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Modeling Tick-Borne Diseases in the Virginia and Middle Peninsula

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Modeling Tick-Borne Diseases in the Virginia and Middle Peninsula

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By

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Abstract

Tick-borne diseases are on the rise, causing concern for human health as ticks continue to expand both in range and numbers. This study sought to assess the prevalence of two tick-borne diseases on the Virginia and Middle Peninsula and to identify the variables that explain their distributions. The two disease-causing bacteria, *Ehrlichia chaffeensis* and *Rickettsia parkeri*, are both transmitted by the lone star tick, the most common human-biting tick in the study area and in the southeastern United States. Nymph ticks were collected at 122 random sites in southeastern Virginia and DNA was extracted from the pooled ticks from each site. Bacterial DNA was detected using both endpoint PCR and Taqman qPCR to compare sensitivities of the assays. The two methods detected *E. chaffeensis* at 48.4% and 52.3% of sites, respectively, while *R. parkeri* was not detected at any sites based on Taqman PCR. We developed two logistic regression models, based on each PCR method, which were spatially applied to develop spatial models of probability of *E. chaffeensis* presence, to determine biotic and abiotic variables explaining heterogeneity in disease occurrence. We selected candidate sets of models based on the information-theoretic approach. The variables identified for both sets of models were linked to white-tailed deer browsing and space use, while tick counts and time of year were not included in the final candidate models. These results suggest that the white-tailed deer, the primary host of lone star ticks and the only known reservoir host of *E. chaffeensis*, is the most important predictor of *E. chaffeensis* distribution.

Introduction

In 2011, the Virginia Department of Health sent a letter to clinicians warning that tick-borne diseases are on the rise, and urging medical providers to educate patients about tick-borne diseases and raise awareness of their risk (Remley 2011). In the southeastern United States, the lone star tick (*Amblyomma americanum*) is the most common human-biting tick (Felz et al. 1996, Merten and Durden 2000) and is found throughout the eastern United States; its range extends as far west as Texas and Oklahoma (Centers for Disease Control and Prevention, 2011). In Virginia, lone star tick populations are concentrated in the eastern half of the state (Sonenshine et al. 1965), and in southeastern Virginia specifically, lone star ticks account for 95% of all ticks collected (Nadolny et al. 2014). This species is of particular interest from a health perspective because of its role in the transmission of several zoonotic diseases (Merten and Durden 2000, Goddard & Varela-Stokes 2009). These include Rocky Mountain Spotted Fever (RMSF), “American Boutoneusse Fever” (or *R. parkeri* rickettsiosis), Human Monocytotropic Ehrlichiosis, Southern Tick-Associated Rash Illness (STARI), and Tularemia, as well as several others that have yet to be confirmed via transmission studies (Goddard and Varela-Stokes 2009, Stromdahl and Hickling 2012). Lone star ticks are increasing both in number and geographic range, as are the number of reported cases of associated diseases, and these numbers are predicted to continue to rise (Childs and Paddock 2003, Paddock and Yabsley 2007).

One of the most important diseases transmitted by lone star ticks is Human Monocytotropic Ehrlichiosis (HME), first described in 1986 (Maeda et al. 1987). The

causative agent of this illness is *Ehrlichia chaffeensis* (Fig. 1), an obligate intracellular bacterium that attacks mononuclear phagocytes in the blood and tissues of the infected person (Anderson et al. 1991, Dawson 1991, Dumler and Bakken 1995). Symptoms of HME are often non-specific and flu-like—including fever, headache, malaise, thrombocytopenia, leukopenia and elevated levels of liver enzymes—and usually present after an incubation period of 7 to 21 days post-tick bite (Fishbein et al. 1989, Dawson et al. 1991, Brown et al. 2005, Pujalte and Chua 2013). HME can be treated with tetracyclines (Olano et al. 2003b, Pujalte and Chua 2013), but if left untreated this disease can be deadly, with an annual case fatality rate as high as 3.7% (Centers for Disease Control and Prevention, 2013). Specific human populations are more sensitive to HME and are at an increased risk of infection. The number of reported cases increases with age; between 2000 and 2010 the age group with the highest average annual incidence was 60-64 (Demma et al. 2005). Additionally, more cases are reported in males than in females (Olano et al. 2003a, Centers for Disease Control and Prevention, 2013). A compromised immune system, resulting from cancer treatments, previous organ transplants or HIV, is a significant risk factor for contracting a more severe case of HME (Dumler and Bakken 1995, Paddock et al. 2001, Bakken et al. 2006). People that participate in outdoor activities are more likely to encounter a human-biting tick, since both nymph and adult lone star tick populations peak during the summer months, a popular time for outdoor recreation (Hair and Howell 1970, Standaert et al. 1995). While cases are reported throughout the year, the majority are reported between May and August, with over 25% recorded in June between 1994 and 2010 (Paddock

et al. 2001, Centers for Disease Control and Prevention, 2013). The national incidence between 2001 and 2002 was 0.6 per million people, and has been on the rise since the early 1990's (McQuiston et al. 1999, Demma et al. 2005).

Geographically, the greatest risk of *E. chaffeensis* infection is in the southeastern United States, from Texas and Oklahoma to Virginia, and all states to the south (Paddock and Childs 2003). In Virginia specifically, there were 46 presumed cases between 1986 and 1997, the fifth highest number in the nation (Paddock and Childs 2003). In 2001, Virginia was one of 11 states reporting greater than 3.3 cases/ 10^6 people (Centers for Disease Control and Prevention, 2013). While the southeastern states pose the greatest risk of encountering *E. chaffeensis*, genetic analyses of tick DNA has revealed the bacterium to be present in most sites where lone star ticks are found, including states north of the highest risk areas (Ijdo et al. 2000, Paddock and Childs 2003). Multiple studies have attempted to measure the prevalence of *E. chaffeensis* within infected lone star ticks using a variety of methods. When tested individually, estimates range from 5 – 15%, compared to estimates from 1-5% using pooled DNA from multiple ticks, suggesting that many disease measures may be underestimates (Lockhart et al. 1997b, Paddock and Childs 2003, Mixson et al. 2004, Schulze et al. 2005, Mixson et al. 2006, Cohen et al. 2010, Fritzen et al. 2011). Additionally, nymph lone star ticks exhibit lower prevalence rates than adults of the same species (Mixson et al. 2004). The prevalence of *E. chaffeensis* infection is highly variable across individual sites and the bacterium is often distributed in large clusters of presence or absence

throughout its range (Yabsley et al. 2005, Whitlock et al. 2000, Burkett 1998, Steiner et al. 1999).

