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Tick-Borne Diseases in North Carolina: Is "*Rickettsia amblyommii*" a Possible Cause of Rickettsiosis Reported as Rocky Mountain Spotted Fever?

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ABSTRACT

Cases of Rocky Mountain spotted fever (RMSF) in North Carolina have escalated markedly since 2000. In 2005, we identified a county in the Piedmont region with high case numbers of RMSF. We collected ticks and examined them for bacterial pathogens using molecular methods to determine if a novel tick vector or spotted fever group rickettsiae (SFGR) might be emerging. *Amblyomma americanum*, the lone star tick, comprised 99.6% of 6,502 specimens collected in suburban landscapes. In contrast, *Dermacentor variabilis*, the American dog tick, a principal vector of *Rickettsia rickettsii*, comprised < 1% of the ticks collected. Eleven of 25 lone star tick pools tested were infected with "*Rickettsia amblyommii*," an informally named SFGR. Sera from patients from the same county who were presumptively diagnosed by local physicians with a tick-borne illness were tested by an indirect immunofluorescence antibody (IFA) assay to confirm clinical diagnoses. Three of six patients classified as probable RMSF cases demonstrated a fourfold or greater rise in IgG class antibody titers between paired acute and convalescent sera to "*R. amblyommii*" antigens, but not to *R. rickettsii* antigens. White-tailed deer, *Odocoileus virginianus*, are preferred hosts of lone star ticks. Blood samples collected from hunter-killed deer from the same county were tested by IFA test for antibodies to *Ehrlichia chaffeensis* and "*R. amblyommii*." Twenty-eight (87%) of 32 deer were positive for antibodies to *E. chaffeensis*, but only 1 (3%) of the deer exhibited antibodies to "*R. amblyommii*," suggesting that deer are not the source of "*R. amblyommii*" infection for lone star ticks. We propose that some cases of rickettsiosis reported as RMSF may have been caused by "*R. amblyommii*" transmitted through the bite of *A. americanum*. Key Words: Rocky Mountain spotted fever—*Amblyomma americanum*—lone star tick—spotted fever group rickettsiae—"*Rickettsia amblyommii*"—*Rickettsia rickettsii* — *Ehrlichia chaffeensis*

INTRODUCTION

SPOTTED FEVER GROUP RICKETTSIAE are obligate intracellular bacteria transmitted by ticks. Tick-borne rickettsioses are an important cause of human morbidity worldwide (Parola et al. 2005). Rocky Mountain spotted fever (RMSF) is a rickettsiosis of significant public health importance, because of the severity of its pathology. It is a zoonotic disease that is maintained in nature in a cycle involving a tick vector and reservoir, with various wildlife species contributing to new lines of ticks infected with the pathogen (Azad and Beard 1998). Humans are incidentally infected. The disease is most prevalent in the eastern United States. In 2006,

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approximately 42% of RMSF cases reported to the Centers for Disease Control and Prevention (CDC) were from North Carolina (NC). The high proportion of cases reported from NC represents an escalating trend, reflecting an approximate 10-fold rise, from 78 cases in 2000 to 842 cases in 2006 (www.epi.state.nc.us/epi/gcdc.html).

Significantly, most reported cases of RMSF in NC have not been confirmed by laboratory tests of paired acute and convalescent sera, but are classified as probable cases, based on exposure to ticks, occurrence of appropriate clinical symptoms and, a single acute phase serum sample producing a titer of $\geq 1/64$ for polyvalent antibodies (all immunoglobulin classes) by indirect immunofluorescence antibody assay or comparable optical density measured by enzyme-linked immunosorbent assay (ELISA). Test results can vary from laboratory to laboratory and standardization of methods is not well established. Consequently, the true burden of disease from RMSF in NC is unknown.

There are several plausible explanations for the observed increases. Tick vectors may be increasing in abundance in residential landscapes (Shriefer and Azad 1994; Childs and Paddock 2002); a novel tick vector could be emerging or increasing in prevalence (Demma et al. 2005); and an unrecognized rickettsial disease could be emerging in NC (Carpenter et al. 1999; Paddock et al. 2004; Whitman et al. 2007). Furthermore, it is conceivable that some people experiencing a summer flu-like illness or reporting exposure to ticks may perhaps exhibit false positive laboratory tests for RMSF because of pre-existing antibody titers resulting from infection from other SFG rickettisae (Paddock 2005).

