases. The range for *A. maculatum* includes the coastal areas of the southeastern US and extends further inland in some states such as Kansas and Oklahoma [37]. Recent entomologic surveys in this region have found *R. parkeri* in 11-43% of Gulf Coast ticks [37-41].

b. *Rickettsia amblyommii*

Rickettsia amblyommii was first isolated from lone star ticks (*Amblyomma americanum*) in Tennessee in 1974 [42]. The range of *A. americanum* covers much of the eastern US, stretching as far north as New England and west to Texas and Oklahoma [2]. *R. amblyommii* is found in a high proportion of lone star ticks, a tick survey done in Missouri and Kansas in 2006 found that greater than 90% of *A. americanum* pools were positive for *R. amblyommii [21].* In studies in North Carolina and Tennessee, *R. amblyommii* was found in 40-56% of lone star ticks [23, 43, 44]. Initial studies of *R. amblyommii* in voles and guinea

pigs determined that the bacteria was nonpathogenic, and for many years it was assumed that it was nonpathogenic for humans as well [42, 45]. More recent studies have provided some evidence that *R. amblyommii* may cause a mild rickettsiosis in humans. Sanchez et al [46] conducted a seroepidemiologic investigation of military personnel from a unit who had trained in Virginia and Kansas, after which two cases of tick-borne illness had been reported. Post-exposure sera was collected from the 109 individuals from that unit and 18 had elevated (≥1:64) titers against *R. rickettsii*, but only 33% reported a febrile illness. A subsequent reevaluation of the sera from 12 of those individuals showed that 5 of those sera had a greater specificity to *R. amblyommii* antigen than to other species of SFGR tested by Western blotting [47]. Billeter et al [16] published a report of a woman from central North Carolina who developed a rash after being bitten by a lone star tick that tested positive for *R. amblyommii*, no other symptoms were reported and no clinical samples were available to confirm the etiology of the rash. Apperson et al [15] conducted active surveillance for tick-borne illnesses in a central North Carolina county in 2006. Through physician-based surveillance, 16 probable cases of RMSF were identified, of which 6 had paired sera available. Since initial testing failed to confirm RMSF in these patients, they were retested against both *R. rickettsii* and *R. amblyommii* antigens, and higher titers to *R. amblyommii* were observed for all 6 patients, and 3 were found to seroconvert against *R. amblyommii*. The illnesses reported by these patients were relatively mild and none had the maculopapular or petechial rash typical of many cases of RMSF.

    c.   Other SFGR found in US tick populations

Several other species of SFGR have been found in tick populations in the US, and are currently considered of unknown pathogenicity for humans. *Rickettsia bellii* was isolated from *D. variabilis* from Arkansas in 1966; and has since been shown to infect a variety of species of ticks and is broadly distributed in the US [48-50]. *Rickettsia montanensis* (formerly *R. montana*) was isolated from *D. variabilis* and *D. andersoni* ticks from Montana in

1963 [51]. This species is commonly found among *Dermacentor* spp. ticks across the US, and recent studies found *R. montanensis* in 10% of *D. variabilis* and 0.3% of *A. americanum* ticks in Tennessee and in 19.4% of *D. variabilis* ticks in North Carolina [23, 44]. To date the only evidence of human infection with *R. montanensis* was a child with an afebrile rash following the bite of an infected *D. variabilis* tick in Georgia [52]. The patient seroconverted to *R. montanensis,* but with a very low convalescent titer of 1:32. *Rickettsia peacockii* was first noted by Parker and Spencer in 1925 when it was found in *D. andersoni* ticks in Montana [53] and is now considered to be a non-pathogenic endosymbiont of these ticks in the western US and Canada. In 1975 *Rickettsia rhipicephali* was isolated from *Rhipicephalus sanguineus* ticks on dogs [54]. This species has also been isolated from several species of *Dermacentor* ticks in California, Montana, and South Carolina. Antibodies to *R. rhipicephali* have been detected in 11% of dogs in North Carolina [55], but no evidence of human infection with this species has yet been documented. [19, 34]. In California, Rickettsia 364D has been implicated in causing an eschar associated illness decades after it was first identified in *Dermacentor occidentalis* ticks [56].

7.   Prior seroepidemiologic studies of SFGR

In addition to the studies described above, several other investigators have compared serologic reactivity of patient samples against multiple SFGR antigens. Philip et al [57] used IgM and IgG IFA to examine serologic responses from RMSF patients and vaccinees against several antigens, including *R. rickettsii, R. akari, R. conorii, R. typhus, R. prowazekii,* and *R. canada*. Cross-reactions against SFGR were observed in many patients, but all culture confirmed RMSF patients produced the highest titers against *R. rickettsii* antigen. Cross-reaction staining characteristics varied, but the authors suggested that more sharp, uniform, and intense staining would likely be seen for the infecting strain than for other strains that have poorer staining. A later study by the same group evaluated cross-reactivity of *Rickettsia* species by IFA in mice [58]. Mice were inoculated with 72 different strains of

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Rickettsia, and the authors found that all strains reacted to their homologous antigen with the highest titer, and with homologous titers at least 4-fold greater than heterologous titers.

Raoult and Paddock [59] evaluated 15 sera from patients that were known to be reactive to *R. rickettsii* using class-specific IFA with *R. rickettsii* and *R. parkeri* antigens. Higher titers (at least 4-fold) of both IgG and IgM antibody against *R. rickettsii* was observed in 4 patients, and against *R. parkeri* in 5 patients. No difference in titer between *R. rickettsii* and *R. parkeri* antigens was observed for 6 patients. Additional evidence of infection with *R. parkeri* was provided by Western blot analysis of the 4 patients that had higher titers against *R. parkeri*, which all reacted with a 120-kD protein of *R. parkeri*. In this study only single samples were tested for each patient, so seroconversion could not be demonstrated, and no clinical information was available.

Paddock et al [36] conducted comparative IgG and IgM serologies by IFA from confirmed (n=6) and probable (n=6) cases of *R. parkeri* rickettsiosis using *R. parkeri, R. rickettsii, R. amblyommii,* and *R. akari* antigens. Paired sera were only available for some of the patients, so reactivity was compared using a single sample from each patient. For patients with more than one sample, the first sample which gave a titer ≥ 1:64 against any antigen was used. IgG geometric mean titers were higher against *R. rickettsii* than for any other antigen and were positive (≥ 1:64) for 11/12 of the patients compared to 10/12 for R. parkeri, and 8/12 for *R. amblyommii* and *R. akari* antigens, although these differences were not statistically significant. IgM geometric mean titers were highest against *R. parkeri* antigen, with 9/12 patients positive for *R. parkeri*, 8/12 for *R. rickettsii*, 7/12 for *R. akari*, and 5/12 for *R. amblyommii* (which was the only statistically significant result). Therefore, comparing single IFA titers from patients with suspected SFGR infections is unreliable for identification of the etiologic agent.

In conclusion, it is clear that while *R. rickettsii* is still circulating and causing disease among people living in the southern and southeastern US, that people are also being

exposed to and infected by other SFGR carried by tick species in these regions. Some of these SFGR have been shown to cause human disease (such as *R. parkeri*), while the pathogenic potential of others has yet to be determined (such as *R. amblyommii*). The fact that the large majority of patients are diagnosed with RMSF (or a SFGR infection) using serologic methods with only *R. rickettsii* antigen combined with the fact that there is serologic cross-reactivity between species of SFGR makes it difficult to determine which agent(s) are responsible for the cases being reported. Until new diagnostic methods are developed which can distinguish between species of SFGR, or the use of molecular methods becomes more popular, the relative contributions of different species of SFGR to human morbidity will remain unclear. Prospective studies of patients with suspected tick-borne illness which include paired serologies, molecular detection and culture from whole blood and skin biopsies are needed to determine the etiologies of SFGR infections in this region. Unfortunately these types of studies would require significant effort and funds to identify a sufficient number of patients and to obtain the necessary clinical specimens within the critical window of time after onset. While it cannot ascertain etiology, a retrospective analysis using species specific IFA on paired sera from patients with suspected RMSF is a simple and inexpensive alternative that may shed some light on this issue.

B. Tick bite prevention among outdoor workers

1. Risk of tick bites and tick-borne diseases among outdoor workers

The growing problem of tick-borne diseases in the US is particularly a concern for people who have frequent and unavoidable exposure to tick infested habitats because of their occupation. The National Institute for Occupational Safety and Health (NIOSH) lists several occupational groups who are at risk for tick-borne diseases including construction workers, landscapers, forestry workers, brush clearers, land surveyors, farmers, railroad workers, oil field workers, utility line workers, and parks and wildlife management workers [60]. Although there is very little published information on the frequency of tick bites among

populations of outdoor workers, from the few studies which collected information on tick exposure it appears that many outdoor workers are exposed to ticks on a daily basis and tick bites are relatively common. A study of employees of the New Jersey Department of Environmental Protection and Energy (primarily outdoor workers) found that almost half of the study participants reported finding ticks on their skin or clothing at the end of the work day and had an overall mean of 3.5 (± 7.6) tick bites in the previous year, although there was considerable geographic variation [61]. In contrast to the paucity of data on tick bites, there has been a substantial amount of research focused on the occupational risk of tick-borne disease. There are more than 25 published studies on the risk of Lyme disease among outdoor workers, primarily among forestry workers, farmers, and park rangers [61-92]. The majority of these studies found that these populations of outdoor workers were at increased risk for exposure to *Borrelia burgdorferi*, the agent of Lyme disease. A recent serosurvey of National Park Service employees from the Great Smoky Mountains (in North Carolina and Tennessee) and Rocky Mountain National Park (in Colorado) showed that 22% of employees were seropositive for spotted fever group rickettsia, 3% were seropositive for *Ehrlichia chaffeensis*, 8% were seropositive for *Anaplasma phagocytophilum*, and none were seropositive for *B. burgdorferi* [93]. Covert and Langley, provided a review of the literature on occupational infectious diseases among forestry workers [67]. Of the twenty infectious diseases covered in the review, six were tick-borne diseases, including Lyme disease, tularemia, ehrlichioses, rickettsial diseases, central European encephalitis, and babesiosis.

2. Prevention strategies for tick bites and tick-borne diseases

The first line of defense against tick bites and tick-borne diseases is to avoid contact with tick habitat during the season for peak tick activity. For outdoor workers, this is not feasible

and since there are no vaccines currently available[1] for any of the tick-borne diseases endemic to the US they must therefore rely on the use of personal protection measures to prevent tick bites and tick-borne disease. The methods of tick bite prevention recommended by NIOSH for outdoor workers include: wearing light colored protective clothing (long sleeves, long pants, and hat), tucking pants into socks or boots, regular application of insect repellant (at least 20% DEET) to exposed skin, spraying work clothing with permethrin (must be reapplied after several washings), thoroughly checking your body for ticks every day with prompt and appropriate removal of the tick, and washing work clothing in hot water to kill any ticks [60]. Consistent use of these tick bite prevention methods has been proven effective, but there is evidence that there is poor adherence to these recommendations. A study of outdoor workers employed by the state of New Jersey found that despite significant tick exposure in an area endemic for Lyme disease, less than a quarter of outdoor workers regularly practiced several of the recommended tick avoidance behaviors including using insect repellent on skin or clothes, tucking pants into socks, or wearing long sleeves in the summer [61]. Another study of federal and state employees with outdoor occupations in Lyme disease endemic areas of New York state had similar findings, where only 20% of employees reported using insect repellent on skin or clothing, and less than 10% tucked their pants into their socks [87]. The only tick bite prevention measures consistently used by the majority of outdoor workers from both studies was wearing long pants and checking oneself for ticks. Among National Park Service employees from the Great Smoky Mountains and Rocky Mountain National Parks, less than half of employees reported using insect repellent while on the job, and only 9% treated their clothing with insecticide or repellent [93]. The need for reapplication of insect repellents

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[1]A recombinant vaccine for Lyme disease was licensed by the FDA in 1998, but was withdrawn from the market in 2002 after slow sales due to cost (many insurance companies would not reimburse) and consumer concerns about the safety of the vaccine.

combined with concerns about the toxicity of the chemicals in the repellants is likely to account for much of the underuse of these preventive measures. In addition, the concentration of DEET needed to prevent tick bites is much higher than required to prevent mosquito bites. Poor adherence to currently available preventive measures by persons at high risk of tick-borne disease indicates that safer and more user-friendly tick-bite prevention strategies are needed.

3. Permethrin impregnated clothing

Permethrin [3-phenoxybenzl (1RS)-cis,trans-3-(2,2-dichlorovinyl)-2,2-dimethylcycloprophanecarboxylate] is a synthetic chemical that is approved by the EPA for use as a contact repellent/insecticide for agricultural, residential, and personal use (on clothing only). Permethrin has the ability to repel, knockdown, and kill many arthropod vectors including but not limited to ticks, mosquitoes, sandflies, fleas, and chiggers [94]. Extensive studies on the toxicity of permethrin have shown that permethrin is safe for humans at exposure levels consistent with proper use of permethrin products, although toxic effects due to overexposure can occur through inhalation or skin contact when self-applied permethrin products are used improperly [94, 95]. The most commonly used form of permethrin for clothing treatment is a 0.5% permethrin aerosol spray commonly known as Permanone. Field trials evaluating the effectiveness of permethrin treated clothing using pressurized sprays and dipping methods have shown that permethrin can provide nearly 100% protection against questing ticks including *Amblyomma americanum* [96-98], *Dermacentor variabilis [96, 98]*, *Ixodes scapularis* [98, 99], and *Ixodes pacificus* [100], although the high rate of protection is not sustained over long periods of wear or after multiple washings [100, 101]. A seroepidemiologic study of military personnel exposed to heavily tick-infested area of Arkansas found that persons who applied permethrin products to their uniforms were significantly less likely to seroconvert to SFGR or *Ehrlichia* species

[102]. Spray and dipping methods for treating clothing with permethrin require regular reapplication by the user to maintain high levels of activity after laundering. The need for reapplication and the possibility of overexposure to permethrin during the application process is burdensome and potentially harmful for the user, which are disincentives for the use of self-applied methods of permethrin treatment for clothing.

4. Long-lasting factory-based permethrin impregnated clothing

In recent years, factory-based methods for permethrin impregnation of clothing have been developed which allow clothing to retain long term insecticidal activity. Insect Shield, LLC, a company based in Greensboro, NC, has developed a process which combines factory-based technology with a proprietary formulation of permethrin that allows clothing to retain effective repellent activity for over 70 washes (the effective lifetime of a garment). Clothing treated by Insect Shield® has undergone extensive safety testing and has been approved by the EPA for use among people of all ages, including pregnant women and children [103]. Insect Shield® treated clothing is commercially available and is sold by several well-known outdoor marketers such as LL Bean and Orvis, and is a key component of the Department of Defense Insect Repellent System. Because the permethrin is very tightly bound to the fabric, there is less dermal absorption of permethrin as compared to clothing treated using spray or dipping methods and less contamination of waste water during laundering [103-105]. The use of permethrin impregnated uniforms for high-risk outdoor workers could be a simple, safe, and cost-effective method to reduce tick bites and exposure to tick-borne diseases.

5. Safety of Insect Shield® permethrin impregnated clothing

The Insect Shield® garment treatment is regulated by the US EPA which reviews repellents to meet their standards for safety and efficacy prior to issuance of the registration required to allow such products to be sold in the US. Insect Shield® received the first ever

EPA registration for insect repellent apparel in 2003. When registering a pesticide, the EPA identifies four levels of toxicity for the product and requires an associated Signal Word on the label for all but Toxicity Category IV, the most favorable of the categories. Insect Shield® apparel is in this most favorable category IV and accordingly requires no Signal Word on its labeling. In addition to meeting EPA standards for safety, Insect Shield® conducted its own human biomonitoring study to determine the relative level of safety on the Insect Shield® products compared to self-applied permethrin products. The results showed a level of safety two orders of magnitude safer than the standard used by the EPA [106].

6. Efficacy of factory-based permethrin impregnated clothing

    a. Knockdown testing

Knockdown testing is a widely accepted scientific laboratory methodology for determining the efficacy of insect repellent-treated clothing and measures the sublethal incapacitation of insects upon exposure to treated fabric. Knockdown testing of Insect Shield® permethrin impregnated clothing against two tick species, *Rhipicephalus sanguineus* and *Ixodes scapularis*, was performed by an independent laboratory, with ten replicates at each wash level [107]. The results of the testing showed that the treated clothing has a 90.4% and 100.0% knockdown rate within 60 minutes of exposure for *R. sanguineus* and *I. scapularis* after 80 washings, respectively (Figure 4). All samples tested against *I. scapularis* resulted in 100% knockdown within 15 minutes. In a comparison of the longevity of knockdown activity against *Aedes aegypti* mosquitoes, Insect Shield® treated clothing retained over 80% knockdown activity after 80 washes, while Permanone sprayed clothing had 55% knockdown activity after only 5 washes. Since ticks are more sensitive to permethrin than mosquitoes [94], it is likely that the contrast in longevity between Permanone sprayed clothing and Insect Shield® impregnated clothing is even more dramatic for tick species. These results indicate factory-based long lasting permethrin

impregnated clothing is highly effective as a tick repellent in a controlled laboratory setting and is likely to provide a high level of protection against tick bites in the field for significantly longer durations than for self-applied permethrin products.
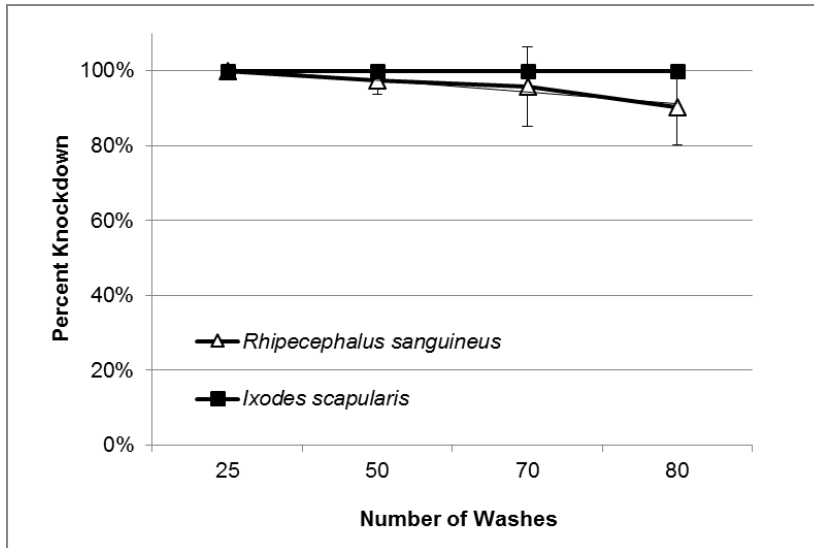
Figure 4. Percent knockdown at 60 minutes for Insect Shield® treated fabric (50% cotton, 50% nylon). [107]

b. Tick attachment studies

Two studies have evaluated the efficacy of factory-based permethrin-impregnated clothing for prevention of tick attachment. In a study by Faulde et al [108], six subjects were exposed to tick-infested areas in the mountains of Germany for a total of 36 person-hours, during which each subject wore permethrin impregnated fabric on one leg and untreated fabric on the other leg. As the subjects walked through the woods, the fabric was examined every 5 minutes and any attached ticks were removed and collected. Only 6 ticks were found attached to the permethrin impregnated fabric, compared to 132 ticks attached to the untreated fabric, which corresponded to a 95.5% protection rate against questing *Ixodes ricinus* ticks for the permethrin impregnated fabric. A small clinical trial assessing the efficacy of self-applied permethrin and factory-based permethrin impregnated casual summer clothing (sneakers, socks, shorts, and t-shirt) for the prevention of tick attachment

was conducted in an indoor setting in which volunteers were artificially infested with 30 nymphal *Ixodes scapularis* ticks and any attached ticks were counted and removed after 2.5 hours [109]. Subjects wearing permethrin treated clothing were 3.4 times less likely to have ticks attach to their body than subjects wearing untreated clothing. Those wearing commercially treated clothing had fewer attachments than those wearing self-applied permethrin, but the difference was not statistically significant.