The lone star tick has also recently been implicated in “American Boutoneusse Fever” (Goddard and Varela-Stokes 2009, Goddard 2004). Also known as *Rickettsia parkeri* rickettsiosis, it is a Spotted Fever Group (SFG) rickettsiosis, a group of illnesses caused by the bacteria of genus *Rickettsia*, including Rocky Mountain Spotted Fever (RMSF) and rickettsialpox (Paddock et al. 2004, 2008). The illnesses often manifest similarly in infected humans, making clinical diagnosis based exclusively on symptoms difficult. In particular, *R. parkeri* rickettsiosis is often diagnosed as RMSF, since both can present as fever, myalgia, malaise, headache and rashes. (Goddard 2004, Paddock 2005, Cragun et al. 2010, Paddock et al. 2008). Paddock et al. found that half of their patients’ cases of *R. parkeri* rickettsiosis had previously been diagnosed as RMSF (2008). *R. parkeri* rickettsiosis is a milder illness than RMSF, with a smaller proportion of patients requiring hospitalization, though patients with either illness tend to respond well to treatment with tetracyclines (Cragun et al. 2010, Whitman et al. 2007, Paddock et al. 2008). *R. parkeri* rickettsiosis can be distinguished from RMSF by the presence of eschars on the skin, often at the site of the tick bite, though eschars can present in several other SFG rickettsioses (Goddard 2004, Chapman et al. 2006, Paddock et al. 2008, Cragun et al. 2010). The agent of this specific rickettsiosis, *R. parkeri* (Fig. 2), was first isolated from a Gulf-Coast tick (*A. maculatum*) in 1937 (Parker et al. 1939) and the first case of *R. parkeri* rickettsiosis in Virginia was identified in 2007 when a 53 year-old man recently bitten by a tick began exhibiting classic Spotted Fever

Group symptoms (Whitman et al. 2007). The tick assumed to be a Gulf Coast tick based on morphological description and the patient had a single eschar at the site of attachment. *R. parkeri* was isolated from a biopsy of this tissue (Whitman et al. 2007). *R. parkeri* has been identified in Gulf Coast ticks throughout their range, with Gulf Coast tick range overlapping with the larger range of the lone star tick (Sumner et al. 2007, Paddock et al. 2008). In 1986 *R. parkeri* was also isolated from a lone star tick (Goddard and Norment 1986) and several studies since then have confirmed that lone star ticks can be infected with *R. parkeri* (Goddard 2003, Cohen et al. 2009, Yabsely et al. 2009). In 2003, a study provided some evidence that the lone star tick cannot only be infected but also act as a vector of transmission when guinea pigs that were fed upon by infected lone star ticks exhibited mild clinical symptoms (Goddard 2003). In Virginia specifically, two regions were found to have *A. americanum* ticks infected with *R. parkeri*, one of which is geographically close to the study area investigated in this project (Gaines et al. 2014). However, since the primary vector of *R. parkeri* is believed to be the Gulf Coast tick, relatively little is known about its prevalence or life cycle in lone star ticks, and several studies testing this tick species for *R. parkeri* have found zero presence (Castellaw et al. 2010, Fritzen et al. 2011).

Lone star ticks feed primarily on large mammals as adults, ground-feeding birds and other mammals of various sizes as larvae and nymphs, and on white-tailed deer (*Odocoileus virginianus*) during all life stages (Hooker et al. 1912, Bishop and Trembley 1945, Hair and Howell 1970). Different hosts can vary in importance depending on the region (Kollars et al. 2000); however, research shows white-tailed

deer to be among the most important hosts (Bishopp and Trembley 1945, Hair and Howell 1970, Paddock and Yabsley 2007). A deer study in Oklahoma counted as many as 700 lone star ticks per animal and found that these ticks were a significant cause of fawn mortality (Bolte et al. 1970). Another study observing lone star ticks described deer as “literally covered with larvae, nymphs and adults in various stages of engorgement.” (Bishopp and Trembley 1945). Additionally, it is the only host of lone star ticks that has been confirmed to be both experimentally susceptible to *E. chaffeensis* infection and a reservoir host of the bacterium (Ewing et al. 1995, Dawson et al. 1996, Steiner et al. 1999, Lockhart et al. 1997a, Little et al. 1998). A serological study of deer found a temporal association between lone star tick establishment and the presence of antibodies in the deer blood reactive to *E. chaffeensis* (Lockhart et al. 1995). Uninfected larval or nymph ticks are exposed to the bacteria by feeding on infected white-tailed deer or other vertebrate hosts (Paddock and Childs 2003). Lone star ticks can pass the *E. chaffeensis* infection transstadially, across life stages, but there is little evidence of transovarial transmission from parent to offspring, unlike *R. parkeri*, which has been shown to be transmittable in both ways (Ewing et al. 1995, Goddard 2003, Walker et al. 2004). Additionally, infected ticks can establish the *E. chaffeensis* infection in susceptible reservoir hosts or humans during the acquisition of a blood meal (Paddock and Childs 2003). The zoonotic life cycle, linking the disease agent to the vector and reservoir host, as well as other ecological factors are important considerations in the emergence of the lone star tick as a vector of human disease.

Multiple factors help to explain the increase in tick-borne diseases, particularly HME, predicted for the coming decades. The geographic ranges of the vector, the lone star tick, and the primary reservoir host, the white-tailed deer, are changing and expanding northward in some areas (McShea et al. 1997, Keirans et al. 1998). Additionally, the numbers of both white-tailed deer and lone star ticks are increasing compared to historic numbers, an important factor leading to increased contact between disease-carrying ticks and humans, particularly those that participate in outdoor activities (Ginsberg et al. 1991, Standaert et al. 1995, McCabe and McCabe 1997, Means and White 1997, Armstrong et al. 2001). Human factors can also influence the emergence of tick-borne diseases, for example through climate change (Gubler et al. 2001) and through conversion of rural land to low-density housing development, which increases the likelihood of tick exposure (Long and DeAre 1982, Bayles et al. 2014).

Humans are also more likely to contract increasing numbers of tick-borne diseases as the size and longevity of susceptible populations increase. Older age groups exhibit higher rates of *E. chaffeensis* infection, though this could be a result of either increased susceptibility to the disease or an increased severity of the disease occurring with age (Fishbein et al. 1994, Centers for Disease Control and Prevention, 2013, Demma et al. 2005, Bayles et al. 2014). Another reason for the anticipated increase in numbers of reported cases of tick-borne diseases is not a factor of the diseases themselves but due to improvements in diagnostics and surveillance (Paddock and Childs 2003).

The anticipated rise of tick-borne diseases has sparked a tremendous interest both basic and medical research. However, much of the research targets better-known illnesses, like Lyme disease. Many studies of tick-borne disease focus on the vector, and though the lone star tick has been less researched than many other tick species, a handful of studies have attempted to analyze the species' habitat preferences (Semtner et al. 1971, Schulze 2002, Civitello et al. 2008, Willis et al. 2012). Few studies have attempted to model *E. chaffeensis* itself, and those have all been developed with presence/absence data at the county level (Yabsley et al. 2005, Wimberly et al. 2008a, b). *R. parkeri* is typically studied in the context of its primary vector the Gulf Coast tick, so very little research has been done on the presence of *R. parkeri* in lone star ticks. A predictive model has not, as far as I am aware, previously been constructed for *E. chaffeensis* or *R. parkeri* in lone star ticks with site-specific presence and absence data. In this study, I developed a predictive model for *E. chaffeensis* distribution in the coastal areas of southeastern Virginia. Specifically, I used two methods of PCR to determine the presence of the bacteria in field-collected ticks. I then related *E. chaffeensis* occurrence to factors influencing the distribution of hosts using statistical modeling to identify key variables influencing the distribution of *E. chaffeensis*. Lastly, I spatially applied the statistical models to delineate areas across the study site with high risk of *E. chaffeensis* occurrence.

Methods

Study Area

This study was conducted on the Virginia and Middle Peninsula between the James and York Rivers, and bordered on the East by the Chesapeake Bay, and on the west by the fault line. Ticks were collected at 122 sites (Fig. 3), randomly chosen based on a development-forest gradient using Arc Map GIS (ESRI, Redlands, CA). Several sites, 14 of the 122 total, were re-sampled a second time later in the summer to observe temporal effects on *E. chaffeensis* occurrence. The study included sites on land owned by the National Park Service, Colonial National Park Service land ($n = 29$), City of Williamsburg, Colonial Williamsburg ($n = 5$) and Waller Mill Park ($n = 6$), James City County, Freedom Park ($n = 7$) and Greensprings Trail Park ($n = 5$), City of Newport News, Newport News Park ($n = 16$), York County, New Quarter Park ($n = 5$), City of Hampton, Sandy Bottom ($n = 3$), Virginia Department of Forestry, Dragon Run State Forest ($n = 11$) and Sandy Point State Forest ($n = 5$), College of William and Mary ($n = 15$), United States Army, Fort Eustis ($n = 8$), and State of Virginia, York River State Park ($n = 7$).