Accordingly in 2005, we initiated a tick-borne disease surveillance and tick ecology project that involved physician-based surveillance for tick-borne illness, coupled with laboratory confirmation, collection of ticks from suburban woodlands, and analysis of sera from hunter-killed white-tailed deer in a single county in the Piedmont region of NC. Our objectives were to determine the comparative abundance of tick species on residential properties, to estimate the prevalence of infection for selected bacterial pathogens in a sample of the ticks, to determine exposure rate of white-tailed deer (*Oedocoileus virginianus*) to selected pathogens, and to confirm through laboratory testing the occurrence of RMSF among patients diagnosed with a tick-borne illness.

MATERIALS AND METHODS

Study area

Chatham County, NC, was chosen as the location for our investigation. The county is representative of other counties in the Piedmont region that have experienced rapid suburbanization (http://quickfacts.census.gov/qfd/ states/37/37037.html). In 2000, the population density averaged 27.9 persons per square kilometer in Chatham County while the statewide average was 63.8 persons per square kilometer. However, from 2000 to 2004, the resident population increased by approximately 15.6% compared to an increase of 6.1% for the entire state. The total number of cases of RMSF reported during this period was 33 cases in Chatham County, while the total number of cases averaged 14.2 cases per county for all 100 counties in NC (http://www.epi.state.nc.us/epi/gcdc/pdf/CDbyDiseasebyYear2000-2005.pdf).

Collection and processing of ticks

Ticks were collected from April 9 to July 24, 2005 in vegetation around residences ($n = 32$) of people expressing concern about heavy infestations on their property. In general, the landscape consisted of oak-pine woodlands with an understory of leaf litter and low-growing shrubs. A flag constructed of corduroy cloth was pulled over and through vegetation, and attached ticks were removed and immediately preserved in 95% ethanol. Quantitative collections were not made, but attempts were made to collect at least 100 ticks at each residence. Subsequently, ticks from each residence were sorted by species and life stage into pools for molecular analyses for pathogens as described below.

Collection and storage of deer blood samples

Exposure of white-tailed deer to tick-borne pathogens was assessed from blood collected from hunter-killed deer. Blood from each deer was adsorbed onto Nobuto blood filter strips (Toyo Roshi Kaisha, Ltd., Tokyo, Japan) within 1 hour of the time the deer was killed. Strips were air dried and then placed in individual labeled plastic envelopes. Within 2 days of collection, strips were stored at –18ºC until they were processed as described below.

Molecular analyses of ticks

Dermacentor variabilis and *Ixodes scapularis* were processed individually, except for one *D. variabilis* pool that contained three ticks. At sites where these tick species were collected, a small number of *A. americanum* pools were also analyzed. Because of the large overall total, a sample of *A. americanum* pools at other sites was randomly chosen and tested. Ticks were macerated individually or in pools of up to 20 ticks/pool in microcentrifuge tubes containing two copper metal pellets and 0.5 mL sterile PBS in a Mixer Mill 300 (Qiagen Inc, Valencia, CA). Total nucleic acids (NA) were extracted from individual samples using a Qiagen Viral RNA Kit (Qiagen Inc, Valencia, CA) according to the manufacturer's instructions. Quality control measures included negative controls (water) that were extracted and amplified in parallel with all specimens. Positive control DNA for each agent was included in each assay run. To minimize the potential for NA contamination, three separate, designated areas were used for NA extraction and preparation of primary and secondary PCRs. Additionally, two thermal cyclers (PTC-100^{TM}, MJ Research, Inc.), designated for either primary or secondary amplification, were used.

The 16S rRNA gene was targeted for the detection of *Ehrlichia chaffeensis* and *Anaplasma phagocytophilum* using 2.5 μL of DNA template in a primary polymerase chain reaction (PCR) with external primers ECC and ECB (Anderson et al. 1991), which amplify all *Ehrlichia* and *Anaplasma* spp. expected to be found in the geographic area of our investigation. For secondary PCR amplification, 1 μL of primary product was used in reactions with species-specific primers HE1and HE3 to detect *E. chaffeensis* (Dawson et al. 1994), producing a 389-bp amplicon, and in reactions with primers GA1UR and GE9F to detect *A. phagocytophilum* (Chen et al. 1994; Little et al. 1998), producing a 400-bp amplicon. External primers FLALL and FLARL and internal primers FLALS and FLARS, which amplify a 330-bp region of the *flaB* gene of all species in the genus *Borrelia*, were used in a nested PCR assay as described elsewhere (Barbour et al. 1994; Moore et al. 2003). Primer pairs 17 kDa1/17 kDa2 (external) and 17 kDa3/17 kDa4 (internal) were used to amplify a 434-bp portion of the 17kDa gene from *Rickettsia* spp. (Skeyova et al. 2001).