7. Evaluating the effectiveness of long lasting permethrin impregnated clothing

The effectiveness of long lasting permethrin impregnated uniforms against ticks over an extended period of time under actual field conditions has not previously been evaluated. We anticipate that the results of a randomized-controlled trial will show that the use of long lasting permethrin impregnated uniforms can significantly reduce the number of tick bites sustained by outdoor workers as compared to existing tick bite prevention methods. As an improved method of tick bite prevention that is simple, safe, and user-friendly, permethrin impregnation of uniforms would likely to appeal to employers who are concerned about the risk of tick bites and tick-borne diseases among their employees who are frequently exposed to tick-infested habitats. If permethrin impregnation of uniforms is implemented as a standard safety policy for outdoor workers the public health benefits would extend not only to outdoor workers but to the environment by the decreased use of self-applied permethrin products which are readily leached into wastewater after laundering.

Chapter 3

PRELIMINARY STUDIES

A. Occupational tick bite logs

Several divisions within the North Carolina Department of Environment and Natural Resources (NCDENR) and the North Carolina Department of Transportation (NCDOT) have instituted a tick bite reporting policy in order to collect information on the frequency of tick bites among employees. The use of these "tick bite logs" was motivated by the difficulties in obtaining workers compensation after developing medical complications due to tick bites. The tick bite logs are intended to serve as a mechanism to confirm an employee's occupational tick bite history in the event of a tick bite related medical claim. Employees are asked to report all tick bites to their supervisor, which are recorded in monthly tick bite logs. The Location and Surveys Unit of the NCDOT was the first division to implement this policy due to the high level of exposure to ticks among field staff and the presence of a highly motivated safety officer. All tick bite logs from 2006 through 2008 were requested from NCDOT Locations and Survey Unit field offices, and data was available for five of the thirteen field offices. Table 1 shows the descriptive statistics by year for all tick bites reported between 2006 and 2008 among employees of these five offices (from a total of approximately 40 employees).

	2006	2007	2008	2006-2008 (Cumulative)
Total tick bites	115	72	77	264
Mean*	4.3	2.5	2.7	3.1
Standard deviation*	4.6	2.8	2.7	3.5
95% CI (Poisson distribution)	3.5, 5.1	1.9, 3.1	2.1, 3.3	2.7, 3.5
Range*	1 - 24	1 - 14	1 - 11	1 - 24

* Tick bites per employee among employees who reported at least one tick bite.

Table 1. Tick bite log data from Locations and Survey Units, NCDOT, 2006-2008.

The peak season for tick bites was consistent across all three years, with most tick bites reported between April and August (Figure 5). We chose study periods of March through September for our study to ensure that we will capture the majority of the active tick season, including an extra month at the beginning and end of the peak season in the case of an early or delayed tick season in the study years.
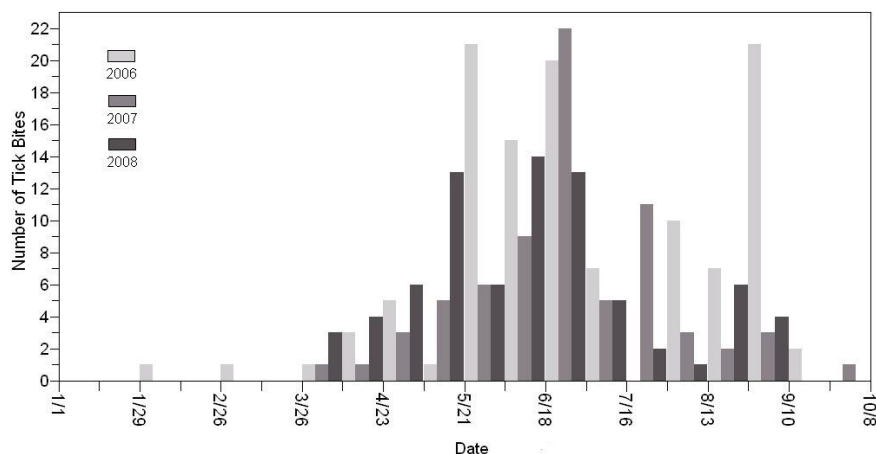
Figure 5. Number of tick bites reported by Locations and Surveys Unit of NCDOT by week, 2006-2008.

Information on the location of the tick bite(s) on the body is also collected on the NCDOT tick bite logs. For all tick bites reported between 2006 and 2008, 11% of bites were on the head or neck, 12% of bites were on the arms, 17% of bites were in the groin area or on the buttocks, 26% of bites were on the legs, and 34% of bites were in the midsection area (chest, waist, and back). These data show that the majority of tick bites take place in areas

that are covered by clothing. Using barrier methods such as tucking pants into socks or boots may prevent many ticks from getting inside pant legs, but previous studies of outdoor workers have shown that only a small percentage practice this method on a regular basis [61, 87]. The use of permethrin impregnated clothing is an alternative strategy that can prevent ticks from getting underneath clothing by immobilizing (and then killing) any ticks that come into contact with treated clothing.

The North Carolina Division of Parks and Recreation (NCDPR) and the North Carolina Division of Forest Resources (NCDPR), two of the sources of the study population for our study, began implementation of the tick bite reporting policy in 2008. The tick bite log data for 2008 was requested from all state parks and county level forest service offices. NCDPR 2008 tick bite log data was compiled by state park and the mean number of tick bites per employee was calculated for each park. The density of tick bites by park was mapped and is displayed in Figure 6a. Similarly, the 2008 tick bite log data from the NCDFR was compiled by county and the mean number of tick bites per county is shown in Figure 6b. Data was unavailable for some NCDFR county offices where the reporting policy had not yet been fully adopted (counties displayed in white). Both maps indicate the low incidence of tick bites in the western part of the state, which is consistent with the low rates of tick-borne disease in this region. Based on this information we chose to limit our study area to the eastern and central parts of the state, corresponding to NCDFR regions 1 and 2, where the incidence of tick bites is highest. The overall mean number of tick bites per employee for NCDPR employees and NCDFR employees within regions 1 and 2 for 2008 was 3.1 and 5.6, respectively.
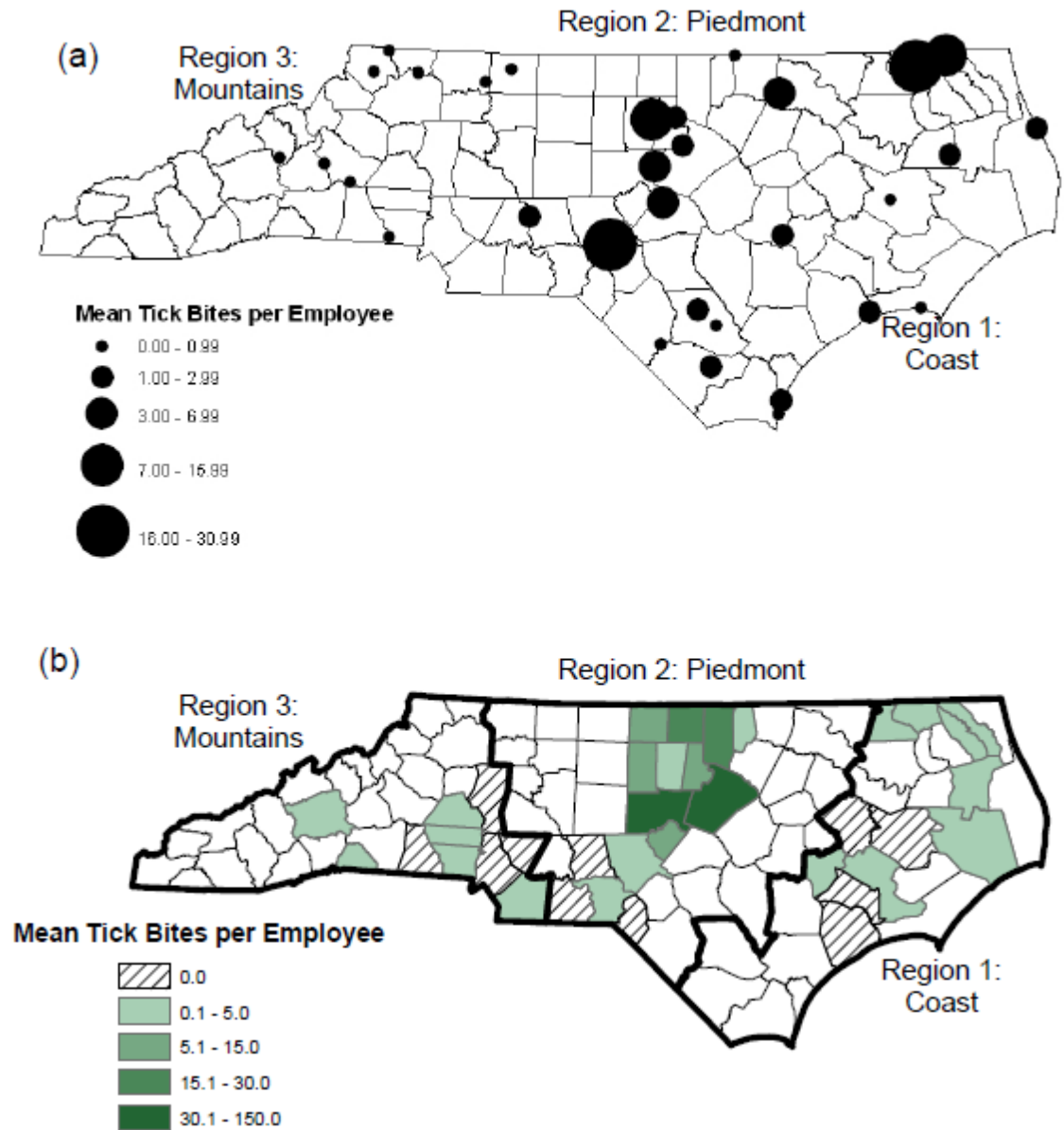
Figure 6. Mean number of tick bites per employee, 2008. (a) Tick bites reported for each state park for employees of the North Carolina Division of Parks and Recreation. (b) Tick bites reported by county for employees of the North Carolina Division of Forest Resources. Data was unavailable for counties in white.

B. North Carolina state employee workers compensation claims related to tick bites

Given the large number of tick bites sustained by North Carolina state employees who perform outdoor work, we were interested in the financial cost of these tick bites and associated illnesses. We requested workers compensation claim data that included the

work "tick" in the claim from 2004-2009. Over the six year period there were 147 claims related to tick bites, with a total payout of over $114,000 (Table 2). Of note, in 2008 and 2009 a much lower percentage of claims were paid, which motivated the implementation of the tick bite logs across many of the divisions in DENR. Overall these claims are likely to grossly underestimate the true "cost" of tick bites among outdoor workers in North Carolina, as it is likely that many employees who seek treatment following a tick bite do not file a claim (personal communication, Chuck Stanfill, DENR Safety Director). Further studies are needed to estimate the medical costs and lost work time due to tick bites and related illnesses.

Year	Tick related claims	Tick related claims paid	Total payout
2004	19	14	$28,841
2005	29	25	$34,643
2006	20	16	$10,824
2007	38	29	$36,786
2008	22	9	$1,699
2009 (thru 9/9/09)	19	8	$1,286
TOTAL (2004-2009)	147	101	$114,079

Table 2. North Carolina state employee workers compensation tick-related claims 2004-2009.

C. Pilot study: Effectiveness of long-lasting permethrin-impregnated clothing for the prevention of tick bites

In 2009, we conducted a nonrandomized open label pilot study to determine the effectiveness of Insect Shield® treated clothing for the prevention of tick bites among sixteen outdoor workers from the North Carolina Division of Water Quality [110]. Treatment status was self-selected, all subjects chose whether to have their clothing treated with permethrin or to serve as "controls". Subjects who chose to be in the treatment group were asked to submit all items of clothing normally worn while performing field work , including

shirts, pants, socks, hats, and boots. All subjects were instructed to launder their clothing as they normally would and to continue with their normal tick bite prevention measures, regardless of their treatment status.

Participants completed questionnaires at the start of follow-up (March 2009) and at the end of follow-up (September 2009), and tick bites and outdoor work hours were reported on weekly tick bite logs for the entire follow-up period.   During the follow-up period there were 68 tick bites reported by the subjects in the control group (mean=9.7 bites per subject), and 6 tick bites reported by the subjects in the treatment group (mean=0.7 bites per subject) (Figure 7). Crude incidence rates and incidence rate ratios were computed using negative binomial regression. Subjects wearing Insect Shield® treated clothing had a 93% reduction ($p < 0.0001$, 2-tailed Chi-square test) in the total incidence of tick bites compared to subjects using standard tick bite prevention measures (Table 3). This pilot study provides preliminary evidence that long-lasting permethrin-impregnated clothing may be highly effective against tick bites.
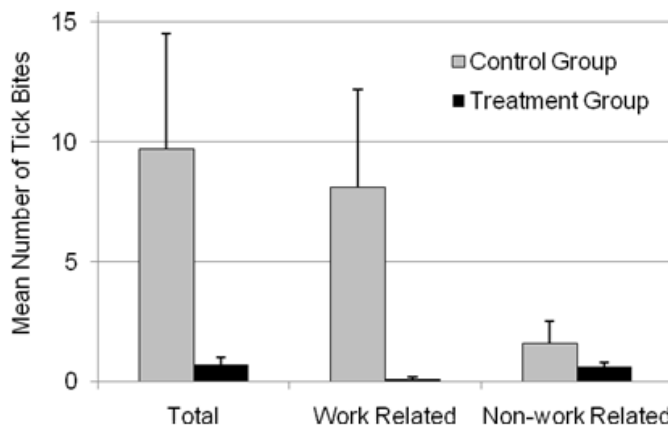
Figure 7. Mean number of tick bites by treatment group, including total, work related, or non-work related tick bites from a pilot study on the effectiveness of Insect Shield® treated clothing for the prevention of tick bites [110].

	Tick Bite Rate		IRR* (95% CI†)	p-value
	Control Group	Treatment Group		
Total (per 100 outdoor hours)	2.32	0.16	0.07 (0.02, 0.24)	<0.0001
Work Related (per 100 outdoor work hours)	4.68	0.05	0.01 (0.001, 0.11)	<0.0001
Non-work Related (per 100 outdoor non-work hours)	0.73	0.31	0.42 (0.10, 1.79)	0.24

*IRR: Incidence rate ratio
†CI: Confidence interval

Table 3. Estimates of tick bite incidence rates and incidence rate ratios from a pilot study on the effectiveness of Insect Shield® treated clothing for the prevention of tick bites [110].

Chapter 4

RESEARCH DESIGN AND METHODS

A. Seroepidemiology of spotted fever group rickettsiae in North Carolina

1. Selection of case patients

Case patients were identified from a database of patients who were tested for RMSF at the North Carolina State Laboratory of Public Health, between 2008 and 2010. This database included any patients for which sera had been submitted for serologic testing against *R. rickettsii*, which does not necessarily mean they had symptoms of RMSF, but just that their physician ordered the test. All sera submitted to the NCSLPH serology unit are retained and stored at -20 ºC for up to 3 years, so all patients listed in the database for which sera was available at the time of the study were candidates for inclusion in the study.

Eligibility criteria for inclusion in the study population consisted of:

- Having been tested for RMSF by IFA at the NCSLPH between 2008 and 2010

- Availability of paired sera (acute and convalescent)

- At least one of the sera had a titer ≥ 1:64 against *R. rickettsii*

Patients were excluded from the study if there was only a single serum specimen available, if there was not sufficient sera remaining for additional testing, or if the sera could not be located.