I selected the study area because of the heterogeneity of the landscape throughout the region. The Virginia Peninsula includes a variety of land cover types, including a gradient of rural to urban development, forest, agriculture, marsh and swampland, as well as patchy areas that feature a mixture of the aforementioned categories. This study area lends itself well to the analyses of zoonotic disease, wherein the contact between humans and disease vectors is highly relevant and can be heavily influenced by landscape factors. Additionally, several factors unique to

the Virginia and Middle Peninsula make it an ideal area to conduct a study of this nature. First, the management of white-tailed deer populations is of concern in the Virginia Peninsula (Knox 1997), and highly relevant to this study, as the white-tailed deer is the only proven reservoir host of *E. chaffeensis* (Lockhart et al. 1997a). The area is experiencing a trend of increased urbanization (Gottman 1957), which is important in that it will likely lead to increased contact between humans and human-biting ticks, specifically lone star ticks, as people move into and develop areas that were previously natural or with little development. Finally, while *R. parkeri* has only been detected in lone star ticks on rare occasions, it has been consistently detected at high levels (41.8-55.7% presence) in Gulf Coast ticks between 2010 and 2012 in nearby areas of southeastern Virginia (Nadolny et al. 2014), suggesting the pathogen is present in the geographical region of the study.

Tick Collection

I collected ticks on two perpendicular 30-m transect crossing at the 15-m midpoint. Transects were centered on the site's GPS coordinates and pointed either east or north. I used two 30-m tapes to delineate each transect. To sample ticks, I dragged a 1 m² square piece of white canvas (flag henceforth) which was attached to a 1.5-m wooden dowel along the ground following the transect in each direction (Ginsberg and Ewing 1989). Every 3 meters, the flag was slowly flipped over and any attached ticks were collected with tweezers, placed in 2 mL plastic tubes containing 95% ethanol, and identified to species and life stage. The canvas was then turned back over and the process repeated a total of 20 times at each site. I

collected and recorded ticks at all sites to control for observer bias. Both nymph and adult ticks were recorded and collected (though only nymph ticks were tested for disease presence and used in further analyses), and larval ticks were not included in site tick counts as the ground-dragging cloth method may not sample this developmental stage well given the clumped distribution of tick nests. Ticks preserved in plastic tubes of ethanol were stored in a -80°C freezer until extraction to prevent degradation of DNA.

DNA Extraction

Nymph tick DNA was extracted using a DNeasy Blood & Tissue Kit (QIAGEN, Valencia, CA). Up to 20 ethanol-fixed ticks from each site were placed in 1.5 mL screw-cap tubes with ~250 µL 1 mm glass beads for bead-beating and DNA extraction. Multiple extractions were performed on sites with more than 20 ticks, with a maximum of five extractions. Bead beating was performed using an Omni Bead Ruptor Homogenizer (Omni International, Kennesaw, GA) for 60 s at 5000 RPM. To each tick sample disrupted by bead-beating, 180 µL of ATL buffer and 20 µL proteinase K were added, and tubes were gently inverted several times before being placed in a centrifuge at 13,200 RPM for 15 s. Subsequently, 200 µL AL buffer was added to each tick sample and tubes were vortexed before centrifuging for 60 s at 13,200 RPM. 200 µL ethanol was added to each tick sample, and all tubes were vortexed. The entire volume of liquid from each tube was pipetted into a DNeasy spin column, inserted in a 2 mL collection tube and centrifuged for 60 s at 8,000 RPM. The flow-through was discarded and spin columns with bound DNA extracted

from ticks were placed in new 2 mL collection tubes. 500 µL AW1 buffer was added to each tube and samples were centrifuged for 60 s at 8,000 RPM. The flow-through was discarded, and spin columns were placed in new 2 mL collection tubes. 500 µL AW2 buffer was added to each sample and tubes were centrifuged for 3 min at 13,200 RPM. The flow-through was discarded, and spin columns were transferred to 1.5 mL microcentrifuge tubes. 100 µL AE buffer was added to each spin column and centrifuged to elute the DNA extracted from ticks. The eluted DNA was stored in the same 1.5 mL microcentrifuge tubes at 4°C short-term, and long-term in a -80°C freezer.

Genetic Analysis

DNA Concentration

The concentration of each extracted tick DNA sample was measured with a Qubit 2.0 Fluorometer (Life Technologies, Grand Island, NY) using the Qubit dsDNA HS (High Sensitivity) Assay Kit (Life Technologies, Grand Island, NY). A working solution was prepared by diluting Qubit dsDNA HS reagent by 200 in Qubit dsDNA buffer. 190 µL working solution was added to each of 2 0.5 mL thin-wall PCR tubes. 10 µL of the standards were added to the tubes (1 of the 2 standards in each). 199 µL working solution was added to each sample tube, 0.5 mL thin-wall PCR tubes, along with 1 µL extracted DNA sample. The final volume of both standard and reaction tubes was 200 µL, and all tubes were mixed by vortexing 2-3 s and incubated at room temperature for 2 min.

The Qubit 2.0 fluorometer was recalibrated, using a freshly prepared pair of standards for each set of samples. The fluorometer was then used to determine the concentration of each tube in ng/mL with a minimum concentration of 0.50 ng/mL. The Dilution Calculator function was then used to determine the concentration of the original extracted gDNA sample in ng/µL.

Endpoint Polymerase Chain Reaction & Gel Electrophoresis

Presence of tick DNA in extracts was confirmed using a polymerase chain reaction (PCR) targeting a specific region of the tick 16s rRNA gene. The specific primers used were 16S+1: 5'CTGCTCAATGATTTTTAAATTGCTGT-3' and 16S-1: 5'-GTCTGAACTCAGATCAAGT-3' resulting in a 454 bp product (Macaluso et al. 2003, Nadolny et al. 2011). The presence of *E. chaffeensis* bacterial DNA was tested using a PCR targeting a region of the 16s bacterial rDNA. The following primers were used: HE1: 5'- CAATTGCTTA TAACCTTTGGTTATAAAT-3' and HE3: 5'-TATAGGTACCGTCATTATCTCCCTAT-3', targeting a 389 bp amplicon (Anderson et al. 1992, Stromdahl et al. 2000). The total PCR reaction volume was 20 µL consisting of 15 µL reaction mix and 5 µL extracted DNA. The reaction mix included 10 µL EconoTaq PLUS GREEN 2x Master Mix (#30033, Lucigen, Middleton, WI), 0.8 µL each forward and reverse primers (10µM) and 3.4 µL H₂O. Both PCR reactions were performed in a 2720 Thermal Cycler (Life Technologies, Grand Island, NY). The cycle parameters for the tick-specific PCR consisted of an initial step at 95°C for 4:00, 35 cycles of 95°C, 50°C, 68°C for 1 min each, followed by a final 10 min step at 68°C. The presence of *E. chaffeensis* was tested using cycle conditions of

3 cycles of 94°C for 60 s, 55°C for 120 s, 72°C for 90 s, 28 cycles of 94°C for 30 s, 55°C for 35 s, 72°C for 40 s followed by 72°C for 7 min.

All PCR products were separated for 20 min at 120 Volts on a 1% agarose gel using either GelRed Nucleic Acid Stain (10 µL/100 mL) (RGB-4103, Phenix Research Products, Candler, NC) or ethyldium bromide (5 µL/100 mL) (Thermo Fisher Scientific, Waltham, MA). Each gel included a negative (no DNA) control to control for potential contamination. All gels were imaged under ultraviolet light with a Canon DSLR camera (Fig. 5).