Products amplified with internal primer sets HE1/HE3, GA1UR/GE9F, FLALS/FLARS, and 17kDa3/17kDa4 were purified with a Qiagen Gel Purification Kit (Qiagen Inc., Valencia, CA) and sequenced in both the 3′ and 5′ directions with a PerkinElmer ABI Prism 3700 automated DNA sequencer at the Molecular Genetics Instrumentation Facility at the University of Georgia. The sequences were assembled and edited with the Sequencher software package, version 4.0.5 (Gene Codes Corp., Ann Arbor, MI), and a nucleotide–nucleotide BLAST (BLASTN) search was performed to determine the most similar GenBank sequences.

Tick-borne disease surveillance and laboratory confirmation of clinical diagnoses

Five physician practices were recruited into our prospective investigation for a 3-month period from June 1 to August 31, 2005. Participating physicians agreed to report all suspected cases of tick-borne illness to the Chatham County Health Department. To be considered a suspect case of tick-borne illness, the affected patient had to have had a reasonable opportunity for tick exposure (bite was not necessary), a fever or rash, and one other compatible symptom not explained by another illness. Self-reported symptoms were considered by physicians in making a diagnosis. Physicians also agreed to provide a clinical assessment for patients, and to collect acute and convalescent serum samples for testing in the North Carolina State Laboratory of Public Health. Sera were tested by an indirect immunofluorescence antibody (IFA) assay against a panel of bacterial

antigens, including *R. rickettsii*, *R. typhi*, and *Ehrlichia chaffeensis* (CDC Biologics Branch, Atlanta, GA). Polyvalent conjugate to both heavy and light chains of IgG was used; therefore, the antibody titers reflect the summary reaction of all immunoglobulin classes. A case was classified as probable when the patient exhibited symptoms of a tick-borne illness (CDC 2006) and the IFA test produced a serologic titer of $\geq$1/64 for an acute phase serum sample (CDC 2006). A confirmed case of tick-borne illness required a $\geq$ 4-fold increase in antibody titer between paired sera (CDC 2006). Serum samples from all patients that were tested for antibodies to rickettsial and ehrlichial pathogens were also sent to the CDC (Fort Collins, CO) for testing by ELISA and Western blot against *Borrelia burgdorferi* antigens. Subsequently, paired sera were sent to CDC (Atlanta) for additional rickettsial testing as described in the Results section.

Testing of deer serum samples

Each Nobuto strip was eluted separately in enough diluent to produce an estimated 1/20 dilution. Eluates were briefly refrigerated until assayed for antibodies to various agents. For screening, the elutions were diluted threefold just prior to testing to make a 1/60 dilution. Subsequent twofold serial dilutions were made into a PBS (pH 7.38) diluent containing 1% rabbit serum and 1% bovine serum albumin. Indirect immunofluorescence antibody tests were carried out as described previously for *A. phagocytophilum* (Petrovec et al. 2002), but with *E. chaffeensis* or rickettsial antigens. Titers are expressed as the reciprocal of the dilution of the final well showing distinct immunofluorescence.

RESULTS

Comparative abundance of tick species

A total of 6,502 ticks were collected from the 32 home sites in Chatham County (Table 1). The lone star tick (*Amblyomma americanum*) was the most abundant species collected on residential property, accounting for 99.6% of the ticks collected. Nymphs comprised almost 83% of the lone star ticks collected. American dog ticks (*Dermacentor variabilis*) and black-legged ticks (*Ixodes scapularis*) were much less abundant, and together accounted for $<$ 1% of the specimens collected. Lone star ticks were collected at 31 (97%) of 32 home sites, and American dog ticks and a black-legged tick were collected at 13 (41%) and 1 (3%) of the 32 sites sampled, respectively.