2. Indirect Immunofluorescence Assay (IFA)

The IFA was conducted according to the protocol used by the Centers for Disease Control and Prevention [111], with minor modifications as per the recommendations of the staff of the special serology unit at the NCSLPH. All testing was completed by trained staff

at the NCSLPH, as well as myself, after completing training by Gaylen Daves, the senior technician of the special serology unit.

a. Preparation of antigen slides

R. rickettsii, R, typhi, R. parkeri, and *R. amblyommii* antigens were provided by William Nicholson, Rickettsial Zoonoses Branch, CDC. Frozen lyophilized antigen was resuspended sterile deionized distilled water. Antigen was spotted onto the wells of glass templated slides using capillary tubes. Each divided well was spotted with either *R. typhi* and *R. rickettsii*, or *R. parkeri* and *R. amblyommii*, to allow for side by side comparison of fluorescence and staining patterns. Antigens spots were allowed to air dry and then fixed in acetone for 15 minutes at room temperature. Slides were then stored in sealed boxes at -70 ºC until they were ready to be used.

b. Sample preparation and dilution

Frozen slides were placed at room temperature and allowed to warm while serial dilutions were being made. Dilutions were prepared in u-bottom 96-well microplates. The primary dilution of 1:64 was prepared in 3% egg yolk for the *R. rickettsii* antigen and 1% bovine serum albumin (BSA) in phosphate buffered saline (PBS) *for R. parkeri, R. amblyommii,* and *R. typhi*. Remaining serial two-fold dilutions were made in PBS with 1% BSA for all antigens. Sera were diluted to a final dilution of 1:2048, except for positive control sera, which were diluted to 1:16384. Dilutions were applied to the slides and incubated in a humid chamber at 37 ºC for 30 minutes. Slides were rinsed in PBS, washed in PBS for 10 minutes, and washed in distilled water for 10 minutes, and air dried. Fluorescein isothiocyanate (FITC)-labeled goat anti-human IgG conjugate (Scimedx, Danville, NJ) with Eriochrome black counterstain was applied to each well and incubated in a humid chamber at 37 ºC for 30 minutes. Slides were washed as described above and once dried a drop of buffered glycerol was added to each well and overlaid with a coverslip. Slides were stored in the dark until they were read (within 4 hours).

c. Reading slides and interpretation of results

Slides were read on a UV epifluorescence microscope, and wells were initially examined at low power (100x) and then high power (400x). After reading the negative control wells, the positive control and test samples were read from highest dilution to lowest dilution. Fluorescence was scored according to brightness and consistency of staining throughout the well on a scale of 4+, 3+, 2+, 1+, +/-, and -, with 4+ being the most intense fluorescence. Endpoint titers were recorded as the reciprocal of the dilution with 1+ fluorescence. If staining intensity did not increase as expected from higher dilutions to lower dilutions or if non-specific staining of the egg yolk, cytoplasm, or nucleus was observed, then non-specific staining was noted and no endpoint titer could be determined. Staining for negative controls was confirmed by the absence of specific fluorescence (either +/- or -). Digital photos were taken of a small subset of samples to illustrate the fluorescence patterns that were observed for each antigen.

d. Quality control

The assays were performed in a CLIA certified laboratory, by trained technicians. Positive control sera from a patient with a known titer against *R. rickettsii* antigen was included in each run (including dilutions from 1:64 to 1:16384). Slides for which the positive control was within one dilution of the known titer were considered acceptable. There were no positive control sera available for *R. parkeri* or *R. amblyommii*, so the positive control sera for *R. rickettsii* was used on these slides. All sera were also tested against *R. typhi* (as per standard procedure) to rule out that the patient had been exposed to a typhus group rickettsia. Negative controls, including PBS only, PBS with BSA and a negative control sera that is non-reactive to *R. rickettsii* (primary dilution only) were also included in each run. If a slide showed any reactivity in the control wells the assay was considered invalid and was repeated. Paired sera were always tested together, in the same set of samples, by the same technician. If a sample had a questionable result or if problems with the staining were

noted, the senior technician reevaluated the slide and either made the final call on the endpoint titer or noted there was nonspecific staining, or suggested that the test be repeated. If a test needed to be repeated, both samples (acute and convalescent) were repeated together. For samples with a high level of debris or filmy appearance, the sera were centrifuged at 10,000 RPM for 5 minutes and/or heat inactivated for 30 minutes at 56 ºC, and the assay was repeated. For samples that did not give a titer against *R. rickettsii* that was within one dilution of the original titer, the assay was repeated and the titer that was found in the majority of the assays was accepted as the correct titer.

3. Surveillance reports

In North Carolina, when a patient tests positive against *R. rickettsii* antigen it is required that the results are reported to the NC Division of Public Health, and in each case, an effort is made by the state vector-borne nurse consultant to collect relevant clinical data from that patient to determine if that patient meets the case definition for "Spotted Fever Group Rickettsiosis (including Rocky Mountain spotted fever)". In some cases a public health nurse will interview the patient or health care provider who saw the patient, in some cases the medical record is reviewed, and other cases no information other than the laboratory report is available. Surveillance reports from the NC Division of Public Health (NCDPH) were requested for all patients included in this study. Sample numbers from the NCSLPH were cross-referenced with event numbers in the North Carolina Electronic Disease Surveillance System (NCEDSS) and all available reports were provided in paper format (deidentified). A form was created in Qualtrics and relevant fields were entered into the form for each patient.

a. Variables

The variables were created based on part 2 of the communicable disease report for Rocky Mountain spotted fever (Appendix A) and modified as needed to reflect the new electronic reporting format and response options. The variables are listed in Table 4.

Variable	Type
DEMOGRAPHIC INFORMATION	
Age	Continuous
Gender	Binary
Race	Nominal
Ethnicity	Nominal
CLINICAL INFORMATION	
Patient symptomatic?	Binary
Symptoms/clinical findings	
Fever	Binary
Headache	Binary
Myalgias	Binary
Skin rash	Binary
Fatigue/malaise/weakness	Binary
Chills/rigors	Binary
Nausea	Binary
Vomiting	Binary
Meningitis	Binary
Encephalitis	Binary
Acute renal failure	Binary
Thrombocytopenia	Binary
Leukopenia	Binary
Anemia	Binary
Elevated liver enzymes	Binary
Took antibiotics for illness?	Binary
Patient hospitalized?	Binary
Discharge/final diagnosis	Nominal
Died from illness?	Binary
SOURCE INFORMATION	
Patient interviewed?	Binary
Health care provider(s) consulted?	Binary
Medical record reviewed?	Binary

Table 4. Variables abstracted from surveillance reports of patients tested for R. rickettsii by IFA at the NCSLPH.

4. Outcome definition: seroconversion

A seroconversion was defined as a four-fold or greater change in IgG titer against an antigen between acute and convalescent samples. This includes not only a four-fold

increase but also a four-fold decrease in titer, which can occur when the acute sample is drawn later after the onset of illness at the peak of IgG production. For each antigen, the patient was classified as either a seroconversion, stationary titer (lack of four-fold or greater change in titer), or unknown (if one or more of the samples had non-specific fluorescence or was unreadable).

5. Sample size

IFA results from patients in the NCSLPH database that had been tested for RMSF between 2008 and 2010 who had at least two sera tested (n= 311) were reviewed. Of these patients, 126 (40.5%) had at least one sample with a titer of ≥1:64. Eight samples could not be located, so those eight patients were excluded, leaving 118 patients in the study. Surveillance reports were available for approximately half of the patients.

6. Human subjects

This research was approved by the Institutional Review Board at the University of North Carolina at Chapel Hill. The use of the data was approved by the North Carolina Division of Public Health through a Data Use Agreement with Meagan Vaughn and Steven Meshnick. Since all data are deidentified, informed consent was not necessary as the risk to subjects was minimal.

7. Data analysis

a. Frequency of seroconversions

Standard descriptive statistics were calculated for demographic and clinical variables. Our principal interest was the frequency of seroconversions against each antigen. Patients who had an unknown outcome for any of the antigens were excluded from the analysis. With three antigens, there are seven possible combinations of seroconversion (excluding those patients who had stationary titers against all three antigens). Each patient who seroconverted to at least one antigen was classified into one of the possible outcomes listed in Table 5. A table and 3-way quantitative Venn diagram were created to illustrate the

36

degree of cross-reactivity and individual reactivity between the antigens. Positive percent agreement and negative percent agreement were calculated for *R. parkeri* and *R. amblyommii* using seroconversion against *R. rickettsii* as the referent comparison.

Outcome	Antigen(s)
1	*R. rickettsii*
2	*R. parkeri*
3	*R. amblyommii*
4	*R. rickettsii* and *R. parkeri*
5	*R. rickettsii* and *R. amblyommii*
6	*R. parkeri* and *R. amblyommii*
7	*R. rickettsii, R. parkeri*, and *R. amblyommii*

Table 5. Possible seroconversion outcomes for each patient, excluding patients who had stationary titers against all three antigens.

b. Comparison of clinical profiles

Based on the theory that many patients in North Carolina are being infected with non-pathogenic or less pathogenic species of rickettsiae, I hypothesized that patients who seroconverted to *R. parkeri* and/or *R. amblyommii* had a less severe illness than those who seroconverted to *R. rickettsii*. Unfortunately, there was insufficient clinical data to make useful comparisons. Instead, the clinical profiles for the 12 patients with clinical data who seroconverted to at least one antigen are presented in a line listing.

B. Permethrin impregnated uniforms for tick bite prevention among outdoor workers

The design for this study is a double-blind randomized controlled trial. The study was conducted for 2 tick seasons (19 months, March, 1, 2011 through Sept, 30, 2012). Participants were randomized into two groups, the treatment group and the control group. Uniforms were collected from all participants, but only the uniforms from members of the treatment group were factory-impregnated with permethrin. All participants were asked to record any tick bites during the study period on weekly tick bite logs. Questionnaires were

administered to all participants at the start and end of the study period which collected general information on tick exposure and tick prevention practices.

1. Study population

The study population includes employees of the NC Division of Forest Resources (NCDFR), the NC Division of Parks and Recreation (NCDPR), NC Wildlife Resources Commission (NCWRC), and NC County and Local Parks and Recreation (NCCLPR). The NCDFR has offices in nearly all of the 100 counties in North Carolina, as well as 13 district offices and 3 regional offices. We recruited NCDFR employees who are based in regions 1 and 2, which correspond to the piedmont and coastal regions of the state. Employees from region 3 (the mountain region of the state) were not included in this study due to the low number of tick bites reported from that region. The NCDPR has staff at each of the 34 state parks and the 4 state recreation areas. We recruited employees from state parks that lie within the boundaries of the NCDFR regions 1 and 2, which includes 26 of the 38 state managed parks. The NCWRC has offices throughout the state including approximately 50 employees in the piedmont and coastal areas. Eligibility criteria included being over 18 years of age, spending an average of 10 or more hours of outdoor work per week during tick season, having to wear a uniform while on the job, and completion of written informed consent. Exclusion criteria included pregnancy or a planned pregnancy during the follow-up period (since exposure to an insecticide is involved), non-English speakers, or having a known allergy to insecticides.

This population was selected for this study because the work duties for most employees of these agencies put them in direct and frequent contact with tick-infested habitats, and thus represent a highly exposed occupational group for tick bites. In addition, most employees of these agencies are required to wear a uniform, which provided a logistical advantage for the study as it was possible to treat the entire set of uniforms owned by each participant, thus ensuring consistent use of the product throughout the study.

a. Recruitment and retention

From the personnel database provided by NCDENR, all potential study participants from NCDFR and NCDPR were sent preliminary announcements with information about the study by email. Informational meetings were conducted at local and regional offices and parks throughout the study area between October 2009 and April 2010. Eligible employees were invited to enroll in the study at the informational meetings. Female employees who were interested in participating in the study were screened for pregnancy by self-report, and were offered a free pregnancy test. Upon enrollment into the study, subjects were given a small LED flashlight and 6 pairs of boot socks as a token of appreciation for their participation. Participants who completed the first year of follow-up were given an additional 6 pairs of boot socks as an incentive to complete the final year of follow-up.

2. Study protocols

a. Baseline questionnaire

After completing the informed consent process, study participants were asked to complete either a paper or web-based questionnaire which collected demographic and occupational information, history of tick-bites and tick exposure in the past year, history of tick-borne disease, and use of tick-bite prevention measures (Appendix B).

b. Randomization

Subjects were assigned a study ID number upon enrollment. Block randomization using block sizes of six generated through random numbers by computer was used to ensure the treatment and control groups had approximately equal numbers of participants in each group. The randomization list was released to the study investigators after all tick bite log data collection was completed in November 2012.

c. Collection and processing of uniforms

Subjects were asked to write their name on all of their work uniforms with a laundry pen. Given that it took several weeks week to ship, treat, and return the uniforms to each subject,

it was impossible to treat the entire set of uniforms for each subject at one time because they need to have a set of uniforms to wear while the other sets were being treated. Thus, the treatment process was conducted in two stages. Subjects were asked to send half of their spring/summer work uniforms, including overshirts, t-shirts, pants, shorts, hats, and socks directly to the Insect Shield facility in Greensboro, NC. Each shipment included a packing list that listed the number and type of garments included. Once the clothing was received at Insect Shield, all items of clothing were labeled with a small heat sealed label with the subject's ID number to ensure proper processing and return to their owner. After determining the assigned treatment group for that identification number according to the randomization list, the clothing was either treated with permethrin according to Insect Shield's® patented process (treatment), or was simply washed and dried in a commercial washer/dryer (control). Clothing was folded and bagged by ID number, after which it was shipped back to the subject. The same process was repeated (using the existing treatment assignments) for any remaining items sent by the subject. Since many subjects ordered new uniforms during the study period, subjects were provided with shipping materials and instructed to send all new uniforms items to Insect Shield® before wearing them. Clothing treated using Insect Shield® technology has no odor, texture, or any other characteristic that would allow treated clothing to be distinguished from untreated clothing, thus subjects were blinded to their treatment status.

    d. Tick bite logs

During the follow-up period, all subjects were asked to keep a diary of all tick bites (attached or embedded ticks) which were recorded on weekly tick bite logs (Appendix C). Upon enrollment each subject was provided with either blank paper tick bite logs (to be submitted by mail) or a blank electronic tick bite log (to be submitted by email). For each entry in the log, subjects recorded the date of the tick bite(s), the number of tick bites, the

location of the tick bites on the body, the county where the tick bite(s) were most likely to have been acquired, whether they were on the job when the tick bite(s) were acquired, whether they had been using insect repellent at the time of the bite(s) and the type of repellent, and whether the tick(s) were collected. If there were no tick bites during a week, there was a checkbox to indicate that there were no tick bites. Subjects were also asked to record the number of work related and non-work related hours spent outdoors each week, which provided an estimate of the amount of potential exposure a subject had to tick-infested areas. At the end of each month subjects were asked to submit all tick logs from that month to the study investigators by mail or email. Email reminders were sent to all subjects the day that the tick bite logs were due (the first day of the month). If a subject did not submit their tick bite logs within one week of the reminder email then a second reminder email was sent.

e. Follow-up questionnaires

At the end of the first and second years, all subjects were asked to complete a follow-up questionnaire. These questionnaires were similar to the baseline questionnaire, but asked questions about work habits, tick exposure, and use of tick bite prevention measures during the study period. These questionnaires also asked about frequency of bites from mosquitoes, chiggers, and midges.

3. Outcome assessment

The incidence of tick bites was assessed using the data collected from the tick bite logs. To ensure that only attached ticks were reported on the tick bite logs, participants were instructed to report tick bites in which the tick was found attached to or embedded in the skin. Subjects were instructed that unattached ticks found crawling on the body did not need to be reported on the tick bite log. Tick bite diaries have been used successfully in other studies to collect tick bite count information [112-114]. By asking participants to complete tick bite logs on a weekly basis, we anticipated that the more frequent reporting

mechanism would encourage subjects to remember to record tick bites. Since they were

also asked to collect all attached ticks they removed from their body and mail them to the

entomology laboratory at North Carolina State University, we attempted to validate the

monthly totals from the tick bite logs by comparing this to the number of ticks found in the

collection vial for each month.

4. Covariates

Information on covariates that could have affected the probability of acquiring tick bites

during the study period (other than treatment assignment) were collected in the baseline and

follow-up questionnaires. Variables that were evaluated as potential covariates are shown

in Table 6.

Variable	Variable type	Data source
Age (at enrollment)	Continuous	Baseline questionnaire
Gender	Dichotomous	Baseline questionnaire
Race/ethnicity	Nominal	Year 1 questionnaire
DENR division	Nominal	Baseline questionnaire
Occupation	Nominal	Baseline questionnaire
Years at current position	Continuous	Baseline questionnaire
Education	Nominal	Baseline questionnaire
Number of tick bites in year prior to enrollment	Continuous	Baseline questionnaire
Previous diagnosis with a tick-borne illness	Nominal	Baseline questionnaire
Average number of hours spent outdoors per week on the job	Continuous	Baseline questionnaire and tick logs
Average number of hours spent outdoors per week (non-work related)	Continuous	Baseline questionnaire and tick logs
Frequency of wearing long pants when working outdoors	Ordinal	Year 1 and Year 2 questionnaires
Frequency of wearing a long sleeved shirt when working outdoors	Ordinal	Year 1 and Year 2 questionnaires
Frequency of wearing a hat when working outdoors	Ordinal	Year 1 and Year 2 questionnaires
Frequency of tucking pants into boots or socks, or taping pants to boots	Ordinal	Year 1 and Year 2 questionnaires
Frequency of insect repellant use on the skin while working outdoors	Ordinal	Year 1 and Year 2 questionnaires

Frequency of insect repellent use on clothing while working outdoors	Ordinal	Year 1 and Year 2 questionnaires
Frequency of checking self for ticks when working outdoors	Ordinal	Year 1 and Year 2 questionnaires
Frequency of checking self for ticks at the end of the work day	Ordinal	Year 1 and Year 2 questionnaires
Use of self-applied permethrin on clothing	Dichotomous	Year 1 and Year 2 questionnaires

Table 6. Covariates to be evaluated for inclusion in multivariable regression analysis.

5. Human subjects

   a. Human subjects involvement and IRB approval

The study population included employees of the NC Division of Forest Resources, the NC Division of Parks and Recreation, NC Wildlife Resources Commission, and NC County and Local Parks and Recreation. Healthy volunteers were recruited from all regional offices within the central and eastern regions of the state. Subjects eligible for the study were at least 18 years of age, spoke English, and spent an average of at least 10 hours per week working outdoors. Participants were not excluded on the basis of race, ethnicity, or gender. Potential participants were excluded on the basis of English-language only because the study resources could not provide for a translator. Potential participants were excluded on the basis of age (<18 years) to assure proper understanding of the consent procedure. Written consent was obtained for all subjects at an enrollment meeting or by mail. This research was approved by the Institutional Review Board at the University of North Carolina at Chapel Hill (IRB# 10-1027).

   b. Potential risks

The risk posed to subjects in the treatment group by wearing permethrin impregnated uniforms is minimal. A potential, but extremely unlikely adverse effect that could be observed among wearers of the permethrin impregnated uniforms was minimal skin

sensitivity. As with all studies involving human subjects, there was a potential risk of breach of confidentiality to all subjects by participation in this study.

    c.   Protection against risk

    The consent forms and tick bite logs were mailed to the subjects in envelopes that had no indication of the study focus. Study subjects were emailed a link and password to access the web-based questionnaires, and web-based questionnaires used 128 bit SSL encryption to protect the privacy of the study subjects. The investigators keep hard copies of data and informed consent forms in a locked file cabinet. To protect privacy, records were filed under code numbers rather than by name. A master list of code numbers relating the participant names to the codes were kept in a separate location in a locked file cabinet. Digital copies of data were maintained in password protected files. Only the study investigators were allowed to look at the data. If a subject experienced any adverse effects that they believed were related to their uniforms (for which they were blinded to the treatment status of), they were asked to report those adverse effects to the study investigators. All participants were instructed to perform self-inspections daily and to remove attached ticks promptly. All subjects were also instructed to continue their usual tick-bite preventive practices. Participants were provided with a wallet-sized card with information about the symptoms of tick-transmitted illnesses and were instructed to seek medical attention immediately if they developed any of the symptoms. Free medical attention for suspected tick-related illnesses was provided by the study physician (Dr. Jonathan Juliano) if requested. If a subject saw Dr. Juliano at UNC Hospitals, all clinic/hospital charges for the visit and fees for his time were covered by the study. Any fees for laboratory tests or treatments were borne by the subject, except for any testing performed by the study investigators (serology for tick-borne pathogens).

d. Potential benefit to the subjects and others

The direct benefit to the subjects was the potential for decreased tick bites (and tick-borne disease) and other insect/arthropod bites among those wearing the permethrin impregnated clothing. Since subjects in the control group did not receive this benefit during the study period, free treatment of uniforms was offered to all control subjects at the end of the study. Information gained in this study could lead to policy recommendations for outdoor workers on how best to prevent tick bites and tick-borne disease in this high-risk population.

e. Data Safety and Monitoring Board (DSMB)

According to the Dan Nelson, Director of UNC's Office of Human Research Ethics, this project did not constitute a clinical trial because it did not involve "drugs, treatments, devices, or new ways of using known drugs, treatments, or devices" as in the NIH definition. Thus, a DSMB was not mandated. However, after the first year of follow-up a DSMB was convened (UNC TraCS DSMB) to determine whether the number of seroconversions observed in year 1 was possibly related to treatment status, and whether the study should be stopped early. Based on the recommendation of the DSMB, the study was allowed to proceed as planned.