Real-time PCR

Real-time Polymerase Chain Reaction (qPCR) was used as a highly sensitive means to detect tick, *E. chaffeensis* and *R. parkeri* bacterial DNA. Three sets of Taqman custom forward and reverse primers were used, targeting the same amplicon as the endpoint PCR amplifications in the case of *A. americanum* and *E. chaffeensis*, with the addition of probes with sequences TCGAGGTCGCAAACTA and CCGCAGGGATTATAC respectively. The *R. parkeri* assay targeted the *ompB* gene, using the primer Rpa129F: CAAATGTTGCAGTCCTCTAAATG, Rpa224R: AAAACAAACCGTTAAAATACCG and Rpa188 probe: CGCGAAATTAATACCCTTATGAGCAGCAGTCGCG, yielding a 96 bp amplicon (Jiang et al. 2012, Nadolny et al. 2014). Reaction mixtures consisted of 10 µL Taqman Gene Expression Master Mix (#4369016, Life Technologies, Grand Island, NY), 1µL Taqman Assay (Life Technologies, Grand Island, NY), 1-5 µL DNA and enough H2O to yield a final reaction volume of 20 µL. DNA was diluted to a concentration of 1 ng/µL, and 1 µL DNA was added. In samples with a concentration below 1 ng/µL,

enough DNA, with a maximum of 5 µL, was added to reach a quantity of 1 ng ($\pm .05$ ng). qPCR amplifications were run in 96-well plates in a Stratagene Mx3005P instrument (Agilent Technologies, Santa Clara, CA), using three replicates of each sample per assay, nine wells total per sample. A negative (no DNA) control was included for each assay on every plate. Cycling conditions followed the Taqman Gene Expression Master Mix Protocol: 1 hold at 95°C for 10 min then 40 cycles of 95°C for 15 s and 60°C for 1 min. MxPro software (Agilent Technologies, Santa Clara, CA) was used to record the Ct score of each curve as well as the average Ct score for the three replicates (Fig. 5).

PCR products were spot checked for accuracy by sequencing to confirm species. Samples were purified and prepared for sequencing using a PCR/Gel Extract Mini Prep Kit (#IB47020, IBI Scientific, Peosta, IA). Sequencing was conducted by Lidia Epp at the College of William & Mary Molecular Core Facility.

Model

Variable Selection

In my models, presence/absence of *E. chaffeensis* is the dependent variable for two primary reasons. First, endpoint PCR and gel electrophoresis allow only for the detection of *E. chaffeensis*, and not a quantifiable measurement of the amount of pathogen present. And second, because ticks from each site were pooled and extracted together, it is impossible to know how many ticks from a given site were infected. A minimum infection rate (MIR), assuming only one disease-carrying tick per pool to be present, could be calculated, but since many more than one tick may be infected, this estimate was not deemed useful to include.

Independent variables included in the model were selected a priori as biologically relevant to the zoonotic system being studied (Burnham and Anderson 2002). I included independent variables that have been shown to influence the distribution of the primary host, white-tailed deer; the vector, lone star ticks; or the pathogen itself, *E. chaffeensis* or *R. parkeri*.

Each independent variable was analyzed at multiple scales, biologically relevant to the dispersal of lone star ticks and their hosts, in order to find the scale with the best fit. A study done on nymph lone star ticks found them capable of dispersing up to 55 feet in a 12-week period (Smittle et al. 1967), approximately 15 meters, the same distance that our transects extended in each direction from the center GPS coordinate. Lone star ticks are capable of using a variety of species as hosts, including small, medium and large mammals, particularly white-tailed deer, as well as ground-feeding birds (Childs and Paddock 2003). The small and medium mammals considered were the eastern gray squirrel (*Sciurus carolinensis*) the Virginia opossum (*Didelphis virginiana*) and the raccoon, (*Procyon lotor*), which have home ranges, assuming a circular home range, of radii 69 m (Riege 1991), 400 m (Rosatte et al. 2010), and 186 m (McNab 1963), respectively. I found the average summer home range of white-tailed deer to be 124 ha (88 (SD), range = 30 – 3500 ha) based on data from four studies conducted within the eastern deciduous forest (Tierson et al. 1985, Holzenbein and Marchinton 1992, Sargent and Labisky 1995, Campbell et al. 2004). I developed three scales based on the mean and ± 1 SD home range size of radii of 340 m, 630 m, and 820 m). I also derived a 60-m scale based on the 1.26 ha average home range of ground-feeding birds: Eastern Towhee (*Pipilo*

erythrophthalmus; Morimoto & Wasserman 1991); Wood Thrush (*Hylocichla mustelina*; Twomey 1945, Weaver 1949, Evans et al. 2009); Northern Cardinal (*Cardinalis cardinalis*; Gould 1961, Kinser 1973, Merritt 1975, Gottfried 1976); Carolina Wren (*Thryothorus ludovicianus*; Simpson 1984, Strain and Mumme 1988); Chipping Sparrow (*Spizella passerina*; Sutton 1937, Bradley 1940, Walkinshaw 1944, Keller 1979, Stull 1986, Albrecht and Oring 1995); and American Robin (*Turdus migratorius* ; Howell 1942, Pitts 1984). Based on the above information, I selected six scales at which to evaluate independent variables: 15 m, 60 m, 180 m, 350 m, 600 m and 800 m. 15 m wasn't used for all variables since some data sets had a cell size greater than 15 m.

Two primary datasets were used to extract independent variables of interest for analysis: the LANDFIRE Existing Vegetation Type (EVT; <http://www.landfire.gov>, accessed 15 December 2014) 2012 raster and LiDAR data for the state of Virginia (<http://virginalidar.com>, accessed 15 December 2014). I used ArcMap 10.1 (ESRI, Redlands CA) to extract the LANDFIRE raster to the study area, which included 58 vegetation types (Appendix Table 1). I reclassified the 58 existing types into 14 new categories (Appendix Table 2), using both NatureServe's Ecological Systems classification (<http://explorer.natureserve.org/classeco.htm>, accessed 15 December 2014) information and the literature to hypothesize which vegetation factors were most relevant to the zoonotic system. Open water and Pocosin were classified as aquatic ecotone. Agricultural and cropland were lumped into one category as agricultural cover has been shown to negatively influence presences of *E. chaffeensis* (Manangan et al. 2007). The 18 forest types were

condensed into seven categories—developed, riparian, mesic, coniferous, deciduous, mixed evergreen/deciduous, and barren/early successional forest—based on canopy openness, dominant tree type, and moisture. Mesic forest was hypothesized to be an important independent variable because lone star ticks are sensitive to desiccation and need moisture and humidity (Hair et al. 1975), though one study found that specific forest type did not affect lone star tick distribution (Sonenshine et al. 1971). Fields and grasslands were merged into an open vegetation category, as open grassland habitats have been shown to support fewer lone star ticks than forested areas (Semtnner et al. 1971, Patrick and Hair 1978). LANDFIRE EVT with high moisture levels like marshes and wetlands were lumped together, as flooded land covers are unsuitable for the vector and host. One study found wetlands to correlate negatively with *E. chaffeensis* presence (Manangan et al. 2007). Similarly, roads and high- and medium-intensity development were re-classified together as one developed category, as non-vegetated land covers do not provide foraging opportunities and are therefore unsuitable for tick hosts. Ruderal and recently disturbed vegetation types were merged into a single category, as were developed herbaceous and developed shrub land.