Prevalence of rickettsial organisms in ticks

A sample of 332 ticks in 52 pools (30 pools of *A. americanum*, 23 pools of *D. variabilis* and 1 *I. scapularis*) representing 18 of the 32 home sites was tested by PCR for *E. chaffeensis*, *A. phagocytophilum*, *Borrelia* spp., and *Rickettsia* spp. *E. chaffeensis* was detected in 1 pool of *A. americanum* and in a single *D. variablis*, *A. phagocytophilum* was detected in 1 pool of *A. americanum*, and *Rickettsia* spp. were detected in 15 pools of *A. americanum*, 5 *D. variabilis* pools, and a single *I. scapularis*. *Borrelia* spp. were not detected in the 52 pools tested (Table 2).

Polymerase chain reaction products of the 21 *Rickettsia*-positive pools were sequenced to identify the species. "*Rickettsia amblyommii*" was identified in 11 *A. americanum* pools and in 2 individual *D. variablis*. The sequence ho-

Table 1. Numbers and Life Stages of Tick Species Collected by Flagging Vegetation on the Home Grounds of Residences (n = 32) in Chatham County, North Carolina, April 9 to July 24, 2005

	Number collected (% of total)			
Tick species	*Females*	*Males*	*Nymphs*	*All stages*
Amblyomma americanum	578 (8.9)	506 (7.8)	5,390 (82.9)	6,474 (99.6)
Dermacentor variabilis	17 (0.3)	10 (0.2)	0	27 (0.4)
Ixodes scapularis	0	0	1 (<0.1)	1 (<0.1)

TABLE 2. RESULTS OF PCR ANALYSES OF TICKS COLLECTED IN CHATHAM COUNTY FOR SELECTED PATHOGENS

	No. positive pools/no. pools (no. ticks)			
Tick species	*Ehrlichia chaffeensis*	*Anaplasma phagocytophilum*	*Borrelia* spp.	*Rickettsia* spp.
Amblyomma americanum	1/30 (308)	1/30 (308)	0/30 (308)	15/30 (308)
Dermacentor variabilis	1/21 (23)	0/21 (23)	0/21 (23)	5/21 (23)
Ixodes scapularis	0/1 (1)	0/1 (1)	0/1 (1)	1/1 (1)
Total	2/52 (332)	1/52 (332)	0/52 (332)	21/52 (332)

mologies for PCR amplicons that were successfully identified to species were 99%–100%. The ticks infected with "*R. amblyommii*" were collected from seven different home sites. Specific identification based on 17 kDa gene sequences could not be made for an additional 4 pools of *A. americanum*. Because of the lack of sequence diversity in the amplified region of the 17 kDa gene, differentiation between *R. rickettsii* and *R. montanensis* could not be made in 2 *D. variablis* pools (4 ticks) and in the single *I. scapularis* tested. In another sample containing a single *D. variablis* female, we could not differentiate between *R. felis* and *R. rickettsii*.

Serological testing—humans

Serum samples were obtained from 79 patients, but only 51 patients met clinical criteria for inclusion in the investigation. Paired sera were obtained for 29 (56.9%) of the 51 patients. Based on polyvalent serological test results of paired sera, 6 patients were classified as probable RMSF cases, and 5 patients were confirmed as HME cases. Another 10 patients from whom only single acute sera were obtained also exhibited polyvalent antibodies reactive with *R. rickettsii* antigens at levels $\geq 1/64$. Thus, 31% (16/51) of patients were classified as probable cases of RMSF. The diagnosis of a single Lyme disease patient based on an erythema migrans lesion was not supported by a positive laboratory test result. Additionally, serum samples from all other patients tested negative for antibodies to *B. burgdorferi*.

We were surprised at the number of patients that were considered probable for RMSF and that laboratory tests failed to confirm any of the patients presumptively diagnosed with this illness. Given the low relative abundance of American dog ticks, the high relative abundance of lone star ticks around residences and, the prevalence of "*R. amblyommii*" in lone star tick pools, we reasoned that the probable RMSF case patients were being exposed to and possibly infected with another SFGR, most likely "*R. amblyommii*." Accordingly, paired sera from the six patients classified as probable RMSF were re-evaluated in the CDC Rickettsial Zoonoses Branch against *R. rickettsii* and "*R. amblyommii*" antigens by class-specific IFA. In general, greater reactivity, as reflected in higher end point titers, was exhibited to "*R. amblyommii*" when tested concurrently against *R. rickettsii* antigens (Table 3). A fourfold increase in antibody titer to "*R. amblyommii*" antigens was found in IgG class antibodies for patients 4, 5, and 6.