6. Data analysis

a. Data management and data quality

Data from the web-based questionnaires were automatically entered into a database linked to the online questionnaire. Logical checks were built into the questionnaire to prevent invalid entries. Two identical databases were created for the tick logs, and study personnel entered data from tick bite logs into both databases, which were compared to ensure accuracy. Discrepancies were resolved by examining the original written or electronic records. Consistency and range checks were implemented during the data cleaning process, and inconsistencies or implausible values were checked by reviewing the original records.

b. Descriptive analyses

Variables derived from the data collected in the baseline questionnaire were used to describe tick exposure and use of tick bite prevention practices among this group of outdoor workers. Standard univariate analysis methods were used to summarize exposure and treatment variables and covariates, including frequencies, means, standard deviations and graphical methods. This data was used to assess the comparability of the treatment and control groups and to determine whether randomization was successful. T-tests were used to compare continuous variables including mean age, mean number of years at current position, mean number of outdoor hours worked per week, mean number of outdoor hours (non-work related) per week, and total number of tick bites in the previous year. Chi-square tests (2-tailed) were used to compare proportions for categorical variables listed in Table 6. Any variables that showed statistically significant differences (alpha level=0.05) between the treatment and control groups, or were significantly associated with the tick bite incidence rate in the control group were to be considered for inclusion as covariates in regression models.

c. Incidence of tick bites

All analyses were conducted on an intent-to-treat basis, assuming that subjects who were assigned to the treatment group adhered to their assignment and wore treated study uniforms while on the job throughout the study period. The start of follow-up was defined as the date the subject started wearing treated uniforms (either as reported by the subject or as determined by the date staff were instructed to switch to wearing summer uniforms). Person-time at risk was computed as the sum of the outdoor work hours reported by each subject on their tick logs between the start of follow-up and September 30th of each year. The incidence rate ratio (and 95% confidence interval) for tick bites comparing the treatment and control groups was estimated using a generalized estimating equations (GEE) approach for Poisson regression [115]. The use of GEE methods made it possible to account for the

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within subject correlation due to repeated weekly measures using a working correlation matrix. The appropriate structure of the working correlation matrix was determined by examining the correlation coefficients when the model is specified using an unstructured working correlation matrix. Protective effectiveness (1 – the incidence rate ratio) [116] and 95% confidence intervals for comparing reported tick bites between the treatment and control groups were calculated using a generalized estimating equations (GEE) approach for Poisson regression. The use of GEE methods accounted for the within subject correlation due to repeated measures using a working correlation matrix.

The primary interest was to evaluate the effectiveness of permethrin-impregnated uniforms to reduce tick-bites that were acquired while on the job (while the subject is wearing study uniforms), therefore we excluded any reported tick bites that the subject believed to have been acquired while off the job. To determine if the effectiveness of the treatment wanes over time with wear and washing, we specified *a priori* that incidence rate ratios would be stratified by year of follow-up. If necessary, any potential covariates that were identified in the descriptive analyses would be included in a fully adjusted model and a backwards elimination strategy based on 10% change-in-estimate would be used to determine which variables would remain in the final model. Coding decisions for continuous variables were made after examining whether the variable(s) had linear or non-linear relationships with the outcome using graphical methods and indicator coding. Regression diagnostics were implemented to assess goodness-of-fit using the quasi-likelihood information criterion (QIC).

d. Missing data

It was anticipated that some subjects would have missing tick bite logs for some months or would be lost to follow-up during the study period. The GEE model assumes that missing observations are Missing Completely at Random (MCAR). The missingness of data was assessed for all covariates listed in Table 6 to determine whether the missing data occur

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MCAR. Since subjects were blinded to their treatment status, informative censoring by treatment status was unlikely, but this was also evaluated. We planned to implement multiple imputation methods if there was more than 5% missing outcome data and it was not MCAR.

7. Sample size and statistical power

To calculate the required sample size, we used the estimated mean number of tick bites per subject during the study period. The mean and standard deviation for the number of tick bites over the study period in the control group was estimated from 2006 – 2008 tick bite logs from the NCDOT (Table 1). These estimates are similar to the estimates from the tick bite logs NC Division of Parks and Recreation for 2008. For a 50% reduction in tick bites in the treatment group, we would expect to see a mean number of 3.10 tick bites per employee over the duration of the 2 year study period. The statistical power to detect a 50%, 40%, and 30% difference in the mean number of tick bites observed per subject with a 2-sided α at 0.05 level over the duration of the study for sample sizes of 100, 120, and 140 subjects is shown in Table 7.   A total of 159 subjects were enrolled in the study, with 127 subjects actively participating in the study during year 1 and 101 subjects active during year 2. Using the estimated difference in means, we had >90% and 88% power to detect a 50% difference in the incidence of tick bites in year 1 and year 2, respectively. The power to detect a difference as low as 30% in the incidence of tick bites between the control group and the treatment group was >50% for year 1 and 47% for year 2.

α	Sample size (total)	Difference in means	SD	Power
0.05	100	3.10 (50%)	4.9	0.879
		2.48 (40%)	4.9	0.707
		1.86 (30%)	4.9	0.466
0.05	120	3.10 (50%)	4.9	0.930
		2.48 (40%)	4.9	0.785
		1.86 (30%)	4.9	0.539
0.05	140	3.10 (50%)	4.9	0.961
		2.48 (40%)	4.9	0.845
		1.86 (30%)	4.9	0.606

Table 7. Statistical power based on mean differences in tick bites between treatment and control groups for total sample size 100, 120, and 140.

Chapter 5

**SEROLOGICAL EVIDENCE FOR HUMAN INFECTIONS WITH SPOTTED FEVER GROUP
RICKETTSIAE DUE TO SPECIES OTHER THAN *RICKETTSIA RICKETTSII* IN NORTH
CAROLINA**

A. Introduction

Rocky Mountain spotted fever (RMSF), caused by the bacteria *Rickettsia rickettsii*, is

the most commonly reported fatal tick-borne disease in the US. The incidence of spotted

fever rickettsioses (including RMSF) has been rising at an unexpected rate in recent years,

from 2.5 cases per million in 2001 to 9.5 cases per million in 2011 [117]. Mounting evidence

suggests that infections with other species of spotted fever group rickettsiae (SFGR) are at

least partly responsible for the increasing incidence. Serosurveys of adults and children in

the US show that seroprevalence to *R. rickettsii* is between 6% and 12% [31, 32]. Since

subclinical infection or mild infections with *R. rickettsii* are considered by experts to be rare

or even non-existent, and there is cross-reactivity among SFGR in serologic tests, exposure

to other species of SFGR could account for this relatively high seroprevalence [18]. For

many years *Rickettsia parkeri* was considered to be a nonpathogenic SFGR, until it was

isolated from a patient in Virginia in 2002 [35]. *R. parkeri* is now recognized as a human

pathogen and causes a milder illness than classic RMSF, often characterized by formation

of an eschar at the site of inoculation [19, 34]. In California, Rickettsia 364D has been

implicated in causing an eschar associated illness decades after it was first identified in

Dermacentor occidentalis ticks [56].   Recent studies have suggested that *Rickettsia*

amblyommii, which is present in a large percentage of lone star ticks (*Amblyomma*

americanum) may cause a mild rickettsiosis in humans [15, 16, 21, 23, 43, 44, 47]. In this

study, we evaluated the reactivity of paired sera from North Carolina patients who had been tested for RMSF to a panel of SFGR, including *R. rickettsii*, *R. amblyommii*, and *R. parkeri*.

B. Methods

Selection of case patients

Case patients were identified from a database of patients who were tested for RMSF at the North Carolina State Laboratory of Public Health, between 2008 and 2010. Samples are submitted from across the entire state, with the majority from patients in the piedmont region (central North Carolina). Eligibility criteria included having paired sera available (acute and convalescent)*,* and at least one sample had a titer ≥1:64 against *R. rickettsii* in the initial test. Patients were excluded from the study if there was only a single serum specimen available, if there were not sufficient sera remaining for additional testing, or if the sera could not be located.

Antigens

R. rickettsii, R, typhi, R. parkeri, and *R. amblyommii* antigens were provided by William Nicholson, Rickettsial Zoonoses Branch, CDC. Frozen lyophilized antigen was resuspended in sterile deionized distilled water. Antigen was spotted onto the wells of glass templated slides. Each divided well was spotted with either *R. typhi* and *R. rickettsii*, or *R. parkeri* and *R. amblyommii*, to allow for side-by-side comparison of fluorescence and staining patterns. Antigens spots were allowed to air dry and then fixed in acetone for 15 minutes at room temperature. Slides were then stored in sealed boxes at -70 ºC until they were ready to be used.

Indirect Immunofluorescence Assay (IFA)

All testing was conducted at the North Carolina State Laboratory of Public Health according to previously published methods for the IFA [118]. Briefly, frozen slides were placed at room temperature and allowed to warm while serial dilutions were prepared. The primary dilution of 1:64 was prepared in 3% egg yolk for the *R. rickettsii* antigen and 1%

bovine serum albumin (BSA) in phosphate buffered saline (PBS) *for R. parkeri, R. amblyommii,* and *R. typhi*. Remaining serial two-fold dilutions were made in PBS with 1% BSA for all antigens. Sera were diluted to a final dilution of 1:2048, except for positive control sera, which were diluted to 1:16384. Positive control sera from a patient with a known titer against *R. rickettsii* antigen was included in each run (including dilutions from 1:64 to 1:16384). Negative controls, including PBS only, PBS with BSA and a negative control sera that is non-reactive to *R. rickettsii* (primary dilution only) were also included in each run. Dilutions were applied to the slides and incubated in a humid chamber at 37 ºC for 30 minutes. Slides were rinsed in PBS, washed in PBS for 10 minutes, and washed in distilled water for 10 minutes, and air dried. Fluorescein isothiocyanate (FITC)-labeled goat anti-human IgG conjugate (Scimedx, Danville, NJ) with Eriochrome black counterstain was applied to each well and incubated in a humid chamber at 37 ºC for 30 minutes. Slides were washed as described above and once dried a drop of buffered glycerol was added to each well and overlaid with a coverslip. Slides were stored in the dark until they were read (within 4 hours).

Reading slides and interpretation of results

The North Carolina State Laboratory of Public Health utilizes a standardized reading system to accurately and consistently measure the intensity of fluorescence of the antigen-antibody complex due to the subjectivity of the IFA test. Paired sera were always tested and read together by the same technician. Slides were read on a UV epifluorescence microscope, and wells were initially examined at low power (100x) and then high power (400x). After reading the negative control wells, the positive control and test samples were read from highest dilution to lowest dilution. Staining for negative controls was confirmed by the absence of specific fluorescence. Slides for which the positive control was within one dilution of the known titer were considered acceptable. Fluorescence was scored according to brightness and consistency of staining throughout the well on a scale of 4+, 3+, 2+, 1+,

+/-, and -, with 4+ being the most intense fluorescence. Endpoint titers were recorded as

the reciprocal of the dilution with 1+ fluorescence. If staining intensity did not increase as

expected from higher dilutions to lower dilutions or if non-specific staining of the egg yolk,

cytoplasm, or nucleus was observed, then non-specific staining was noted and no endpoint

titer could be determined. If a sample had a questionable result or if problems with the

staining were noted, the senior technician reevaluated the slide and either made the final

call on the endpoint titer or noted there was nonspecific staining, or suggested that the test

be repeated. If a test needed to be repeated, both samples (acute and convalescent) were

repeated together. For samples that did not yield a titer against *R. rickettsii* that was within

one dilution of the original titer, the assay was repeated and the titer that was found in the

majority of the assays was accepted as the correct titer.

Surveillance reports

Surveillance reports from the NC Division of Public Health (NCDPH) were requested for

all patients included in this study. Sample numbers from the NCSLPH were cross-

referenced with event numbers in the North Carolina Electronic Disease Surveillance

System (NCEDSS) and all available reports were provided in paper format (deidentified) by

the NC Public Health Data Group. A form was created in Qualtrics and relevant fields were

entered into the form for each patient.

Outcome definition: seroconversion

A seroconversion was defined as a four-fold or greater change in IgG titer against an

antigen between acute and convalescent samples. For each antigen, the patient was

classified as either a seroconversion, stationary titer (lack of four-fold or greater change in

titer), or unknown (if one or more of the samples had non-specific fluorescence or was

unreadable).

Data analysis

Standard descriptive statistics were calculated for demographic and clinical variables. Positive percent agreement and negative percent agreement were calculated for each antigen using seroconversion against *R. rickettsii* as the referent comparison. For patients with surveillance reports available, clinical characteristics were compared for those who seroconverted to *R. rickettsii* and those who seroconverted to *R. parkeri* and/or *R. amblyommii*. All analyses were performed using SAS (version 9.2, SAS Institute Inc., Cary, NC, USA).

Human subjects

This research was approved by the Institutional Review Board at the University of North Carolina at Chapel Hill. The use of the data was approved by the North Carolina Division of Public Health through a Data Use Agreement. Since all data were deidentified, informed consent was not required as the risk to subjects was minimal.

C. Results

Case patients

IFA test results from patients in the NCSLPH database with paired sera that had been tested for RMSF between 2008 and 2010 were reviewed (n= 311). Of these patients, 126 (40.5%) had at least one sample with a titer of ≥1:64. Samples from 8 patients were no longer available, and 12 patients were excluded from the analysis due nonspecific fluorescence or an unreadable result for at least one of the antigens. Of the 106 patients included in the study, surveillance reports were available for 53. The majority of these patients were white males, with a median age of 50 (range 1-80) (Table 8). The demographic profile was similar for patients who seroconverted to at least one of the SFGR antigens and patients with stationary titers.

	All patients (n=53)	Seroconverters (n=12)	Non-seroconverters (n=41)
DEMOGRAPHIC INFORMATION			
Age – median (range)	50 (1 – 80)	49 (1 – 70)	51 (7 – 80)
Gender			
Male	29/47 (61.7%)	7/10 (70.0%)	22/37 (59.5%)
Female	18/47 (38.3%)	3/10 (30.0%)	15/37 (40.5%)
Race			
White	45/53 (84.9%)	10/12 (83.3%)	35/41 (85.4%)
Black	3/53 (5.7%)	0/12 (0%)	3/41 (7.3%)
Other	2/53 (3.8%)	2/12 (16.7%)	0/41 (0%)
Hispanic Ethnicity	3/42 (7.1%)	1/11 (9.1%)	2/31 (6.4%)
CLINICAL INFORMATION			
Symptoms/clinical findings			
Fever	27/46 (58.7%)	8/11 (72.7%)	19/35 (54.3%)
Headache	14/36 (38.9%)	4/10 (40.0%)	10/26 (38.5%)
Myalgias	24/40 (60.0%)	6/10 (60.0%)	18/30 (60.0%)
Skin rash	16/41 (39.0%)	3/11 (27.3%)	13/30 (43.3%)
Acute renal failure	2/29 (6.9%)	1/5 (20.0%)	1/24 (4.2%)
Thrombocytopenia	7/28 (25.0%)	3/6 (50.0%)	4/22 (18.2%)
Leukopenia	5/26 (19.2%)	3/5 (60.0%)	2/21 (9.5%)
Anemia	4/28 (14.3%)	2/6 (33.3%)	2/22 (9.1%)
Elevated liver enzymes	8/28 (28.6%)	2/5 (40.0%)	6/23 (26.1%)
Took antibiotics for illness	36/45 (80.0%)	9/9 (100%)	27/36 (75.0%)
Patient hospitalized	7/48 (14.6%)	2/12 (16.7%)	5/36 (13.9%)
Died from illness	0/20 (0)%	0/5 (0%)	0/15 (0%)

Table 8. Demographic and clinical characteristics of all patients with a surveillance record available, patients with a surveillance record who seroconverted to a SFGR, and patients with a surveillance record who did not seroconvert.

Seroconversions

Of the 106 eligible pairs tested, 10 patients seroconverted to *R. rickettsii* antigen in the original testing. In our subsequent testing, only 8 of the 10 seroconverted to *R. rickettsii* antigen. The two patients that seroconverted to *R. rickettsii* antigen in the original testing but did not meet the criteria for seroconversion to *R. rickettsii* in subsequent testing both produced titers that were within the one dilution allowance for intra-assay variability of the

IFA. In our subsequent testing, one of these patients seroconverted to *R. amblyommii* antigen and the other did not seroconvert to any of the SFGR antigens.

Overall, 21 patients seroconverted to one or more of the SFGR antigens in the testing performed for this study. The frequency of seroconversions to each antigen and cross-reactivity between antigens is depicted in Figure 8. Of the eight patients that seroconverted to *R. rickettsii*, seven also seroconverted to both *R. parkeri* and *R. amblyommii*. Eleven patients had seroconversions against a single antigen, one against *R. rickettsii*, four against *R. parkeri*, and six against *R. amblyommii*. Three patients had seroconversions against both *R. parkeri* and *R. amblyommii* antigens.