I used Geospatial Modeling Environment (GME; Beyer 2011) to extract the proportion of each land cover type at each sampling point in the study at all scales 60 m and greater, as the literature suggests that proportion of forest and agricultural land cover can impact the presence or absence of *E. chaffeensis* (Yabsley et al. 2005, Manangan et al. 2007). The 15 m scale could not be used to analyze the LANDFIRE data because it has a resolution of 30 m. Additionally, given that edge

favorably impacts white-tailed deer (Alverson et al. 1988) but that research has found forest fragmentation to have negative relationship with *E. chaffeensis* presence (Wimberly et al. 2008a, b), amount of edge was deemed potentially important to this study. The proportion of edge at each sampling site was calculated at the same five scales. I developed seven sets of edges: forest-agriculture, forest-developed herbaceous/shrub land, forest-recently disturbed, forest-open/marsh, forest-urban, forest-water and urban-agriculture. Edges were analyzed by creating a new binary raster for each of the seven edge types from the reclassified LANDFIRE raster. I used GME's "extractedge" tool to create a line shapefile to delineate edges, and then used density tool in ArcMap to calculate edge density (length of edge (km)/(km²) at scales \geq 60 m.

Virginia LiDAR was the second source of data and was used to analyze vegetation density, canopy and elevation using LASTools (<http://rapidlasso.com/lastools>; accessed February 2, 2015). I created three vegetation density rasters from 30 cm to a height of 1.5 m, 2 m and 3 m (Allombert et al. 2005, Bressette et al. 2012). All three rasters were included to see which vegetation height related best to white-tailed deer browsing. Because canopy cover has been shown to correlate positively with both *E. chaffeensis* (Wimberly et al. 2008a, b, Bayles et al. 2014) and lone star ticks (Semtner et al. 1971), I included maximum canopy height and percent canopy cover. Lastly, I included elevation as an independent variable as Yabsley et al. (2005) found *E. chaffeensis* presence to decrease with increasing elevation. The data from these six rasters were extracted for each sampling point at all six scales because the LiDAR data has a cell size of 3 m

Statistical & Spatial Modeling

In my model I only analyzed sites with tick presence. Including tick-absent sites as zeros in the model would have the potential to introduce false negatives because deer infected with *E. chaffeensis* could still be present. I developed two sets of models, one using presence/absence data from endpoint PCR and one using data from qPCR, since the sites found positive for *E. chaffeensis* differed between the two tests. I used program R (R Core Team 2014) to run a univariate analysis of all variables at each of the selected scales, and compared them to the null model. Any variable with an AIC less than that of the null model by two delta AIC was included in the analysis, at the scale with the lowest AIC value. I used the spearman's rank correlation to test for multicollinearity between variables. For variable pairs with a correlation greater than 0.7 (Leu et al. 2011), I used the variable that was most biologically reasonable and which appeared in more model sets. Additionally, variables were excluded if more than 80% of data points were zeros. Each variable was plotted against the dependent variable, *E. chaffeensis* occurrence, to see if any exhibited non-linear associations.

I used the dredge function in R (R Core Team 2014) on the variables remaining after the initial filter, limiting the models to include a maximum of four variables for the endpoint PCR-based model and five variables for the qPCR-based model, or 10% of presence sites, to avoid model over fitting (Hosmer et al. 2013). The Akaike Information Criterion (AIC) weight of each model was summed, and all models whose cumulative AIC weight summed to 0.95 were included in the final

model (Burnham and Anderson 2002). I also used the AIC weight of each model to determine the strength of evidence for each variable included in the final model (Burnham and Anderson 2002). I model averaged the coefficients of each variable in each model based on its respective AIC model weight (Burnham and Anderson 2002). Finally, I used R (R Core Team 2014) to calculate the standard error (SE) of the coefficient for each variable in each model. Because the final endpoint PCR model was based on 163 candidate models, I model averaged the standard errors based on enough models such that a standard error was calculated at least once for each variable. The standard errors for each variable were multiplied by the weight of each model and these weight-adjusted standard errors were summed in order to find a weighted standard error for each variable.

Final models were internally validated by calculating a receiver operating characteristic (ROC) (Hand and Till 2001), and then spatially applied across the study area using ArcGIS to create a predictive model of *E. chaffeensis* distribution.

Results

Tick Collection

I sampled a total of 122 sites for ticks across the Virginia and Middle Peninsula. Ticks were present at 99 of 122 (81.1%) sites, with 1284 nymph lone star ticks collected in total, and 1220 during the first round of sampling (Fig. 3, Appendix Table 3). The maximum number of ticks found at any single transect was 177. Of the 14 sites sampled again later in the summer, a total of 64 nymph ticks were found compared to 245 found at the same sites during the first round of

sampling. Of the 14 re-sampled sites that had ticks, 12 also had ticks during the second round of sampling.

Genetic Analysis

DNA Extraction

DNA was extracted from pooled ticks from 97 sites. The ticks from sites with approximately 20 or fewer were pooled and extracted together. Of the 97 unique sites, 8 had enough ticks to warrant multiple extractions on multiples of 20 ticks, up to a maximum of 5 extractions per site. A total of 128 extractions were performed.

DNA Concentration

The DNA concentration of all 128 extracted DNA samples was measured and recorded (Appendix Table 4). Six samples had a concentration that was too low for the assay to measure, one of which was one of the two extracts that did not successfully amplify tick DNA on the gel. The remaining 122 samples ranged from concentrations of 0.13 ng/ μ L to 77.00 ng/ μ L, with a mean concentration of 8.52 ng/ μ L (SD = 12.8) and a median of 3.75 ng/ μ L.

Endpoint Polymerase Chain Reaction & Gel Electrophoresis

Presence of *E. chaffeensis* was tested for in all 128 extracted gDNA samples using endpoint PCR. Of the 128, 126 (98.4%) samples successfully amplified tick DNA. For the two sites for which tick DNA was not successfully observed on the gel, *E. chaffeensis* presence/absence data were not included in the analysis. Of the total 126 samples, 50 (39.7%) tested positive for *E. chaffeensis* (Appendix Table 5). For

the 95 individual sites, considering only data from the first round of sampling and only the first extraction in cases where multiple extractions were performed, 46 (48.4%) tested positive for *E. chaffeensis*.

Considering the eight sites for which multiple extractions were performed, 4 were positive in the first extraction. Only one of these sites tested positive in subsequent extractions, and none were found positive in later extractions that were not positive in the first extraction (Table 1).

I analyzed 11 of the re-sampled sites for *E. chaffeensis* presence. When analyzing ticks from the first round of sampling, seven of the 11 sites tested positive for *E. chaffeensis*, but only four of these seven sites tested positive again when based on ticks that were collected during re-sampling. None of the sites tested positive for *E. chaffeensis* based on the second sampling that had not tested positive in the first round (Table 2).

Real-time Polymerase Chain Reaction

Taqman real-time PCR was performed on 117 DNA extracts; 11 of the original 128 samples did not have enough DNA extract left to run a qPCR after endpoint PCR was performed. All 117 amplified *A. americanum* DNA (100%). In four of the runs, the negative, no-DNA control amplified. For this reason, any sample with a Cycle Threshold (Ct) score higher than the lowest contaminated control value (36.48) was excluded as a false positive signal. Additionally, at least two of the three replicates for each sample had to amplify to be considered positive. Based on these criteria, 49 of the total 117 (41.9%) samples tested were positive for

E. chaffeensis (Appendix Table 6). When considering the individual sites themselves, 45 of 86 (52.3%) were *E. chaffeensis* positive.

Of the eight sites for which multiple extractions were performed, two sites tested positive based on the first extraction. One site tested positive for *E. chaffeensis* in both extraction samples, and five sites were negative for each extraction performed. One site was found to be negative for the first and third extraction, but positive for the second. One site was found to be positive for the first extraction but negative for the subsequent four (Table 1).