Serological testing—deer

Phosphate buffered saline extracts of dried blood samples from 32 white-tailed deer were tested by IFA for antibodies to *E. chaffeensis* and *A. phagocytophilum* at a screening dilution of 1/60. Twenty-eight (87.5%) samples tested positive for antibody to *E. chaffeesis*, and 3 (9.4%) tested positive to *A. phagocytophilum*. The *R. rickettsii* and "*R. amblyommii*" antigens used in IFA tests of human sera were also used in IFA assays of PBS extracts of dried blood samples from deer. Interestingly, none of the 32 samples exhibited reactivity to *R. rickettsii*, and only 1 (3%) sample was reactive to "*R. amblyommii*" antigens at a dilution of 1/480.

TABLE 3. RESULTS OF DIAGNOSTIC TESTS OF PROBABLE RMSF PATIENTS. VALUES REPRESENT THE RECIPROCALS OF END-POINT DILUTIONS GIVING STRONG FLUORESCENCE IN IFA TESTS

	Sera drawn (days after onset)		*Rickettsia rickettsii antigens*				*"Rickettsia amblyommii" antigens*			
			IgM (mu)		*IgG (gamma)*		*IgM (mu)*		*IgG (gamma)*	
Patient	*acute*	*conv.*	*acute*	*conv.*	*acute*	*conv.*	*acute*	*conv.*	*acute*	*conv.*
1	0	24	28	128	<16	16	512	256	128	64
2	5	59	28	128	64	32	256	128	32	64
3	1	51	28	128	32	32	128	128	256	256
4	9	64	64	64	16	16	16	256	256	1024
5	1	14	28	128	16	64	256	256	<16	512
6	21	46	32	32	64	64	128	64	<16	512

Clinical findings

Onset of acute symptoms for these patients occurred between June 30 and August 8, 2005. Age of patients ranged from 2 years to 83 years of age, with a median age of 54 years. Clinical presentations for probable RMSF patients (for whom paired sera were available) suggested a mild illness. Symptoms included sudden onset (6/6 patients self-reported), fever (5/6 patients self-reported, 3/6 patients on examination), headache (4/6 patients self-reported, 2/6 patients on examination), rash (2/6 patients on examination), and probable exposure to ticks (5/6 patients self-reported). The reported rash was not described for patient 5, but that for patient 4 was described as an erythematous maculopapular rash on trunk, arms, and legs. However, the rash was attributed to an adverse drug reaction. Elevated hepatic transaminases were reported for patient 1. When clinical examinations were performed, fevers for patients 1, 2, and 4 ranged between 37.3°C and 37.8°C. Routine hematological tests were completed for these patients, and mild thrombocytopenia was reported. No eschars were reported at the site of the tick bite. No patients required hospitalization. No other abnormalities were documented. Doxycycline was prescribed as an anti-rickettsial therapy, and the illnesses of all patients resolved.

Of the 79 people suspected of having tick-borne illnesses, 5 were confirmed as *Ehrlichia chaffeensis* infections. The confirmed HME patients experienced a more severe illness. Four case patients' records were available for review. All reported extensive tick exposure and an acute onset of febrile illness with moderate to severe headache and body aches but no skin rash. One patient was hospitalized with a temperature of 40°C and subsequently experienced a myocardial infarction during the acute illness. Complete blood counts were performed on 3 case patients: 2 had thrombocytopenia (platelet counts of 28 and 111×10^9/L, normal $130–400 \times 10^9$/L); and 3 had leucopenia (total white blood cell counts of 2.7, 3.3, and 3.7×10^9/L; normal $4.3–10.8 \times 10^9$/L). The blood chemistries performed on 2 patients revealed elevated hepatic transaminases, approximately 6 times the upper limit of normal, in both patients. All patients received doxycycline and recovered.