Antigen	Number of seroconversions
R. rickettsii only	1
R. parkeri only	4
R. amblyommii only	6
R. rickettsii and *R. parkeri*	0
R. rickettsii and *R. amblyommii*	0
R. parkeri and *R. amblyommii*	3
R. rickettsii, R. parkeri, and *R. amblyommii*	7
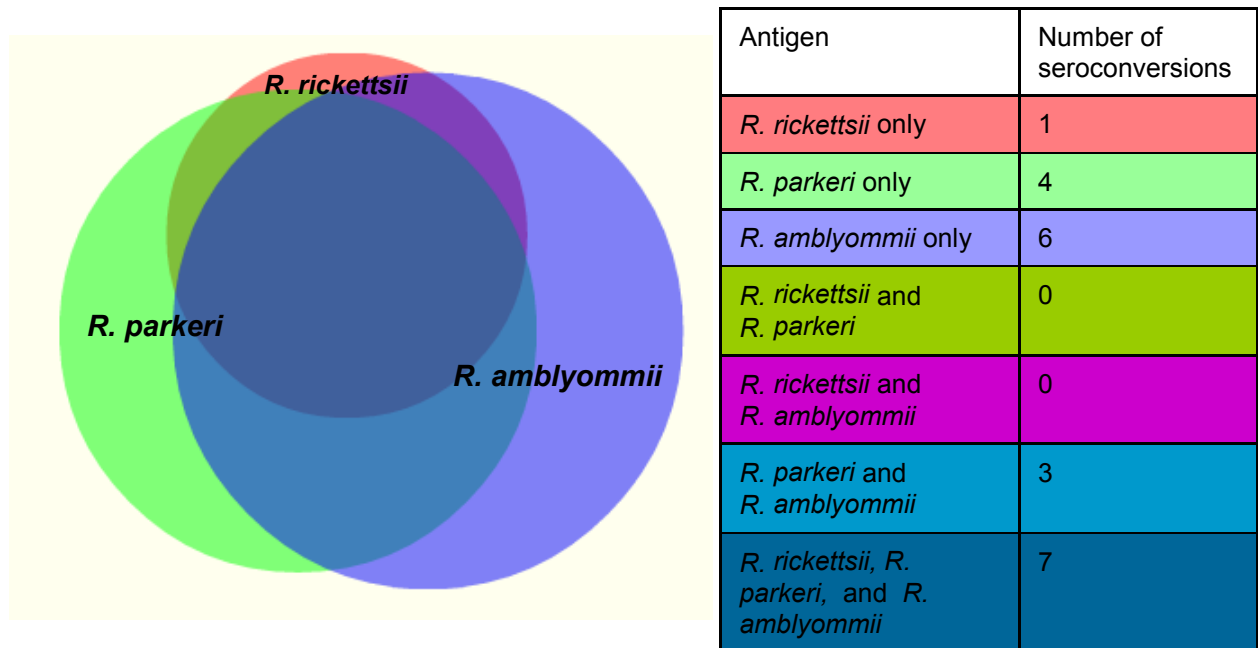
Figure 8. Proportional Venn diagram showing seroconversions to *R. rickettsii, R. parkeri,* and *R. amblyommii* antigens and degree of cross-reactivity by indirect immunofluorescent assay (generated by http://venndiagram.tk/).

The ability to detect seroconversions to *R. parkeri* and *R. amblyommii* using *R. rickettsii* antigen was poor, as measured by positive percent agreement of 50% and 43.8%, respectively (Table 9). The majority of positive agreement between seroconversion

classifications was due to broad cross-reactivity to all three antigens. The negative percent

agreement with *R. rickettsii* antigen was equally high for both *R. parkeri* and *R. amblyommii*

antigens (98.9%). There was very strong agreement between the seroconversion

classification of the original test and the retesting with *R. rickettsii* antigen (100% positive

percent agreement and 98% negative percent agreement).

Comparison	Positive Percent Agreement	Negative Percent Agreement
R. rickettsii and *R. parkeri*	50.0%	98.9%
R. rickettsii and *R. amblyommii*	43.8%	98.9%
R..rickettsii (retest) and *R. rickettsii* (original test)	100%	98.0%

Table 9. Assessment of seroconversion classification agreement between *R. rickettsii, R. parkeri,* and *R. amblyommii* antigens.

Clinical characteristics of seroconverters

Surveillance reports were available for half of the patients (n=53). Of these, 12 reports

were available from patients who seroconverted to at least one of the SFGR antigens.

Fever, thrombocytopenia, leukopenia, elevated liver enzymes, and acute renal failure

occurred more frequently among seroconverters, while skin rash was more common among

patients who did not seroconvert (Table 8). Headache and myalgias occurred with similar

frequency in both groups. Hospitalization was more common in seroconverters, however

no deaths were reported among any of the patients included in this study.

Similar frequencies of signs and symptoms was observed between patients who

seroconverted to all three antigens compared to those who seroconverted to *R. parkeri*

and/or *R. amblyommii*, with the exception of skin rash (Table 10). None of the patients who

seroconverted exclusively to *R. parkeri* and/or *R. amblyommii* antigens reported a rash, as compared to three of five patients with seroconversions to all three antigens. Hospitalization occurred in two seroconverters; in a patent who seroconverted to *R. parkeri* (patient 70) and in another patient who seroconverted to *R. amblyommii* (patient 31, who also seroconverted to *Ehrlichia chaffeensis in the original testing)*. Both patients suffered from multiple symptoms suggestive of infection with a viral or rickettsial agent.

	IFA Titers (acute, convalescent)			Symptoms						Prescribed Abx	Hospitalized	Discharge diagnosis
Pt No.	*R. rickettsii*	*R. parkeri*	*R. amblyommii*	Fever	Rash	H/a	Myalgia	Other				
3	**<1:64, 1:256**	**<1:64, 1:1024**	**<1:64, 1:512**	+	+	-	-	-		+	-	Presumed RMSF
42	**1:128, 1:1024**	**1:512, >1:2048**	**1:128, >1:2048**	+	-	-	+	Leukopenia		na	-	na
56	**<1:64, 1:512**	**<1:64, ≥1:2048**	**<1:64, ≥1:2048**	+	+	+	+	Lethargy, thrombocytopenia		+	-	RMSF
58	**<1:64, 1:1024**	**<1:64, 1:512**	**1:64, 1:512**	+	-	+	+	Nausea, vomiting		+		Febrile illness, possible RMSF
68	**<1:64, 1:2048**	**<1:64, ≥1:2048**	**<1:64, 1:2048**	-	+	-	-	Cough		+	-	Rash, unspecified
88	1:512, 1:256	**1:1024, 1:256**	**1:1024, 1:256**	+	-	+	+	Multiple[1]		+	-	na
109	1:256, 1:128	**1:256, 1:1024**	**1:256, 1:1024**	+	-	na	na	na		na	-	na
70	1:256, 1:256	**1:256, 1:1024**	1:256, 1:256	+	-	+	+	Multiple[2]		+	+	na
93	1:512, 1:512	**1:2048, 1:512**	1:1024, 1:1024	-	-	-	-	-		+	-	na
31	1:128, 1:256[3]	1:256, 1:256	**1:256, 1:1024**	+	-	-	-	Multiple[4]		+	+	Tick-borne illness
44	1:128, 1:256	1:512, 1:1024	**1:64, 1:512**	na	na	na	na	na		na	-	na
76	1:512, 1:256	1:1024, 1:2048	**1:512, 1:2048**	-	-	-	+	Fatigue		+	-	na

[1]Nausea, diarrhea, weakness, cough, sweats. [2]Elevated liver enzymes, thrombocytopenia, leukopenia, and anemia. [3]In the original testing this patient seroconverted to *R. rickettsii* and *E. chaffeensis*. [4]Elevated liver enzymes, acute renal failure, thrombocytopenia, leukopenia, and anemia.
Table 10. Clinical characteristics for patients with surveillance reports available who seroconverted to *R. rickettsii, R. parkeri,* and/or *R. amblyommii* antigens (n=12). Seroconversions are highlighted in bold.

D. Discussion

This is the first study to evaluate reactivity of paired sera from suspected RMSF patients against *R. rickettsii, R. parkeri,* and *R. amblyommii* antigens concurrently. These antigens are likely to represent the species of SFGR that occur in tick vectors most frequently in North Carolina based on current knowledge. Although the seroconversions observed cannot be used to infer etiology due to cross-reactivity, the greater number of unique seroconversions to *R. parkeri* and *R. amblyommii* than to *R. rickettsii* indicate that species of SFGR other than *R. rickettsii* may be causing infections among North Carolina residents. These results also suggest that serologic testing of paired samples using *R. rickettsii* antigen may result in missed cases of spotted fever rickettsioses caused by other species of SFGR, and the specificity of *R. rickettsii* antigen for RMSF may be better than expected. Thus, even the current "gold standard" for serologic diagnosis of SFGR has significant limitations.

The large relative frequency of seroconversions to *R. amblyommii* was not unexpected in the context of previous work by Apperson et al [15]. Active surveillance for tick-borne diseases in a central North Carolina county resulted in the identification of several patients with mild illness in which initial testing failed to confirm RMSF. Upon further testing with both *R. rickettsii* and *R. amblyommii* antigens three patients seroconverted against *R. amblyommii* in that study. At this time, *R. amblyommii* has not been recognized as a human pathogen, but the high prevalence in lone star ticks creates ample opportunity for exposure and potential infection of human hosts [21, 23, 43]. Seroconversions to *R. amblyommii* among ill patients described in this report provides further evidence that infections and illness caused by *R. amblyommii* may be occurring. We also anticipated that we would detect seroconversions to *R. parkeri*, a known human pathogen that was found in 29% of gulf coast ticks (*Amblyomma maculatum*) in a recent entomologic survey in North Carolina [40].

It is well known that there is serologic cross-reactivity between species of SFGR, and as a result, serologic tests cannot be used to infer etiology [119]. Serologic cross-reactivity between *R. rickettsii* and *R. parkeri* antigens has been described previously for patients with single IFA titers. Raoult and Paddock retested sera from 15 patients diagnosed with RMSF using class-specific IFA with *R. rickettsii* and *R. parkeri* antigens [59]. Equal titers were observed for six patients, while 4 patients had higher titers to *R. rickettsii* and 5 patients had higher titers to *R. parkeri*. Western blot analysis of 4 patients with higher titers against *R. parkeri* provided additional evidence of infection with *R. parkeri*. Paddock et al conducted comparative class specific IFA from 6 confirmed and 6 probable cases of *R. parkeri* rickettsiosis using *R. parkeri, R. rickettsii, R. amblyommii,* and *R. akari* antigens [36]. IgG geometric mean titers were higher against *R. rickettsii* than for any other antigen. These studies illustrate that comparison of single titers is unreliable for distinguishing cases of RMSF and *R. parkeri* rickettsiosis. By evaluating reactivity of acute and convalescent paired sera, we found that some patients showed rising or falling titers to multiple rickettsiae which is consistent with the cross-reactivity observed in previous studies. It is also possible, but highly unlikely that patients could have been co-infected with multiple species of rickettsiae. Notably, we found that some patients seroconverted to only a single antigen, indicating that extensive cross-reactivity between SFGR antigens may not be present in all cases.

Limitations of this study include the lack of clinical data for all patients and the variability in the level of completeness of those for which data are available. The small number of patients with surveillance reports available prevents us from making conclusions on the association between seroconversion to specific antigens and clinical signs and symptoms. Due to the eligibility requirement of having paired sera, the patients in this study are likely to have suffered from more severe illnesses, which led the medical provider to order repeated testing for tick-borne pathogens. By limiting our study to these patients, it is possible that we excluded many cases of mild disease caused by SFGR. Curiously, there were some

patients with paired sera who had no reported signs or symptoms. In a few surveillance reports, it was noted that testing was done as a precaution after multiple tick bites, although none of those patients seroconverted in this study.

It is clear that while *R. rickettsii* is still circulating and causing disease among people living in the southern and southeastern US, residents are also being exposed to and infected by other SFGR carried by ticks in these regions. Some of these SFGR, such as *R. parkeri*, have been shown to cause human disease, while the pathogenic potential of others such as *R. amblyommii, R. montanensis, and R. rhipicephali* has yet to be determined. Until more specific serologic diagnostic methods are developed which can distinguish between species of SFGR, or the use of molecular detection techniques becomes routine, the relative contributions of different species of SFGR to human morbidity will remain unclear. Active surveillance for mild cases of suspected tick-borne illness, which include paired serology and molecular detection, are needed to determine the etiologies of SFGR infections in this region. If current serological methods continue to be the standard for diagnosis and surveillance of spotted fever group rickettsioses, inclusion of antigens for all species known to cause human disease in the relevant geographic region should be considered to prevent missed infections.

Chapter 6

EFFECTIVENESS OF LONG-LASTING PERMETHRIN IMPREGNATED UNIFORMS FOR TICK BITE PREVENTION IN FORESTRY, PARKS, AND WILDLIFE WORKERS

A. Introduction

In the US, over 34,000 cases of tick-borne illnesses including Lyme disease, spotted fever group rickettsioses, ehrlichiosis, and anaplasmosis were reported in 2010 [7]. The true incidence is likely to be higher due to under-reporting [1]. The incidence of tick-borne diseases is on the rise and new tick-borne pathogens are emerging.

Tick-borne diseases are an occupational risk for outdoor workers, particularly among forestry workers [67]. A recent serosurvey of National Park Service employees showed that 22% of employees were seropositive for previous exposure to spotted fever group rickettsiae, 3% for *Ehrlichia chaffeensis*, and 8% for *Anaplasma phagocytophilum* [120].

Tick bite prevention methods recommended by the National Institute for Occupational Safety and Health for outdoor workers, include: wearing light colored protective clothing (long sleeves, long pants, and hat), tucking pants into socks or boots, regular application of insect repellent (at least 20% DEET) to exposed skin and clothing, spraying work clothing with permethrin, and thoroughly checking your body for ticks daily [60]. The most commonly used form of permethrin for clothing treatment is a self-applied permethrin aerosol spray. Under controlled conditions, self-application of permethrin to clothing can provide nearly 100% protection against questing ticks including *Amblyomma americanum* [96, 98, 101, 121], *Dermacentor variabilis* [96, 98], *Ixodes scapularis* [98, 99, 121], and *Ixodes pacificus* [100]. This high rate of protection, however, is not sustained over long periods of wear or

after multiple washings [100, 101]. Furthermore, adherence to these recommendations, even among those who work in highly endemic areas for tick-borne disease, appears to be poor [61, 62, 87, 120]. Thus, more effective and user-friendly tick-bite prevention methods are needed.

A factory-based method for long-lasting permethrin impregnation (LLPI) of textiles using a proprietary formulation of permethrin has been shown to retain tick-repellent activity over 70 washes in laboratory studies [107, 122]. Clothing treated with this method is commercially available at many outdoor retailers, and is a key component of the Department of Defense Insect Repellent System [123, 124]. An open-label pilot study was conducted to assess the effectiveness of this LLPI clothing for the prevention of tick bites among 16 outdoor workers from the North Carolina Division of Water Quality [110]. Subjects wearing LLPI clothing had 93% fewer tick bites compared to subjects using standard tick bite prevention measures. To evaluate this intervention in a more rigorous manner, a double blind randomized-controlled trial of LLPI uniforms was conducted among outdoor workers from North Carolina's Divisions of Parks and Recreation, Forestry, and Wildlife.

B. Methods

Study design

A double-blind randomized intervention was conducted to determine whether wearing long-lasting permethrin impregnated uniforms results in fewer tick bites among outdoor workers. The study included follow-up over two tick seasons (March through September 2011 and 2012). The institutional review board at the University of North Carolina at Chapel Hill approved the study protocol (IRB# 10-1027). All subjects completed written informed consent.

Participants

Eligible participants included employees of the North Carolina Forest Service, North Carolina Division of Parks and Recreation, North Carolina Wildlife Resources Commission,

and North Carolina County and Local Parks and Recreation who worked in the eastern or central North Carolina, who were over 18 years of age, who spent an average of ten or more hours of outdoor work per week during tick season, and who were required to wear a uniform while on the job. Exclusion criteria include pregnancy, non-English speakers, or having a known allergy to insecticides. Informational meetings were conducted at state and local parks and forestry and wildlife offices throughout the study area between October 2010 and April 2011, and eligible employees were invited to enroll. Subjects were assigned a study ID number upon enrollment and given a small flashlight and six pairs of boot socks as a token of appreciation for their participation. After the first year of follow-up, subjects were given an additional six pairs of socks as incentive to complete the final year of follow-up.

Randomization and masking

Prior to the start of enrollment, each ID number was assigned to either the treatment or control group through block randomization using block sizes of six, which were generated through random numbers by computer, with 1:1 allocation for the treatment or control interventions. The randomization list was generated by the study statistician and none of the study personnel or investigators had access to this list until after data collection was completed.

Intervention

All subjects were asked to send all of their spring and summer work uniforms, including shirts, T-shirts, pants, shorts, hats, and socks directly to the treatment facility. Once the uniforms were received, all items were labeled with the subject's ID number and either treated with permethrin according to the factory-based proprietary process of long-lasting permethrin impregnation of clothing (treatment), or were simply washed and dried in a commercial washer/dryer (control). In addition, according to their treatment group, subjects were either given six pairs of treated or untreated boot socks at start of each tick season. Subjects were instructed to send any new uniforms purchased during the study period to the

treatment facility before wearing them. All subjects were instructed to launder their clothing as they normally would and to continue with their usual tick bite prevention measures (including use of repellents).

Data Collection

After completion of informed consent, participants completed a baseline questionnaire, which collected demographic and occupational information, history of tick-bites and tick exposure in the past year, history of tick-borne disease, and use of tick-bite prevention measures. The follow-up periods consisted of two consecutive tick seasons (2011 and 2012), starting the week of March 15, 2011 or the week the subject started wearing their study uniforms and continuing through the last week of September. During the follow-up periods, all subjects were asked to keep a diary of all tick bites (attached ticks) which were recorded on weekly tick bite logs. For each entry in the log, subjects recorded the date of the tick bite(s), the number of tick bites, the location of the tick bites on the body, the county where the tick bite(s) were most likely to have been acquired, whether they were on the job when the tick bite(s) were acquired, whether they had been using insect repellent at the time of the bite(s) and the type of repellent, and whether the tick(s) were collected. Subjects also recorded the number of work hours and non-work hours spent outdoors each week on their weekly tick bite logs. Subjects were provided with a tick removal kit, including forceps and collection vials, and were encouraged to submit any attached ticks for identification. At the end of the first and second years, all subjects were asked to complete a follow-up questionnaire. After all data collection was completed, subjects were asked to guess whether they were in the treatment or control group, and provide the reason for their guess.