Nine re-sampled sites were analyzed by qPCR. Four of the nine sites tested positive when testing ticks from the first round of sampling. None of these nine sites were found to be positive when testing ticks from the second round of sampling. Two sites had enough extracted DNA left from endpoint PCR to run qPCR on DNA from ticks collected during the re-sampling, but not for the first round of sampling, so no comparisons could be made. One was positive and one was negative for *E. chaffeensis* (Table 2).

None of the sites tested positive for *R. parkeri* based on qPCR. However, the positive control, a *R. parkeri*-positive tick DNA extract (provided by Holly Gaff, Old Dominion University), did amplify, confirming the validity of the Taqman assay.

Endpoint PCR vs. qPCR

When comparing the two methods and only considering the 117 samples that both tested, I found that the prevalence of *E. chaffeensis* was lower (37.8%, n = 45 sites) based on endpoint PCR compared to 41.9% (n = 49 sites) with qPCR.

When examining individual sites, of the 86 that both methods tested, 40 sites were positive (46.5%) according to endpoint PCR, compared to 46 via qPCR (54.7%). The two methods found the same results for 93 of the 117 extracted samples (79.5%). Concerning the 24 samples where *E. chaffeensis* presence/absence differed, 14 tested positive by qPCR, while with endpoint PCR all tested negative. Ten sites tested positive with endpoint PCR but not qPCR.

Model

Endpoint PCR-Based Model

Based on the univariate analysis, 12 of the 31 possible variables had AIC values at least two delta AIC below that of the null model, 135.7. The 19 remaining variables—canopy cover, tick density, Julian date, maximum canopy height, elevation, land cover of developed herbaceous/shrubland, developed forest, developed (high, medium, roads), open habitat, agriculture, wetland/marsh, mesic forest, deciduous forest, mixed evergreen deciduous forest, forest-agriculture edge density, forest-developed edge density, forest-open/marsh edge density, forest-urban edge density, urban-agriculture edge density—were excluded from additional analyses. Of the twelve potential variables—vegetation density up to a height of 1.5 m (800 m scale), vegetation density up to a height of 2 m (800 m), vegetation density up to a height of 3 m (800 m), proportion of ruderal/recently disturbed land cover (180 m), proportion of aquatic ecotone (600 m), proportion of riparian forest (600 m), proportion of coniferous forest (600 m), proportion of mixed evergreen/deciduous forest (60 m), proportion of barren/early successional forest (600 m), proportion of total forest cover (800 m), density of edge between forest

and ruderal/recently disturbed land cover (60 m) and density of edge between forest and aquatic ecotone (180 m)—the best were selected for the next step of modeling. Of the three vegetation density variables, zero to three meters was selected because it had the lowest AIC value, and the other two were excluded. Two variables were excluded because they had more than 80% of sites with a zero value: proportion of mixed evergreen/deciduous forest and density of edge between forest and aquatic ecotone. None of the final candidate variables were found to strongly correlate in the spearman's rank test, and when plotted against the presence/absence of *E. chaffeensis*, none appeared to have non-linear associations.

The candidate set included 131 models with variable weighted AIC values ranging from 0.212 to 0.683 (Table 3, Appendix Table 7). Four of the variables related positively with *E. chaffeensis* presence: edge density between forest and ruderal/recently disturbed land cover, ruderal/recently disturbed land cover, aquatic ecotone and riparian forest. The other four variables related negatively with *E. chaffeensis* presence: vegetation density, coniferous forest, barren/early successional forest and total forest cover. The model receiver operating characteristic, ROC, was equal to 0.7 (SE = 0.05) (Fig. 6, 7).

qPCR-Based Model

The null model created by the univariate analysis had an AIC value of 120.5. Analysis was performed on the same 31 variables as for endpoint PCR, and four variables had a delta AIC value of two or greater. However one did not converge—proportion of open habitat at the 60 m scale—and so was excluded from further

analyses. The three remaining variables were: density of vegetation up to a height of 3 m (15 m scale), proportion of ruderal/recently disturbed land cover (180 m) and proportion of barren/early successional forest (350 m). None of the variables strongly correlated with any others, and none had a non-linear relationship with *E. chaffeensis* presence/absence when plotted.

Vegetation up to a height of 3 m (15 m scale), proportion of ruderal/recently disturbed land cover (180 m) and proportion of barren/early successional forest (350 m) had AIC-weighted values of 0.768, 0.531 and 0.960, respectively (Table 4). Both vegetation density and proportion of barren/early successional forest related negatively with *E. chaffeensis* occurrence, while proportion of ruderal/recently disturbed land cover related positively with *E. chaffeensis* occurrence. The ROC was calculated to be 0.74 (SE=0.05) (Fig. 6, 8).

Discussion

Tick Collection

While lone star tick numbers are reported to be on the rise (Paddock and Yabsley 2007), the number collected during the summer of 2013 was less than the number found when sampling the same sites in 2010 and 2012. A study on black-legged ticks (*Ixodes scapularis*) found significant variation in tick numbers both seasonally and among years (Schulze and Jordan 1996), suggesting that variation in tick populations is not uncommon and that our results are not anomalous, even given the trend of rising tick numbers. Additionally, it has been suggested that while nymph tick numbers from one season can predict adult tick numbers for the

following year, there is little correlation between nymph tick populations from year to year (Sonenshine 1971).

The variation in tick numbers collected during the first and second rounds of sampling are not surprising, given the population peaks of the three tick life stages. As described by Hair and Howell (1970), nymphs peak first in June and then again in late August or early September, while larvae activity is highest later in the summer. This accounts for the decreased number of nymph ticks collected during the second round of sampling, which took place in late-July.

Disease Distribution

Generally, Taqman qPCR appeared to be a more sensitive tool than endpoint PCR for detecting *E. chaffeensis* in tick DNA. When comparing either all 117 DNA extracts, or the individual sites (considering only the first round of sampling and first extraction, when relevant), the qPCR method detected *E. chaffeensis* more often than endpoint PCR, suggesting that estimates of prevalence of *E. chaffeensis* based on endpoint PCR detection may be underestimated. This was supported by comparing the 2013 results to data collected in 2010 and 2012 (Weeks 2013). In each of the previous years, only eight total sites were found to be positive for *E. chaffeensis*, four in 2010 and four in 2012. None of these sites were at the same location among the two years, but seven of the sites were found to be positive in 2013 (zero ticks were collected at the eighth site in 2013 so disease presence could not be analyzed) using qPCR. However, when using endpoint PCR, only four of the

seven were found to be positive in 2013, providing additional evidence of the relative detection strength of the qPCR method.

Many studies have found high variability in terms of prevalence of disease (Lockhart et al. 1997b, Paddock and Childs 2003, Mixson et al. 2004, Schulze et al. 2005, Mixson et al. 2006, Cohen et al. 2010, Fritzen et al. 2011), and this variation could be the result of several factors. Because my model suggests that the deer host is the main driver of *E. chaffeensis* occurrence, it is reasonable to hypothesize that shifts in space use by deer would heavily influence the distribution of the bacterium. Additionally, 2013 saw unusually heavy spring rains, which could have negatively impacted tick populations (Koch 1986). Since lone star ticks have been shown to be a significant source of fawn mortality (Bolte et al. 1970), fewer ticks could have translated into less fawn mortality than typically observed, resulting in a deer population increase. Therefore, increased populations of deer could at least partially explain the large jump in *E. chaffeensis* presence observed in 2013.