DISCUSSION

Dermacentor variabilis and *Ixodes scapularis*, the established vectors of *R. rickettsii* and *B. burgdorferi*, respectively, were infrequently collected in suburban landscapes of Chatham County, NC. Although the American dog tick occurs in North Carolina, quantitative surveys of the statewide distribution and local abundance of this species are lacking. Brown dog ticks have been shown to be important local vectors of *R. rickettsii* in eastern Arizona (Demma et al. 2005), but we did not identify *Rhipicephalus sanguineus* in our surveys. Interestingly, *Amblyomma americanum* was the most frequently collected species of tick around dwellings, indicating that residents would likely experience the highest attack rates from

this species. The probable risk of pathogen acquisition from lone star and other tick species is likely correlated with their distribution and local abundance. *Amblyomma americanum* is the vector of *E. chaffeensis* (Childs and Paddock 2003), and laboratory confirmation of HME patients is congruent with the high frequency of occurrence of lone star ticks around the residences included in our tick survey. Indeed, some patients thought to be RMSF cases may have actually been infected with *Ehrlichia chaffeensis*. Ehrlichial and rickettsial infection can have similar acute clinical appearances and can be confused if laboratory testing is not completed (Carpenter et al 1999). *Rickettsia parkeri* has been recently identified as another SFGR that may be misclassified as RMSF, particularly since cross-reactive antibodies may be seen (Paddock et al 2004; Paddock 2005; Whitman et al. 2007).

In view of the large numbers of RMSF cases from North Carolina reported to CDC, we were surprised that none of the patients presumptively diagnosed with RMSF were confirmed through initial testing completed in the NC State Laboratory of Public Health. Clinical evaluations of patients presumptively diagnosed as RMSF indicated that they experienced a mild febrile illness without a maculopapular or petechial rash and did not require hospitalization. All patients reported sudden onset of illness, and 5 of 6 patients reported a tick bite. Additionally, serological testing confirmed that these patients had been infected with a SFGR.

All six patients who reported a tick bite showed higher endpoint titers to "*R. amblyommii*" over those to *R. rickettsii* in assays run at the same time, using the same reagents, and read by the same investigator. Patients 4, 5, and 6 demonstrated clear IgG seroconversions to "*R. amblyommii*." Class-specific antibody testing clarified the results of the initial laboratory testing (via polyvalent conjugate); notably, significant seroconversions against *R. rickettsii* antigens were still lacking. These results provide support for infection with "*R. amblyommii*" or a closely related SFGR as the cause of these illnesses, while lessening evidence for *R. rickettsii* infection. It also should be noted that patient 5 exhibited a fourfold change in titer to IgG class antibodies to *R. rickettsii* antigen. This patient would have been classified as a confirmed RMSF patient if additional testing with "*R. amblyommii*" antigen had not been carried out. Notably, Burgdorfer et al. (1981) reported that "*R. amblyommii*"–infected voles and guinea pigs did not develop antibodies that cross-reacted with *R. rickettsii* antigen. "*Rickettsia amblyommii*"–infected mice produced antibodies that cross-reacted only with *R. rickettsii* antigen and at lower titer (homologous titer: 1/4,096 versus heterologous titer: 1/16). Antisera to *R. rickettsii* and *R. parkeri* cross-reacted with "*R. amblyommii*" antigen only at low titers (1/16 and 1/8, respectively).

Patients 1, 2, and 3 showed high titers of IgM and IgG class antibodies in acute specimens, but they did not exhibit significant rises in titers in convalescent sera. One or more of the following explanations might account for this observation. First, the patients could have been infected during the preceding year but saw their physicians for an unrelated illness. Second, patients might have been infected with a SFGR other than "*R. amblyommii*" or *R. rickettsii*. Third, the patients could have developed an immune response during an asymptomatic period prior to their perceived date of "onset," but acute sera was still drawn and antibiotic treatment given before a full immune response occurred. Generally, it is suggested that antibody levels in RMSF sera will not reach diagnostic levels before one week of onset and early antibiotic therapy can interfere with antibody production.

Spotted fever group rickettsiae (Goddard and Norment 1986; Goddard et al. 2003; Kardatzke et al. 1992; Solberg et al. 1996), including "*R. amblyommii*" (Burgdorfer et al. 1981; Stromdahl et al. 2008; Labruna et al. 2004, 2007; Kelly et al. 2005; Mixson et al. 2006), have been detected previously in *Amblyomma* spp. ticks. *Amblyomma americanum* is apparently commonly infected with "*R. amblyommii*". When this SFGR was first identified, prevalences as high as 42% were noted (Burgdorfer et al. 1981). In a recent survey encompassing 9 U.S. states, prevalence estimates of "*R. amblyommii*" in lone star ticks ranged from 0% to 84%, and averaged 41.2% (Mixson et al. 2006). "*Rickettsia amblyommii*" was detected in lone star ticks collected from 7 (38.8%) of the 18 residences for which

ticks were tested in our survey, indicating that the organism is prevalent in *A. americanum* populations in central NC.