Adverse events and tick-borne illnesses

An adverse event was defined as any report of adverse effects that a participant believed to be related to their uniforms. In the case of an adverse event report, the study physician would be unblinded so it could be determined whether that subject was in the

treatment or control group.   If an adverse reaction occurred related to a subject's treated

uniforms, the study would pay to replace all of the participants' uniforms. All subjects were

also instructed to report any illnesses they believed might be related to a tick bite, including

fever, rash, headache, muscle aches, or extreme tiredness within three weeks of a tick bite.

In the case of an illness the subject consulted with the study physician or their own

physician and sera were collected for testing against tick-borne antigens.

Statistical analysis

The analyses, conducted in 2013, followed the intent to treat principle. Baseline

characteristics of the treatment and control groups were compared using the Pearson chi-

square test for dichotomous variables, Mantel-Haenszel chi-square test for ordinal variables,

and Student's *t* test for continuous variables. P-values less than 0.05 were considered

statistically significant. Protective effectiveness (1 – the incidence rate ratio) [116] and 95%

confidence intervals for comparing reported tick bites between the treatment and control

groups were calculated using a generalized estimating equations (GEE) approach for

Poisson regression. The use of GEE methods accounted for the within subject correlation

due to repeated measures using a working correlation matrix. The GEE model used to

calculate the protective effectiveness of the LLPI clothing used a Poisson distribution with a

log link, and included terms for treatment, year of follow-up and the interaction of treatment

and year of follow-up, with an offset variable for log outdoor work hours.. No other

covariates were included in the model based on our evaluation of possible confounding by

baseline variables using a 10% change in estimate criteria. The incidence of tick bites was

calculated as the total number of work-related tick bites per 100 hours spent working

outdoors. Estimates were stratified by year of follow-up to examine whether the treatment

effect waned with continued wear/washing. Incidence rate differences and confidence

intervals were calculated by inverse weighting of tick bites by the average outdoor work

hours in the corresponding treatment group, so that the variance of the rate difference could

be estimated using normal approximation. Secondary outcomes, including chigger (larval stage mites in the family Trombiculidae) bites and mosquito bites, were compared using the Pearson chi-square test for dichotomous variables. The success of blinding was assessed using Bang's blinding index, with values between -0.2 and 0.2 used as the threshold for successful blinding.[125] All analyses were performed using SAS (version 9.2, SAS Institute Inc., Cary, NC, USA) or Stata (Release 12, StataCorp LP, College Station, TX).

C. Results

Study population

Over two years of recruitment 159 subjects were enrolled and randomized. Twenty-six (16%) subjects never sent in their uniforms and were excluded (Figure 9). At baseline there were no significant differences in demographic and other characteristics between the treatment and control group (Table 11). The majority of subjects were white males, with a college degree and had been working in their current position for an average of 8 years. The number of tick bites reported in the year prior to enrollment as reported on the baseline questionnaire was similar for both groups. Twenty-six subjects dropped out or were lost to follow-up after the first year, including three subjects that did not submit any tick bite logs. Drop out was primarily due to subjects who moved or were transferred to jobs outside the study area (n=12). Subjects who were lost to follow-up or dropped out of the study did not differ from those who remained in the study according to treatment status, demographic characteristics, and outcome (mean tick bites per week), so we felt that the missing completely at random assumption was reasonable and hence missing data due to dropout were treated as noninformative and ignorable (Table 12).
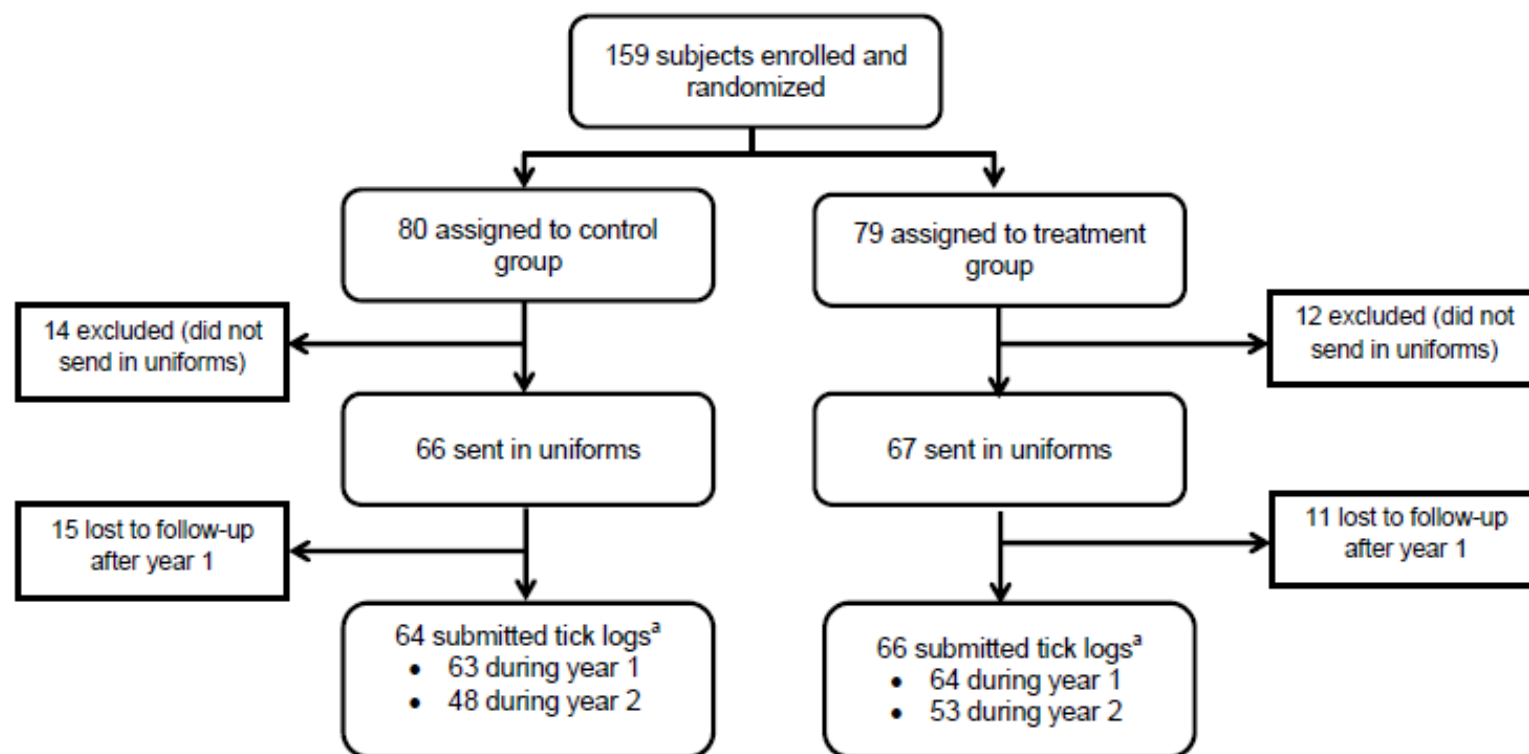
Figure 9. Randomization and follow-up of study participants. [a]Six subjects which were enrolled after the first tick season were only followed for one year. Two subjects did not submit any ticks logs during year 1 but began submitting logs in year 2. Three subjects did not submit any tick logs.

	Treatment group (n=67)	Control group (n=66)
Sex – no. (%)		
Male	52/66 (78.8)	55/66 (83.3)
Female	14/66 (21.2)	11/66 (16.7)
Age – mean±SD	39.1 ± 9.2	38.8 ± 9.3
Race – no. (%)		
White	56/58 (96.6)	58/59 (98.3)
Black	2/58 (3.4)	0/59 (0.0)
Other	0/58 (0.0)	1/59 (1.7)
Education – no. (%)		
High school or less	5/65 (7.7)	10/66 (15.2)
Some college	20/65 (30.8)	14/66 (21.2)
Bachelor or graduate degree	40/65 (61.5)	42/66 (63.6)
Work Division – no. (%)		
NC Forest Service	21/66 (31.8)	27/66 (40.9)
NC Division of Parks and Recreation	31/66 (47.0)	25/66 (37.9)
NC Wildlife Resources Commission	9/66 (13.6)	10/66 (15.2)
NC Local or County Parks and Recreation	5/66 (7.6)	4/66 (6.1)
Years in current position – mean±SD	7.9 ± 6.7	8.2 ± 6.8
Number of tick bites in previous year – mean±SD	19.3 ± 32.0	19.6 ± 39.3
Previous diagnosis with a tick-borne illness[c] – no. (%)		
Lyme disease	4/67 (6.0)	4/66 (6.1)
Rocky Mountain spotted fever	6/67 (9.0)	7/66 (10.6)
Ehrlichiosis	2/67 (3.0)	3/66 (4.6)
Anaplasmosis	0/67 (0.0)	0/66 (0.0)
Babesiosis	0/67 (0.0)	0/66 (0.0)
Use of tick bite prevention measures[d] – no. (%)		
Long pants	62/66 (93.9)	62/65 (95.4)
Long sleeves	48/57 (84.2)	52/59 (88.1)
Hat	43/63 (68.2)	38/64 (59.4)
Tucked or taped pant legs	5/60 (8.3)	7/59 (11.9)
Repellent on skin	22/64 (34.4)	22/61 (36.1)
Repellent on clothing	38/65 (58.5)	35/65 (53.8)
Tick checks after working outdoors	58/66 (87.9)	60/65 (92.3)
Use of self-applied permethrin on clothing – no. (%)	30/67 (44.8)	34/66 (51.5)
Number of uniforms submitted – mean±SD		
Shirts	15.4 ± 10.6	13.9 ± 9.9
Pants	9.1 ± 5.1	9.2 ± 6.5
Socks	8.4 ± 6.0	8.5 ± 6.7
Hats	1.7 ± 2.0	1.3 ± 1.5

Table 11. Demographic characteristics and history of tick bites, tick-borne illness and usage of preventive measures by treatment group. [a]Based on self-report. [b]Reported using these measures at least 50% of the time while working outdoors.

	Active subjects (n=107)	Lost to follow-up (n=26)[a]	Did not send uniform (n=26)[b]
Assigned intervention			
Treatment	56/107 (52.3)	11/26 (42.3)	12/26 (46.2)
Control	51/104 (47.7)	15/26 (57.7)	14/26 (53.8)
Mean bites/week of follow-up - mean±SD	0.187±0.337	0.229±0.533	n/a
Sex – no. (%)			
Male	87/106 (82.1)	20/26 (76.9)	18/20 (90.0)
Female	19/106 (17.9)	6/26 (23.1)	2/20 (10.0)
Age – mean±SD	39.7 ± 9.4	35.7 ± 8.0	45.7 ± 12.2*
Race – no. (%)			
White	94/97 (96.9)	13/13 (100.0)	n/a
Black	2/97 (2.1)	0	n/a
Other	1/97 (1.0)	0	n/a
Education – no. (%)			
High school or less	11/105 (10.5)	4/26 (15.4)	3/18 (16.7)
Some college	27/105 (25.7)	7/26 (26.9)	7/18 (38.9)
Bachelor or graduate degree	67/105 (63.8)	15/26 (57.7)	8/18 (44.4)
Work Division – no. (%)			
NC Forest Service	43/106 (40.6)	5/26 (19.2)	7/20 (35.0)
NC Division of Parks and Recreation	42/106 (39.6)	14/26 (53.8)	10/20 (50.0)
NC Wildlife Resources Commission	14/106 (13.2)	5/26 (19.2)	0/20 (0)
NC Local or County Parks and Recreation	7/106 (6.6)	2/26 (7.7)	3/20 (15.0)
Years in current position – mean±SD	8.3 ± 7.1	6.9 ± 5.3	6.0 ± 4.8
Number of tick bites in previous year – mean±SD	18.9 ± 27.6	21.4 ± 58.1	8.1 ± 12.7**
Use of tick bite prevention measures[c] – no. (%)			
Long pants	99/105 (94.3)	25/26 (96.2)	13/18 (72.2)**
Long sleeves	14/94 (14.9)	2/22 (9.1)	4/15 (26.7)
Hat	64/103 (62.1)	17/24 (70.8)	13/18 (72.2)
Tucked or taped pant legs	12/97 (12.4)	0/22 (0)	3/15 (20.0)
Repellent on skin	33/99 (33.3)	11/26 (42.3)	10/17 (58.8)*
Repellent on clothing	59/105 (56.2)	14/25 (56.0)	11/19 (57.9)
Tick checks after working outdoors	95/105 (90.5)	23/26 (88.5)	17/20 (85.0)
Use of self-applied permethrin on clothing – no. (%)	52/106 (49.1)	12/26 (46.2)	4/20 (20.0)*

[a]Subjects lost to follow-up after the first year. [b]Baseline questionnaires were available from 20 of the 26 subjects that did not submit uniforms. [c]Reported using these measures at least 50% of the time while working outdoors.*p<0.05, **p<0.01, as compared to active subjects.

Table 12. Comparison of assigned intervention, mean observed outcome, and baseline characteristics of active subjects, those lost to follow-up after the first year and those who never submitted uniforms.

Protective effectiveness against tick bites

The mean number of person-weeks of follow-up was similar for the treatment group and control group (41.1 person-weeks and 42.2 person-weeks, respectively). The number of work-related tick bites (bites reported as having been acquired on the job) reported by group and incidence of tick bites per person-week stratified by year of follow-up are shown in Table 13. In total, 1,045 work-related tick bites were reported over 5,251 weeks of follow-up. The incidence of tick bites in year one was 1.37 bites per 100 outdoor work hours in the control group and 0.24 bites per 100 outdoor work hours in the treatment group, with an incidence rate difference of -1.13 (95% CI: -1.78, -0.50). In year two the incidences were 1.05 and 0.69 tick bites per person-week in the control and treatment groups, respectively, with an incidence rate difference of -0.36 (95% CI: -1.12, 0.40). The incidence of tick bites was significantly less among subjects in the treatment group during the first year of follow-up, with a protective effectiveness of 0.82 (95% CI: 0.66, 0.91, p<0.001) against tick bites for subjects wearing LLPI uniforms compared to subjects using their usual tick bite prevention measures. During the second year of follow-up the protective effectiveness was 0.34 (95% CI: 0.67, 0.74, p=0.38). The overall protective effectiveness for both years of follow-up was 0.65 (95% CI: 0.29, 0.82, p=0.004). Two outliers were observed in which a subject reported ≥50 tick bites in a single week. These were likely to be larval ticks and it is uncertain whether they represented true bites (attachment). When these outliers were excluded, the protective effectiveness for year one was 0.78 (95% CI: 0.60, 0.88) and 0.52 (95% CI: 0.01, 0.77) for year two (Table 14).

	N	Total tick bites/total outdoor work hours	Tick bite incidence per 100 outdoor work hours	Incidence rate difference (95% CI)	Protective effectiveness (95% CI)	p-value
Year 1						
Treatment	64	84/34628.0	0.24	-1.13 (-1.78, -0.50)	0.823 (0.655, 0.910)	<0.001
Control	63	493[a]/35750.8	1.37			
Year 2						
Treatment	53	181[b]/26171.5	0.69	-0.36 (-1.12, 0.40)	0.341 (-0.670, 0.740)	0.379
Control	48	287/27353.0	1.05			
Years 1 & 2						
Treatment	66	265[b]/60799.5	0.44	-0.79 (-1.34, -0.26)	0.646 (0.288, 0.824)	0.004
Control	64	780[a]/63103.8	1.23			

Table 13. Incidence of work related tick bites, by treatment group and year of follow-up.
[a]One subject reported 102 tick bites in one week. [b]One subject reported 50 tick bites in one week.

	N	Total tick bites/total outdoor work hours	Tick bite incidence per 100 outdoor work hours	Protective effectiveness (95% CI)	p-value
Year 1					
Treatment	64	84/34628	0.243	0.777 (0.602, 0.876)	<0.0001
Control	63	391/35720.8	1.09		
Year 2					
Treatment	53	131/26151.5	0.501	0.523 (0.010, 0.770)	0.047
Control	48	287/27353	1.05		
Years 1 & 2					
Treatment	66	215/60779.5	0.354	0.670 (0.423, 0.811)	<0.0001
Control	64	678/63073.8	1.07		

Table 14. Sensitivity analysis of incidence of work related tick bites, excluding weeks with ≥50 tick bites reported by a subject.

Secondary outcomes

In support of the tick bite log data, 867 ticks were collected and submitted by subjects over both years, more from subjects in the control group (581 ticks) than from subjects in the treatment group (286 ticks). More than 90% of the submitted ticks were lone star ticks (*A. americanum)*. Nearly all subjects reported having chigger bites and mosquito bites in the year prior to enrollment, with no significant differences between those assigned to the treatment or control groups. During both years of follow-up, the risk of having any chigger bites was significantly reduced among subjects in the treatment group (year one RR=0.66, p<0.001 and year two RR=0.71, p=0.002) (Table 15). Almost all subjects continued to report having been bitten by mosquitoes during follow-up, although the proportion who reported having frequent mosquito bites was less among those in the treatment group (RR=0.66, p=0.08 in year one and RR=0.56, p=0.08 in year two) (Table 16).

Year	Treatment group	Control group	Risk Ratio (95% CI)	p-value
Baseline	65/66 (98.5)	65/66 (98.5)	1.00 (0.96, 1.04)	1.0
Year 1	32/57 (56.1)	50/59 (84.8)	0.66 (0.51, 0.85)	<0.001
Year 2	32/50 (64.0)	45/50 (90.0)	0.71 (0.57, 0.89)	0.002

Table 15. Proportion of subjects reporting any chigger bites by treatment group and year of follow-up.

Year	Treatment group	Control group	Risk Ratio (95% CI)	p-value
Baseline	43/66 (65.2)	47/66 (71.2)	0.91 (0.72, 1.16)	0.46
Year 1	18/57 (31.6)	28/59 (47.5)	0.66 (0.42, 1.06)	0.08
Year 2	10/50 (20.0)	18/50 (36.0)	0.56 (0.28, 1.08)	0.08

Table 16. Proportion of subjects reporting frequent mosquito bites by treatment group and year of follow-up.