In addition to variation of *E. chaffeensis* between years, variation was observed even within a single field season. Of sites sampled twice, more were found positive for *E. chaffeensis* when testing ticks from the first round of sampling (May-June) than were found positive from the second round (July). This was true for both endpoint PCR and qPCR results. However, the few found to be positive from round two by endpoint PCR (zero were found positive from round two by qPCR) were all among the positive sites based on the first round of sampling. This suggests that infection is not spatially variable over a short time scale because no new sites were found to be positive during the second round, and suggests that the

decrease in positive sites from the first to the second sampling could be function of deer-tick dynamics that change throughout the field season, as opposed to spatial variation in *E. chaffeensis* distribution. When considering individual sites, there was variation in detecting *E. chaffeensis* when multiple extractions were performed on ticks from a single site with both PCR methods. Differences in *E. chaffeensis* presence/absence across extractions were observed at half of the sites for which multiple analyses were performed. This suggests that testing a small sample of ticks collected from a single site with high tick densities may not be sufficient to accurately determine the presence or absence of the bacterium at that site.

Considering *R. parkeri*, the zero detection in the study area was not unexpected, given the similar failure of several other studies to detect *R. parkeri* in lone star ticks (Castellaw et al. 2010, Fritzen et al. 2011). However, *R. parkeri* has been previously isolated from this tick species (Yabsley et al. 2009), and the study area was in geographical proximity to areas with large populations of the primary vector, the Gulf Coast tick (Nadolny et al. 2014), suggesting that infection of lone star ticks with *R. parkeri* in our study area was possible.

Model Output

Given the zoonotic system being studied, the models produced by the statistical modeling are logical. White-tailed deer populations and their browsing behavior appear to be the principal driving force behind the distribution of *E. chaffeensis* based on the variables that were found to be important in the two model sets. Because qPCR and endpoint PCR differed in detecting bacterial presence, the resultant models included different candidate sets of variables. However, models

agreed in variables included in candidate sets because all three explanatory variables included in the qPCR-based model were also identified as important in the endpoint-based model, although the scales differed in some cases.

The density of vegetation up to a height of 3 m was found to be an important variable in both models, at the 800 m scale in endpoint-based model and the 15 m scale in the qPCR-based model. Additionally, in the univariate analyses run for both sets of data, all six radii (15, 60, 180, 350, 600 and 800 m) had AIC values below the null model, suggesting that the vegetation density is an important variable at a broad range of biological scales. This variable likely serves as a proxy for deer browsing, because there was negative relationship between vegetation density and *E. chaffeensis* presence. Preliminary analysis showed a negative correlation between deer pellet density and liDAR vegetation density (Leu et al. unpublished data). Areas with low vegetation density have likely experienced significant deer browsing, as multiple studies have shown deer presence and browsing pressure to correlate to reduced stem counts, herbaceous plants and seedlings (Rooney and Waller 2003, Russell et al. 2001). Areas with low vegetation density may also indicate high deer use sites, prominently found in areas where deer-hunting pressure is low, such as in areas near human development. Because this variable is indicative of high use by the only known reservoir host of the bacterium, it is logical to expect an increased presence of *E. chaffeensis* in these areas.

Furthermore, it is well established that the white-tailed deer is a species that thrives in disturbed land covers, which explains several of the other important variables identified by the models. Total forest cover had a negative relationship

while ruderal/recently disturbed land cover had a positive relationship with *E. chaffeensis* presence. While the negative correlation with total forest cover may seem counter-intuitive, studies have shown that disturbance in large forest patches increases the browsing opportunity for white-tailed deer (Masters et al. 1993). Additionally, clear-cutting or other forest disturbance can result in a greater diversity of food sources available for deer than are found in mature forests (Johnson et al. 1995). This explains the white-tailed deer preference for disturbed, less-forested areas as opposed to large contiguous patches of mature forest where less browsing may be available, and why the most important scale for this variable was the largest—800 m, or the scale of white-tailed deer home ranges. Additionally, the edge density between forest cover and disturbed habitat correlated positively to *E. chaffeensis*, suggesting that not only amount of disturbed cover, but its distribution, is an important factor. This is likely for similar reasons: more edge would signify more sunlight and greater successional opportunity for young, more palatable plant growth, which would result in the increased variety of food sources predicted by Johnson et al. (1995).

One surprising variable identified as important in the endpoint-based model was aquatic ecotone, which correlated positively to *E. chaffeensis* presence. Aquatic ecotone was redefined to include the LANDFIRE EVT classifications of open water and Pocosin (Appendix table 2), and it was hypothesized that flooded land cover would not be usable by either the vector or host. However, it is possible that if there is seasonal or temporal variation in the amount of water in these areas, they could provide important food and/or water resources at certain times, if for example, deer

forage along the forest-water boundary. It is important to note that in the initial univariate analyses, edge density between forest and water land covers was identified as an important variable, but was excluded from further analysis on the grounds that it had fewer than 20% non-zero values. Lone star ticks are especially sensitive to dessication (Hair and Howell 1970) and have been found in river or stream-bottom vegetation in prairie habitat (Bishopp and Trembley 1945). This body of evidence suggests that these ecotone areas are more important in the zoonotic lifecycle of *E. chaffeensis* than initially hypothesized.

The moisture/humidity requirements of lone star ticks (Hair and Howell 1970), could also explain the positive relationship between riparian forest and *E. chaffeensis* presence. While numbers of lone star ticks themselves did not come out as important predictive variable, both the vector lone star tick and the host white-tailed deer must be able to survive in an area to transmit complete the zoonotic life cycle. For this reason, it is not unsurprising that coniferous forest was found to have a negative relationship with *E. chaffeensis*, since coniferous forests tend to be much drier and have less understory than the forest types favored by lone star ticks (Bishopp and Trembley 1945, Hair and Howell 1970). However, given that the scale at which the relationship was strongest was 600 m (the estimated summer home range of white-tailed deer), it's likely that this variable is more tied to white-tailed deer. Patrick and Hair (1978) found that oak hickory forest was the favored summer habitat for white-tailed deer, and deciduous tree species are browsed more than evergreen species during the summer (Rooney and Waller 2003). It is

apparent that coniferous forest does not provide an ideal habitat to either vector or tick species.

Another variable that correlated negatively to *E. chaffeensis* presence was barren/early successional land cover. Similarly to coniferous forest, the likely explanation for the negative relationship is a lack of suitable habitat provided to the host, given that the most important scale was again 600 m. This is probably a result of insufficient foraging opportunity afforded to the deer by this land cover type. Additionally, open habitats have shown to poorly support lone star tick populations (Patrick and Hair 1978), so even if deer were able to survive in this habitat type, there would likely be no vector to transmit the bacterium among the deer population.

There are several other variables worth discussing due to their absence from the model output. The most notable is the number of lone star ticks found, which was hypothesized a priori to be an important predictive variable of *E. chaffeensis* presence. There are several reasons that could explain its absence from the models. First, lone star ticks have been found to be more tolerant than other tick species of varying microclimate types (Schulze and Jordan 2002) and forest types (Sonenshine 1971). This would partially explain the seemingly random distribution of the tick species throughout the landscape. Additionally, Yabsley et al. (2005) suggest that small numbers of lone star ticks are sufficient to introduce and maintain *E. chaffeensis* infection into a population of white-tailed deer, implying that low tick numbers are not indicative of a decreased risk of *E. chaffeensis*. Several other variables hypothesized to correlate to the bacterium's distribution did not end up

being important factors in the final models. Julian date was hypothesized to be influential based on the summer dynamics of populations of the three tick life stages (Hair and Howell 1970), but was not identified as important during the modeling process. Given that lone star ticks need humid environments and have been shown to prefer forested as opposed to open habitats (Hair and Howell 1970, Koch and Burg 2006, Schulze 2002, Semtner et al. 1971), canopy cover and open habitat were predicted to be among the important explanatory variables. These variables were all predicted to play a role based on their relationship with the lone star tick, and therefore it is not surprising that these were not included, given that lone star tick numbers were not an important variable. Another surprising variable excluded was agricultural cover, which has been shown in another study to correlate negatively with *E. chaffeensis* (Manangan et al. 2007). However, that study was conducted with presence/absence data at the county scale, which likely explains that differences observed in this study.