When the SFGR known now as "*R. amblyommii*" was originally isolated from *A. americanum* ticks and designated WB-8-2, the investigators (Burgdorfer et al. 1981) discussed the pathogenic potential of this agent. In laboratory studies, it was nonpathogenic to laboratory animals. Because of this finding and the frequent exposures of humans to commonly infected ticks with no apparent subsequent illness, they suggested that the organism was nonpathogenic to humans (Burgdorfer et al. 1981). However, nonpathogenic strains may be important in the evolution of pathogenic SFG rickettsiae through changes in genes encoding antigenic surface proteins (Weller et al. 1998). Stromdahl and coworkers (2008) speculate that bites from *"R. amblyommii"*–infected lone star ticks might account for the seroprevalence of antibodies to SFGR, which often exceeds reported cases of RMSF. Patients experiencing mild febrile illnesses following tick bites and developing antibodies reactive with SFGR have been reported previously in NC (Carpenter et al. 1999; Sexton et al. 1998) and other eastern states (Sanchez 1992; Dasch et al. 1993; Yevich et al. 1995; McCall et al. 2001). Anecdotally, "*R. amblyommii"* infections have been suggested as the cause of unexplained fever following tick bite, based on serologic reactivity of patient sera with rickettsial antigens in Western blot analyses (Dasch et al. 1993). Recently, "*R. amblyommii*" DNA was detected by PCR in an engorged tick of the species *A. americanum* that was removed from a woman who subsequently developed a macular rash at the site of the tick bite (Billeter et al. 2008).

The high prevalence of antibodies to *E. chaffeensis* in white-tailed deer sampled during our investigation is concordant with its status as a wildlife reservoir and its close association with lone star ticks, which are vectors of the pathogen (Paddock and Childs 2003). White-tailed deer seropositve for antibodies to *E. chaffeensis* have been reported previously from NC (Yabsley et al. 2005). For the deer that we tested, a smaller percentage (9.4%) were positive for antibodies to *A. phagocytophilum*. A previous serosurvey reported finding 19% of white-tailed deer samples from NC positive for antibodies to *A. phagocytophilum* (Dugan et al. 2006). In contrast to the high prevalence of antibodies to *E. chaffeensis* in white-tailed deer, we found only 1 (3%) of 32 deer serum samples to be positive for antibodies reactive with "*R. amblyommii*" and none to be positive for *R. rickettsii*. The lack of antibodies to *R. rickettsii* was expected, as deer are not commonly hosts of *D. variabilis*. However, the low prevalence of antibodies to "*R. amblyommii*" is surprising because *A. americanum* feeds heavily on deer (Childs and Paddock 2003). The lack of antibodies to "*R. amblyommii*" was likely not due to failure of ticks to transmit the organism, because Burgdorfer et al. (1981) reported finding disseminated infections of "*R. amblyommii*" in lone star ticks. It is more likely that "*R. amblyommii*" is not infectious to deer, that deer exhibit immune tolerance, or that the production of antibodies is downregulated by heavy exposure to the agent (Semu et al. 2001). White-tailed deer are established reservoirs for *E. chaffeensis* (Paddock and Yabsley 2007), but the reservoir status of deer for "*R. amblyommii*" is unknown. Spotted fever group rickettsiae are maintained vertically by transovarial transmission (Azad and Beard 1998). It may be that there is no vertebrate reservoir for "*R. amblyommii*," as Burgdorfer et al. (1981) reported that 100% of lone star tick progeny from infected females are also infected.

CONCLUSIONS

Our findings suggest that an emerging SFGR may be the cause of some cases of rickettsiosis reported as RMSF in NC. *Amblyomma americanum*, an aggressive tick that readily attacks humans, is the most abundant tick species in rural and suburban landscapes. High prevalence of infection of lone star ticks with "*R. amblyommii*" suggests that this SFG rickettsial species might be responsible for some of the illness reported as RMSF, and it could account for the recent escalation in numbers of cases reported as RMSF. Our findings support previous suggestions that the pathogenicity of "*R. amblyommii*" to humans warrants further investigation (Mixson et al. 2006; Labruna et al.

2007). These studies would include cell culture and molecular evaluation of human specimens from clinically ill patients to provide specific identity of the etiologic agent.

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