Blinding

At the end of the study, all subjects were asked to guess their treatment status. Of the 97 subjects who responded, 41/51 (80.4%) of subjects in the treatment group and 27/46 (58.7%) in the control group guessed correctly (Table 17). Most subjects related their guess to the frequency of tick and chigger bites they experienced and the behavior of ticks on their uniforms. Bang's blinding index, which can be interpreted as the proportion of unblinding in each group, was 0.74 for the treatment group and 0.28 for the control group, indicating unblinding was high for both groups, although less so for the control group.

	Guess			Blinding
	Treatment	Control	Don't Know	Index
Assignment				
Treatment (n=51)	41	3	7	0.745
Control (n=46)	14	27	5	0.283

Table 17.   Subjects' guesses of treatment assignment and Bang's blinding index by group.

Adverse events and tick-borne illnesses

There were no adverse events reported that were related to the subjects' uniforms. Five subjects reported illnesses suspected to be tick-related, two were confirmed (one case of ehrlichiosis and one case of spotted fever rickettsiosis), both among subjects in the control group. The other reported illnesses were a local reaction to a tick bite, viral mononucleosis, and a mild viral illness.

D. Discussion

Prevention of tick bites is critically important among outdoor workers and others with extensive exposure to ticks. This study demonstrated that in the first year of wear, LLPI uniforms significantly reduced tick bites by greater than 80% among outdoor workers even when usual tick bite prevention measures were employed by both groups. The effectiveness of the LLPI uniforms declined in year two. Based on laboratory knockdown

studies of mosquito and tick species after exposure to treated fabric, the repellency of the

LLPI clothing used in this study is registered by the US Environmental Protection Agency to

last through 70 launderings. The estimated numbers of launderings of the subjects'

uniforms in this study, based on questionnaire data, were generally below 70-washes after

the first year (Table 18). Therefore, we believe that environmental conditions in the field

(not present in laboratory studies) may also play a role in loss of effectiveness. Subjects

spent many outdoor hours in their uniforms; this continued exposure to various

environmental conditions (sunlight, rain, heat) and heavy wear of uniforms may have

contributed to the observed loss of effectiveness. Future studies should focus on the effects

of environmental factors on loss of permethrin and knockdown capacity of LLPI clothing.

	Treatment group	Control group
Number of uniforms submitted – mean±SD		
Shirts	15.4±10.6	13.9±9.9
Pants	9.1±5.1	9.2±6.5
Socks	8.4±6.0	8.5±6.7
Hats	1.7±2.0	1.3±1.5
Frequency of washing (no. wears before washing) – mean±SD		
Shirts	1.34±0.60	1.30±0.52
Pants	1.66±0.90	1.72±0.84
Socks	1.01±0.12	1.08±0.28
Estimated number of washes per year[a] – mean±SD		
Shirts	44.3±33.6	44.2±28.0
Pants	28.6±21.7	28.6±31.5
Socks	20.1±8.7	19.6±8.8

Table 18. Number of uniforms submitted, frequency of washing, and estimated number of washes per year, by group. [a]Estimated number of washes calculated assuming that subjects only wore the uniforms submitted for "treatment" and that they were worn 50 weeks of the year (assuming 2 weeks vacation).

Adherence to the assigned treatment (wearing only "study" uniforms), was likely to be

highest in first year of follow-up. The workers in this study typically purchase a number of

new uniforms every year and we asked that any new uniforms purchased during the study be sent for "treatment" before being worn. Approximately half of the subjects in each group submitted additional uniforms after the first year; therefore it is likely that some subjects wore "non-study" uniforms during the second year, which could bias the treatment effect toward the null. Permethrin-treated socks have been shown to be particularly effective in preventing tick bites [109], as questing ticks will often encounter socks and footwear first after finding a human host. Subjects were only provided with six pairs of treated (or sham-treated) socks at the start of each year, and while most subjects submitted additional socks of their own at the start of the study, only 12 subjects submitted additional socks in the second year. Since socks tend to wear out more quickly than other pieces of clothing, it is unlikely that all subjects wore "study" socks exclusively. Failure to wear permethrin-treated socks could also have contributed to the loss of measured effectiveness in year two.

The high degree of unblinding among subjects is a potential source of bias. However, there were no significant differences observed in the proportion of subjects in each group performing regular tick checks and using other tick bite prevention measures during follow-up. The control group tended to use self-applied repellents more frequently, which could have led to an underestimation of protective effectiveness of the LLPI uniforms (Table 19).

Tick bite prevention measure	Treatment group	Control group	p-value
Long pants			
Baseline	62/66 (94.9%)	62/65 (95.4%)	0.71
Year 1	54/57 (94.7%)	56/59 (94.9%)	0.97
Year 2	47/49 (95.9%)	45/50 (90.0%)	0.25
Long sleeves			
Baseline	9/57 (15.8%)	7/59 (11.9%)	0.54
Year 1	6/53 (11.3%)	5/56 (8.9%)	0.68
Year 2	5/46 (10.9%)	4/48 (8.3%)	0.68
Hat			
Baseline	43/63 (68.2%)	38/64 (59.4%)	0.30
Year 1	32/53 (60.4%)	33/58 (56.9%)	0.71
Year 2	30/45 (66.7%)	23/47 (48.9%)	0.09
Tuck pant legs/tape pants to boots			
Baseline	5/60 (8.3%)	7/59 (11.9%)	0.52
Year 1	3/54 (5.6%)	3/56 (5.4%)	0.96
Year 2	1/44 (2.3%)	3/47 (6.4%)	0.34
Repellent on skin			
Baseline	22/64 (34.4%)	22/61 (36.1%)	0.84
Year 1	9/55 (16.4%)	15/56 (26.8%)	0.18
Year 2	6/48 (12.5%)	11/48 (22.9%)	0.18
Repellent on clothing			
Baseline	38/65 (58.5%)	35/65 (53.8%)	0.60
Year 1	15/56 (26.8%)	23/57 (40.4%)	0.13
Year 2	11/46 (23.9%)	22/50 (44.0%)	0.04
Tick check after working outdoors			
Baseline	58/66 (87.9%)	60/65 (92.3%)	0.40
Year 1	52/57 (91.2%)	55/57 (96.5%)	0.24
Year 2	47/50 (94.0%)	43/50 (86.0%)	0.18

Table 19. Proportion of subjects who reported using tick bite prevention measures at least 50% of the time during spring and summer, by year of follow-up and group.

While there is potential for bias due to exclusion of subjects who did not submit uniforms, the proportions excluded from those assigned to the treatment and control group were similar. Subjects who were excluded tended to be older and had significantly fewer tick bites in the year prior to enrollment (Table 12). Excluded subjects also were less likely to wear long pants, use repellent on their skin, and use self-applied permethrin on their clothing. We

speculate that these subjects may have chosen not to send in their uniforms due to low perceived risk of tick bites and reluctance to use repellents. Given the evidence that those who opted to participate had more frequent exposure to ticks, our estimates of protective effectiveness are most relevant for those at high risk of tick bites.

The vast majority of the ticks collected by subjects were lone star ticks (*A. americanum*), the most common human-biting tick in North Carolina. Clothing that has been freshly treated with permethrin provides high rates of protection against several species of ticks, including *I. scapularis, I. pacificus, I. ricinus, D. variabilis,* and *A. americanum* [96-101, 109, 121, 126]. Thus, LLPI clothing is likely to be protective against different tick species but additional long-term studies are needed in other locations.

This study is the first randomized-controlled trial to evaluate the effectiveness of LLPI clothing with follow-up of subjects over an extended period of time. Efficacy of permethrin impregnated clothing against ticks has been demonstrated in the laboratory and in short-duration field trials [96-101, 105, 108, 109, 121, 126]. However, traditional self-applied spray and dipping methods lose effectiveness unless reapplied every three to five washes [100, 101, 104]. In an open-label pilot study, subjects wearing LLPI clothing had 93% fewer (p<0.0001) tick bites compared to subjects using standard tick bite prevention measures over one tick season [110]. The current study was designed to provide a more accurate and precise estimate over a longer duration of follow-up. The results of this study demonstrate that among high-risk individuals, LLPI uniforms are highly effective for at least one year against tick bites compared to existing tick bite prevention measures. Based on these findings, we recommend that wearing LLPI uniforms or clothing should be included as a standard tick bite prevention measure in addition to other recommended prevention measures for outdoor workers with substantial exposure to ticks, with retreatment or replacement of garments after one year if they are worn on a regular basis.

Chapter 7

SUMMARY

A. Seroepidemiology of spotted fever group rickettsiae in North Carolina

1. Summary of findings

An increasing number of entomologic and epidemiologic studies suggest that SFGR other than *R. rickettsii* are responsible for cases of spotted fever rickettsioses in the US, sparking a change in the surveillance case classification from "Rocky Mountain spotted fever" to "Spotted Fever Group Rickettsiosis (including Rocky Mountain spotted fever)". We conducted a retrospective seroepidemiologic study on paired sera that had been submitted for testing for tick-borne illnesses to the North Carolina State Laboratory of Public Health and evaluated the serologic reactivity to *R. rickettsii, R. parkeri and R. amblyommii* antigens. While cross-reactivity between antigens was observed in 10 of the 21 seroconverters, we found a greater number of unique seroconversions to *R. parkeri* and *R. amblyommii* than to *R. rickettsii,* which suggests that species of SFGR other than *R. rickettsii* may be causing infections among North Carolina residents. Among the patients in this study, our ability to detect seroconversions to *R. parkeri* and *R. amblyommii* using *R. rickettsii* antigen was poor, indicating that using *R. rickettsii* antigen for serologic diagnosis may result in missed cases of spotted fever rickettsioses caused by other species of SFGR.

2. Findings in context of current literature

This is the first study to evaluate reactivity of paired sera from suspected RMSF patients against *R. rickettsii, R. parkeri,* and *R. amblyommii* antigens concurrently. Our discovery of patients who seroconverted to *R. amblyommii* but not *R. rickettsii* is consistent with a previous study of patients presenting to primary care physicians in central North Carolina

with symptoms suggestive of a tick-borne illness [15]. Serologic cross-reactivity to *R. rickettsii* and *R. parkeri* antigens among patients with probable or confirmed SFGR infections has been described previously [36, 59]. While cross-reactivity was also observed in our study, the use of more stringent criteria (seroconversion vs. comparison of single titers) allowed us to distinguish between seropositivity due to previous exposure and a probable recent infection.

3. Public health implications

This study highlights the limitations of the *R. rickettsii* IFA (the current gold standard), for serologic diagnosis of spotted fever rickettsioses in the US. Unless more specific serologic diagnostic methods are developed which can distinguish between species of SFGR or the use of molecular detection techniques becomes routine, the relative contributions of different species of SFGR to human morbidity will remain unclear. For the time being, it is likely that the IFA will continue to be the standard for diagnosis and surveillance for spotted fever group rickettsioses, and based on the results of this study I recommend that *R. parkeri* antigen should be considered for inclusion in addition to *R. rickettsii* to prevent missed cases.

4. Future research

Prospective studies of patients with suspected tick-borne illness are needed to better describe the etiologies of SFGR infections in this region. These studies should include paired serologies as well as attempts at molecular detection of rickettsia from blood, serum, and skin biopsies (and tick specimens if available). Improved real-time PCR assays with lower limits of detection may provide the increased sensitivity needed for these types of studies [117, 127].

B. Preventing tick bites among outdoor workers using permethrin impregnated uniforms

1. Summary of findings

Prevention of tick bites is critically important among outdoor workers and others with extensive exposure to ticks, as it is currently the only way to avoid infection with tick-borne pathogens in the US. This study demonstrated that in the first year of wear, effectiveness of long-lasting permethrin impregnated uniforms for the prevention of work-related tick bites was 0.82 (95% CI: 0.66, 0.91) and 0.34 (95% CI: -0.67, 0.74) for the second year of wear. LLPI uniforms significantly reduced tick bites among outdoor workers even when usual tick bite prevention measures were employed by both groups. The effectiveness of the LLPI uniforms declined during the second year of follow-up, which is likely due to wash/wear out of the permethrin or non-adherence. For recreational users of LLPI clothing, the persistence of high levels of protection against ticks is likely to be much longer than observed in this study.

2. Findings in context of current literature

Nearly all previous studies which have evaluated the efficacy of permethrin treated clothing against ticks were based on field trials with a limited number of subjects walking through tick infested areas for a short time period, usually several hours. The efficacy of permethrin impregnated clothing against ticks has been demonstrated to be near 100% in many laboratory studies and short duration field trials [96-101, 105, 108, 109, 126]. Efficacy estimates from these field trials are based on the total number of ticks found on the subjects wearing treated or untreated garments. While these field studies provide valuable information on the performance of permethrin treated clothing under controlled conditions, the results of these studies cannot be used to infer the effectiveness of the treated clothing against tick bites under actual field conditions and usage, and over an extended period of time. In reality, users of permethrin treated clothing are exposed to various types of outdoor environments and weather conditions, and have varying levels of usage of other tick bite

prevention measures, all which can affect the probability of being bitten by a tick. Traditional self-applied spray and dipping methods require regular reapplication every 3-5 washes to maintain high levels of activity after laundering [100, 101, 104]. With the availability of improved formulations and methods for impregnating fabrics with long-lasting permethrin, studies investigating the longevity, effectiveness, and safety of LLPI clothing under regular wearing and washing conditions are warranted. In a pilot study, we found that subjects wearing LLPI clothing had a 93% reduction (p<0.0001) in the total incidence of tick bites compared to subjects using standard tick bite prevention measures over one tick season [110]. The larger randomized study was designed to provide a more accurate and precise estimate over a longer duration of follow-up. Our findings indicate that among high risk outdoor workers, LLPI uniforms are highly effective for at least one year against tick bites compared to existing tick bite prevention measures.

3. Public health implications

Based on our findings we recommend that wearing LLPI garments (including pants, shirts, socks, and hats) should be included as a standard tick bite prevention measure in addition to other recommended prevention measures for outdoor workers with substantial exposure to ticks. Wearing LLPI uniforms could be a practical solution for tick bite and tick-borne disease prevention because it is simple, safe, effective, and requires no additional effort other than getting dressed. It will appeal to employers who are concerned about the risk of tick bites and tick-borne diseases among their employees, and also as a cost-saving strategy in an effort to reduce lost time and medical claims due to tick-related illnesses. Efforts are currently being made to inform policymakers from NCDENR and other divisions about the results of the study, and to discuss options for making treated uniforms available to employees.

4. Future research

Since this is the first study of its kind, additional studies are needed in other study populations to evaluate the effectiveness against other species of ticks or disease vectors. Of particular value would be studies to demonstrate efficacy against *Ixodes scapularis*, the tick vector for Lyme disease, babesiosis and anaplasmosis. Future studies should also examine whether treatment of full uniforms is needed to achieve high levels of protection, or whether partial treatment of uniforms (such as pants and socks only) can provide good protection. In addition, the cost-effectiveness of treating whole or partial uniforms for tick bite prevention needs to be assessed to assist policymakers in decisions on providing treated uniforms to employees. Future studies should also incorporate testing to quantify the concentration of permethrin and knock-down activity in "worn out" treated uniforms.

APPENDIX A: RMSF CASE REPORT FORM

North Carolina Department of Health and Human Services
Division of Public Health • Epidemiology Section
Communicable Disease Branch

North Carolina Public Health

ROCKY MOUNTAIN SPOTTED FEVER
Confidential Communicable Disease Report—Part 2
NC DISEASE CODE: 35

Patient's First Name	Middle	Last	Suffix	Maiden/Other	Alias	Birthdate (mm/dd/yyyy) / /

SSN

Medical provider completing clinical component of surveillance form:
Name/Title: _____
Telephone: (____) ____ - _____ Fax: (____) ____ - _____
Date completed ____/____/____

LHD CD nurse/designee completing form for submission to DPH:
Name/Title (print): _____
Telephone: (____) ____ - _____ Date completed ____/____/____
LHD CD nurse/designee signature _____

GENERAL DIAGNOSTIC INFORMATION

Is/was patient symptomatic?............☐Y ☐N ☐U
Date of illness onset (mm/dd/yyyy): ____/____/____
Date of diagnosis (mm/dd/yyyy): ____/____/____
Patient's health care provider for this illness
_____
Name of provider's practice or facility
_____
Telephone number for health care provider
(____) ____ - _____

CLINICAL FINDINGS

Fever...☐Y ☐N ☐U
Headache.....................................☐Y ☐N ☐U
Muscle aches/pains (myalgias).........☐Y ☐N ☐U
Skin rash.....................................☐Y ☐N ☐U
Location:
☐ All over the body (generalized)
☐ Generalized, predominately central/torso/back (centripetal)
☐ Generalized, predominately face/hands/feet (centrifugal)
☐ Localized/Focal
☐ Palms and soles
☐ Unknown
Appearance of rash (choose all that apply):
☐ Macular ☐ Papular
☐ Petechial ☐ Unknown
Further appearance of rash (select one):
☐ Discrete ☐ Confluent ☐ Unknown
Nausea.......................................☐Y ☐N ☐U
Vomiting......................................☐Y ☐N ☐U
Thrombocytopenia.........................☐Y ☐N ☐U
Leukopenia..................................☐Y ☐N ☐U
Anemia.......................................☐Y ☐N ☐U
Elevated liver enzymes...................☐Y ☐N ☐U
Acute respiratory distress syndrome
(ARDS).......................................☐Y ☐N ☐U
Acute renal failure........................☐Y ☐N ☐U
Disseminated intravascular
coagulation.................................☐Y ☐N ☐U
Specify: _____
Other symptoms, signs, clinical findings,
or complications consistent with
this illness..................................☐Y ☐N ☐U
Specify: _____
_____

PREDISPOSING CONDITIONS

Any immunosuppressive conditions .☐Y ☐N ☐U
Please specify: _____
_____

REASON FOR TESTING
Why was the patient tested for this condition?
☐ Symptomatic of disease
☐ Screening of asymptomatic person with reported risk factor(s)
☐ Exposed to organism causing this disease (asymptomatic)
☐ Household/close contact to a person reported with this disease
☐ Other, specify_____
☐ Unknown

TREATMENT
Did patient take an antibiotic as
treatment for this illness?...............☐Y ☐N ☐U
If yes:
Check all antibiotics that apply:
☐ Doxycycline ☐ Chloramphenicol
☐ Unknown
☐ Other (specify)_____
Date antibiotic began (mm/dd/yyyy)
_____
If no:
Did patient refuse treatment?..........☐Y ☐N ☐U
Comments/details:
_____

HOSPITALIZATION INFORMATION
Was patient hospitalized for
this illness >24 hours? ....☐Y ☐N ☐U
Hospital name: _____
City, State: _____
Hospital contact name: _____
Telephone: (____) ____ - _____
Admit date (mm/dd/yyyy): ____/____/____
Discharge date (mm/dd/yyyy): ____/____/____
ICU admission?............................☐Y ☐N ☐U

LOCAL HEALTH DEPARTMENT USE ONLY

Check one:
☐ Case definition is met.
(Complete Part 1 and Part 2 and submit to state)
☐ Case definition is not met.
(Complete Part 2 only and submit to state)

CLINICAL OUTCOMES
Discharge/Final diagnosis:_____
Survived?....................................☐Y ☐N ☐U
Died?...☐Y ☐N ☐U
Died from this illness?...................☐Y ☐N ☐U
Date of death (mm/dd/yyyy): ____/____/____

TRAVEL/IMMIGRATION
The patient is:
☐ Resident NC
☐ Resident of another state or US territory
☐ None of the above
Did patient have a travel history during the
14 days prior to onset?.................☐Y ☐N ☐U
List travel dates and destinations _____
_____
Does patient know anyone else with similar
symptom(s) who had the same or similar
travel history?.............................☐Y ☐N ☐U
List persons and contact information:
_____
_____
Additional travel/residency information:
_____

OTHER EXPOSURE INFORMATION
Does the patient know anyone else with
similar symptoms? ....☐Y ☐N ☐U
If yes, specify:

OUTDOOR ACTIVITY & ANIMAL EXPOSURE

During the 14 days prior to onset of symptoms, did the patient participate in outdoor activities: .... ☐Y ☐N ☐U
If yes, specify and give details:

ANIMALS

During the 14 days prior to onset of symptoms, did the patient have exposure to household pets? .... ☐Y ☐N ☐U
If there was exposure to more than one household pet, please enter each pet separately.

Pet #1: ☐ Dog ☐ Cat ☐ Other
   Were ticks seen on pet? .... ☐Y ☐N ☐U
Pet #2: ☐ Dog ☐ Cat ☐ Other
   Were ticks seen on pet? .... ☐Y ☐N ☐U
Pet #3: ☐ Dog ☐ Cat ☐ Other
   Were ticks seen on pet? .... ☐Y ☐N ☐U

GEOGRAPHICAL SITE OF EXPOSURE

In what geographic location was the patient MOST LIKELY exposed?
Specify location:
☐ In NC
   City _____
   County _____
☐ Outside NC, but within US
   City _____
   State _____
   County _____
☐ Outside US
   City _____
   Country _____
☐ Unknown
Is the patient suspected of being part of a common source outbreak? .... ☐Y ☐N ☐U

Notes:

VECTORBORNE EXPOSURES

During the 14 days prior to onset of symptoms, did the patient have an opportunity for exposure to ticks? .... ☐Y ☐N ☐U
Exposed on (mm/dd/yyyy): ____/____/____
Until (mm/dd/yyyy): ____/____/____
Frequency
   ☐ Once
   ☐ Multiple times within this time period
   ☐ Daily
Exposure setting _____
City/county of exposure _____
State of exposure _____
Country of exposure _____
Was the tick embedded? .... ☐Y ☐N ☐U
How long? _____
   ☐ Hours
   ☐ Days
   ☐ Unknown
Notes:

CASE INTERVIEWS/INVESTIGATIONS

Was the patient interviewed? .... ☐Y ☐N ☐U
Date of Interview (mm/dd/yyyy): ____/____/____
Were interviews conducted with others? .... ☐Y ☐N ☐U
Who was interviewed?

Were health care providers consulted? .... ☐Y ☐N ☐U
Who was consulted?

Medical records reviewed (including telephone review with provider/office staff)? .... ☐Y ☐N ☐U
Specify reason if medical records were not reviewed:

Notes on medical record verification:

VACCINES

Has patient/contact ever received Rocky Mountain Spotted Fever Vaccine or any other vaccine or immune globulin related to this disease? .... ☐Y ☐N ☐U
Vaccine type: _____
Date of administration (mm/dd/yyyy): _____
Source of this vaccine
   Information: _____

Patient's First Name	Middle	Last	Suffix	Maiden/Other	Alias	Birthdate (mm/dd/yyyy) __/__/__
						SSN

LABORATORY

Name of laboratory _____ City _____ State _____ ZIP _____

SEROLOGIC TESTS Indicate Y(es) or N(o) ONLY if the test was performed.	SEROLOGY 1 Collection Date (mm/dd/yyyy) _____		SEROLOGY 2 Collection Date (mm/dd/yyyy) _____		Other Diagnostic Tests?	Positive?
	Titer/Result	Positive?	Titer/Result	Positive?	PCR	☐Y ☐N
					Immunostain	☐Y ☐N
IFA-IgG	(_____)	☐Y ☐N	(_____)	☐Y ☐N	Culture	☐Y ☐N
IFA-IgM	(_____)	☐Y ☐N	(_____)	☐Y ☐N	Comments/details:	
Other test:_____	(_____)	☐Y ☐N	(_____)	☐Y ☐N		
Was there a fourfold change in antibody titer between the two serum specimens? ☐Y ☐N						

2008 CDC/CSTE CASE DEFINITION

CLINICAL PRESENTATION: Rocky Mountain spotted fever (RMSF) is an illness caused by Rickettsia rickettsii, a bacterial pathogen transmitted to humans through contact with ticks. Dermacentor species of ticks are most commonly associated with infection, including Dermacentor variabilis (the American dog tick), Dermacentor andersoni (the Rocky Mountain wood tick), and more recently Rhiphicephalus sanguineus (the brown dog tick). Disease onset averages one week following a tick bite. Age-specific illness is highest for children and older adults. Illness is characterized by acute onset of fever, and may be accompanied by headache, malaise, myalgia, nausea/vomiting, or neurologic signs; a macular or maculopapular rash appears 4-7 days following onset in many (~80%) patients, often present on the palms and soles. RMSF may be fatal in as many as 20% of untreated cases, and severe, fulminant disease can occur.

Acute illness is best detected by polymerase chain reaction (PCR) and immunohistochemical methods (IHC) in skin biopsy specimens, and occasionally by PCR in appropriate whole blood specimens taken during the first week of illness, prior to antibiotic treatment. Serology can also be employed for detection, however an antibody response may not be detectable in initial samples, and paired acute and convalescent samples are essential for confirmation.

CLINICAL EVIDENCE: Any reported fever and one or more of the following: rash, headache, myalgia, anemia, thrombocytopenia, or any hepatic transaminase elevation.

LABORATORY EVIDENCE: For the purposes of surveillance,

Laboratory confirmed: Serological evidence of a fourfold change in immunoglobulin G (IgG)-specific antibody titer reactive with Rickettsia rickettsii antigen by indirect immunofluorescence assay (IFA) between paired serum specimens (one taken in the first week of illness and a second 2-4 weeks later), or detection of R. rickettsii DNA in a clinical specimen via amplification of a specific target by PCR assay, or demonstration of spotted fever group antigen in a biopsy or autopsy specimen by IHC, or isolation of R. rickettsii from a clinical specimen in cell culture.

Laboratory supportive: Has serologic evidence of elevated IgG or IgM antibody reactive with R. rickettsii antigen by IFA, enzyme-linked immunosorbent assay (ELISA), dot-ELISA, or latex agglutination. *Note:* Current commercially available ELISA tests are not quantitative, cannot be used to evaluate changes in antibody titer, and hence are not useful for serological confirmation. IgM tests are not strongly supported for use in serodiagnosis of acute disease, as the response may not be specific for the agent (resulting in false positives) and the IgM response may be persistent. Complement fixation (CF) tests and other older test methods are neither readily available nor commonly used. CDC uses in-house IFA IgG testing (cutoff of ≥1:64), preferring simultaneous testing of paired specimens, and does not use IgM results for routine diagnostic testing.

EXPOSURE: Exposure is defined as having been in potential tick habitats within the past 14 days before onset of symptoms. A history of a tick bite is not required.

CASE CLASSIFICATION: *Confirmed:* A clinically compatible case (meets clinical evidence criteria) that is laboratory confirmed; *Probable:* A clinically compatible case (meets clinical evidence criteria) that has supportive laboratory results; *Suspect:* A case with laboratory evidence of past or present infection but no clinical information available (e.g. a laboratory report).

Tick-borne Illness and Clothing Study (TICS)
Baseline Questionnaire

Subject ID#: _____

Thank you for participating in this research study. This survey should take 20 to 25 minutes to complete, and will be used to collect information about your exposure to ticks and history of tick bites over the past year. You will also be asked about your use of tick bite prevention measures. You may skip any question you do not wish to answer. Your answers to all questions will be kept strictly confidential, and will not be shared with anyone other than the study investigators at UNC.

We appreciate your time and thank you for your participation.

PREVENTION IS KEY

TICStudyNC

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IMPORTANT NOTE: For the purposes of this questionnaire, "tick bites" are defined as ticks found attached to or embedded in the skin. "Unattached ticks" are defined as ticks found crawling on the skin or clothing.

PART 1: OCCUPATIONAL TICK EXPOSURE

1. On average, approximately how many hours per week did you spend outdoors as part of your work duties for each of the following seasons? (check one per season)

	0 hours per week	1 – 10 hours per week	11 – 20 hours per week	21 – 30 hours per week	More than 30 hours per week
This spring (March-May 2010)	◯	◯	◯	◯	◯
This summer (June-August 2010)	◯	◯	◯	◯	◯
This fall (September-November 2010)	◯	◯	◯	◯	◯

2. Please take your best guess as to the average number of **unattached** ticks that you found on your skin or clothing on days that you worked outdoors for each of the following seasons. (check one per season)

	0 unattached ticks per week	1 – 5 unattached ticks per week	6 – 10 unattached ticks per week	More than 10 unattached ticks per week
This spring (March-May 2010)	◯	◯	◯	◯
This summer (June-August 2010)	◯	◯	◯	◯
This fall (September-November 2010)	◯	◯	◯	◯

3. Did you have any tick bites (ticks attached to or embedded in your skin) this year (2010)?

    ◯ Yes   ◯ No (skip to Q9)

4. Approximately how many tick bites did you have this year? _____

5. In which seasons did you have tick bite(s) this year?
   (check all that apply and indicate approximate number of bites if known)

    ◯ Winter (January – February)   Approximate number of bites: _____
    ◯ Spring (March – May)   Approximate number of bites: _____
    ◯ Summer (June – August)   Approximate number of bites: _____
    ◯ Fall (September – November)   Approximate number of bites: _____

6. As best as you can remember, where were the tick bite(s) you had this year located on your body?
   (check all that apply)

    ◯ Head/neck
    ◯ Arms
    ◯ Chest/back
    ◯ Buttocks/groin
    ◯ Legs/ankles

7. Of the tick bite(s) you had this year, how many occurred while you were on the job?

    ◯ All   ◯ Most   ◯ Some   ◯ None

8. Of the tick bite(s) you had this year, how many did you report on a tick bite log or to your supervisor?

    ◯ All   ◯ Most   ◯ Some   ◯ None

9. Have you ever sought medical attention for a tick bite?

    ◯ Yes   ◯ No

10. Have you ever developed a rash within 4 weeks after being bitten by a tick?

⭘ Yes　　　⭘ No　　　⭘ Don't Know

11. Have you ever experienced fever within 4 weeks after being bitten by a tick?

⭘ Yes　　　⭘ No　　　⭘ Don't Know

12. Have you ever been diagnosed with a tick-borne illness?

⭘ Yes　　　⭘ No　　　⭘ Don't Know

12a. If yes, do you know which one(s) you were diagnosed with? (check all that apply)

⭘ Lyme disease

⭘ Rocky Mountain spotted fever

⭘ Ehrlichiosis

⭘ Anaplasmosis

⭘ Babesiosis

⭘ Other: _____

13. How often do you get bitten by mosquitoes while working outdoors at your job?

⭘ Frequently　　　⭘ Occasionally　　　⭘ Rarely　　　⭘ Never

14. How often do you get bitten by chiggers while working outdoors at your job?

⭘ Frequently　　　⭘ Occasionally　　　⭘ Rarely　　　⭘ Never

PART 2: TICK BITE PREVENTION MEASURES

15. Did you practice any of the following tick-bite prevention measures while working outdoors at your job during the spring and summer this year? (check all that apply):

- ◯ Wore long pants
- ◯ Wore long sleeve shirt
- ◯ Wore hat
- ◯ Tucked pants into socks or boots , or taped pants to boots
- ◯ Used insect repellent on skin
- ◯ Used insect repellent on clothes
- ◯ Checked yourself for ticks during the day while working outdoors
- ◯ Checked yourself for ticks at the end of the day after working outdoors

16. For each of the following tick-bite prevention measures, indicate how often you practiced each while working outdoors at your job during the spring and summer this year. (check one per row)

	Almost always (76 – 100% of the time)	Most of the time (51 – 75% of the time)	Sometimes (26 – 50% of the time)	Rarely (1 – 25% of the time)	Never (0% of the time)
Wore long pants	◯	◯	◯	◯	◯
Wore long sleeved shirt	◯	◯	◯	◯	◯
Wore hat	◯	◯	◯	◯	◯
Tucked pants into socks or boots , or taped pants to boots	◯	◯	◯	◯	◯
Used insect repellent on skin	◯	◯	◯	◯	◯
Used insect repellent on clothes	◯	◯	◯	◯	◯
Checked yourself for ticks during the day while working outdoors	◯	◯	◯	◯	◯
Checked yourself for ticks at the end of the day after working outdoors	◯	◯	◯	◯	◯

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17. If you used insect repellent on your skin while working outdoors at your job during the spring and summer this year, which repellents did you regularly use? (check all that apply)

- ○ DEET
- ○ Permethrin
- ○ Picaridin
- ○ Lemon eucalyptus oil
- ○ Other(s): _____

18. If you used insect repellent on your clothing while working outdoors at your job during the spring and summer this year, which repellents did you regularly use? (check all that apply)

- ○ DEET
- ○ Permethrin
- ○ Picaridin
- ○ Lemon eucalyptus oil
- ○ Other(s): _____

19. If you use a permethrin spray (such as Permanone) to treat your work uniforms, how often do you reapply the treatment?

_____ times per week/month/year (circle one)

○ I do not treat my uniforms with permethrin

20. On average, how often do you wash your work uniforms?

Shirts: After being worn _____ (number) of times

Pants: After being worn _____ (number) of times

Socks: After being worn _____ (number) of times

PART 3: NON-OCCUPATIONAL TICK EXPOSURE

21. On average, how many hours per week did you spend outdoors that were not work-related (such as recreational outdoor activities, yard work, etc.) for each of the following seasons? (check one per season)

	0 hours per week	1 – 10 hours per week	11 – 20 hours per week	21 – 30 hours per week	More than 30 hours per week
This spring (March-May 2010)	○	○	○	○	○
This summer (June-August 2010)	○	○	○	○	○
This fall (September-November 2010)	○	○	○	○	○

22. For each of the following tick-bite prevention measures, indicate how often you practiced each while spending time outdoors that was not work-related during the spring and summer this year. (check one per row)

	Almost always (76 – 100% of the time)	Most of the time (51 – 75% of the time)	Sometimes (26 – 50% of the time)	Rarely (1 – 25% of the time)	Never (0% of the time)
Wore long pants	○	○	○	○	○
Wore long sleeved shirt	○	○	○	○	○
Wore hat	○	○	○	○	○
Tucked pants into socks or boots , or taped pants to boots	○	○	○	○	○
Used insect repellent on skin	○	○	○	○	○
Used insect repellent on clothes	○	○	○	○	○
Checked yourself for ticks during the day while working outdoors	○	○	○	○	○
Checked yourself for ticks at the end of the day after working outdoors	○	○	○	○	○

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23. Did you participate in any of the following recreational activities this year? (check all that apply)

    ◯ Fishing   ◯ Camping

    ◯ Hunting   ◯ Backpacking

    ◯ Hiking   ◯ Gardening

24. Do you own any household pets that spend time both indoors and outdoors?

    ◯ Yes   ◯ No

SECTION 4: DEMOGRAPHIC INFORMATION

25. Name: _____

26. Date of birth: ___/___/_____ (dd/mm/yyyy)

27. Gender: ◯ Male   ◯ Female

28. DENR Division:

    ◯ NC Division of Forest Resources   County, District, or Region: _____

    ◯ NC Division of Parks and Recreation   Park or Region: _____

29. Job title: _____

30. Are you a full time or part-time employee?   ◯ Full time   ◯ Part-time

    28a. If part-time, how many hours per week do you work? _____

31. How long have you been working in your current position? ____/____ (years/months)

32. What is the highest level of education that you have completed?

    ◯ Some high school
    ◯ Completed high school
    ◯ Some college
    ◯ Associates degree
    ◯ Bachelors degree
    ◯ Graduate level degree

Thank you for your time! If you have any questions please contact the study coordinator at (919) 450-7783 or TICStudyNC@gmail.com.

Tick-borne Illness and Clothing Study (TICS): WEEKLY TICK BITE LOG

Name: _____

Week ending: _____
(Sunday)

Total number of _work_ hours spent outdoors this week: _____

Total number of _non-work_ hours spent outdoors this week: _____

Instructions: For each week during the study period, use this log to record all tick bites (attached ticks only). Do not record unattached ticks found on your skin or clothing. Please record the date you located the tick bite, and if there were multiple tick bites found on the same day, indicate the number of tick bites and the location of the tick bite(s) on your body. If known, please indicate the county where the tick(s) were most likely to have been acquired from. Also indicate whether you had been using insect repellant when the tick was acquired and if you saved the tick in one of the vials provided to you.

If you did not receive any tick bites during the week, please check the "Did not have any tick bites this week" box below.

DATE TICK BITE(S) LOCATED	NUMBER OF TICK BITE(S)	LOCATION(S) OF TICK BITE(S) ON BODY	COUNTY WHERE TICK(S) WERE MOST LIKELY ACQUIRED	TICK BITE(S) ACQUIRED ON THE JOB?	REPELLANT USED? (Indicate type and whether applied to skin or clothing)	TICK(S) COLLECTED? (Indicate vial #)

☐ Did not have any tick bites this week

If you are bitten by a tick you should remove the tick from your skin as soon as you notice it. Use fine-tipped tweezers to firmly grasp the tick at the site of attachment, as close to the skin as possible. With a steady motion, pull the tick's body away from your skin. Avoid crushing the tick if possible and save it in one of the vials provided to you. Then wash your skin with soap and warm water. You should monitor the affected area daily and report any change to the bite area to your supervisor, who will assist you in seeking medical attention.

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