In conclusion, the white-tailed deer was found to be the principle factor in influencing the distribution of *E. chaffeensis*, and important model variables could be attributed to characteristics of the host's life history traits or foraging behavior. The vector, the lone star tick, was not found to be an influential variable and no correlation was observed between tick point counts and *E. chaffeensis* presence or absence at study sites.

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Figures

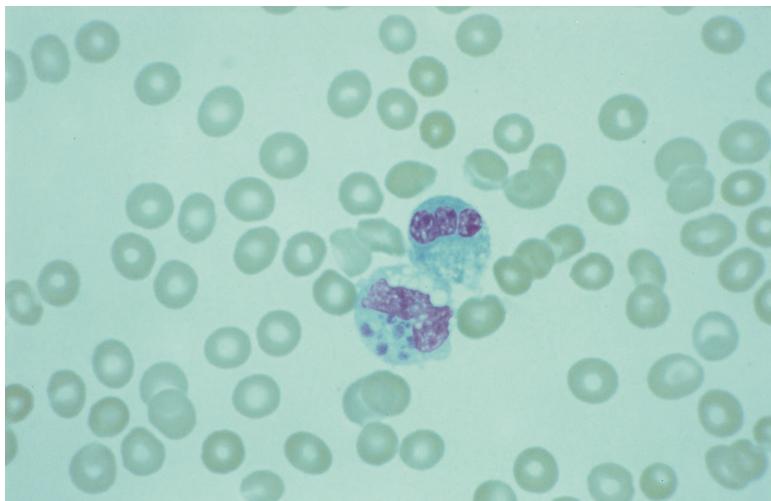


FIG. 1. Peripheral blood smear from a patient infected with Human Monocytotropic Ehrlichiosis (HME). Each morula consists of a cluster of *E. chaffeensis* (purple) contained within a vacuole. Figure developed by Paddock and Childs (2003).

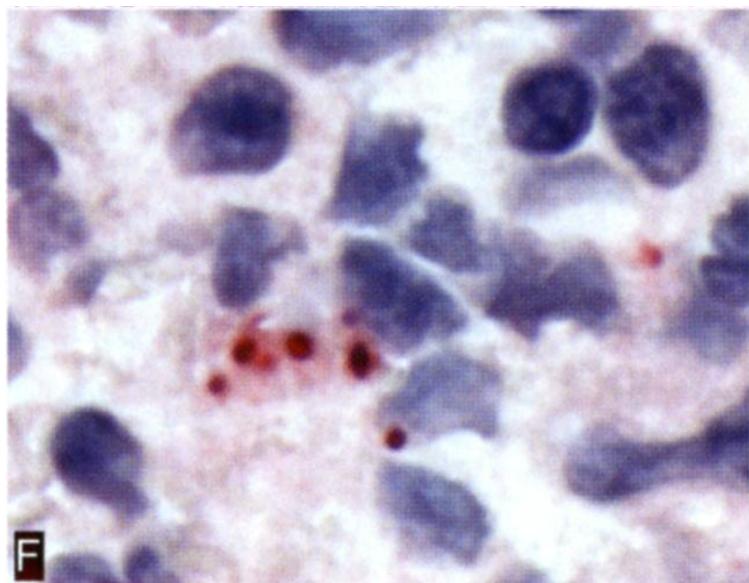


FIG. 2. *R. parkeri* in human mononuclear inflammatory cells isolated from an eschar. Figure developed by Paddock et al. (2008).

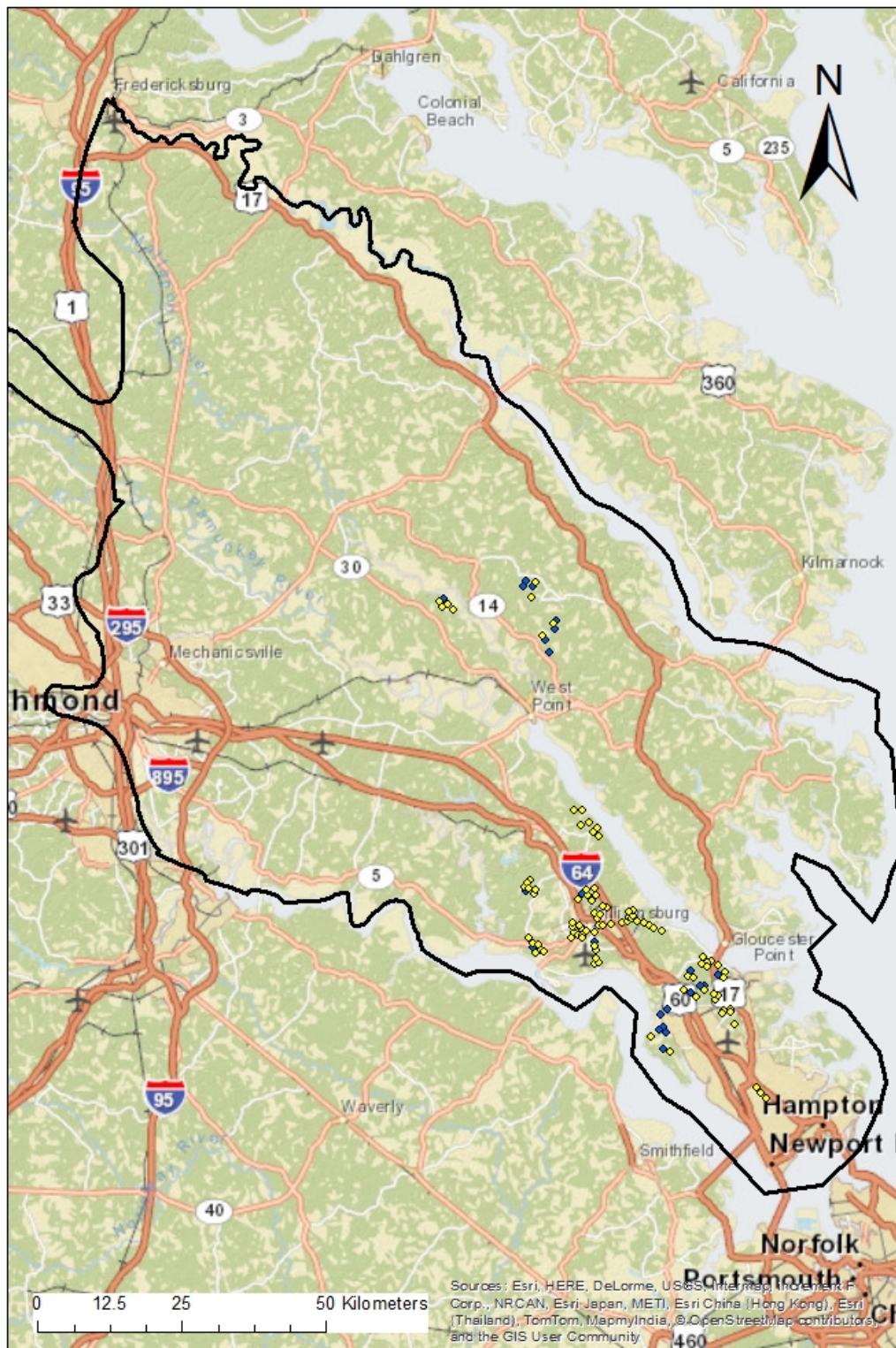


FIG. 3. Study area of the Virginia and Middle Peninsula outlined in black. Each dot represents the location of a tick-sampling site ($n = 122$). Yellow dots are sites with ticks present, while ticks were absent from sites marked by blue dots.

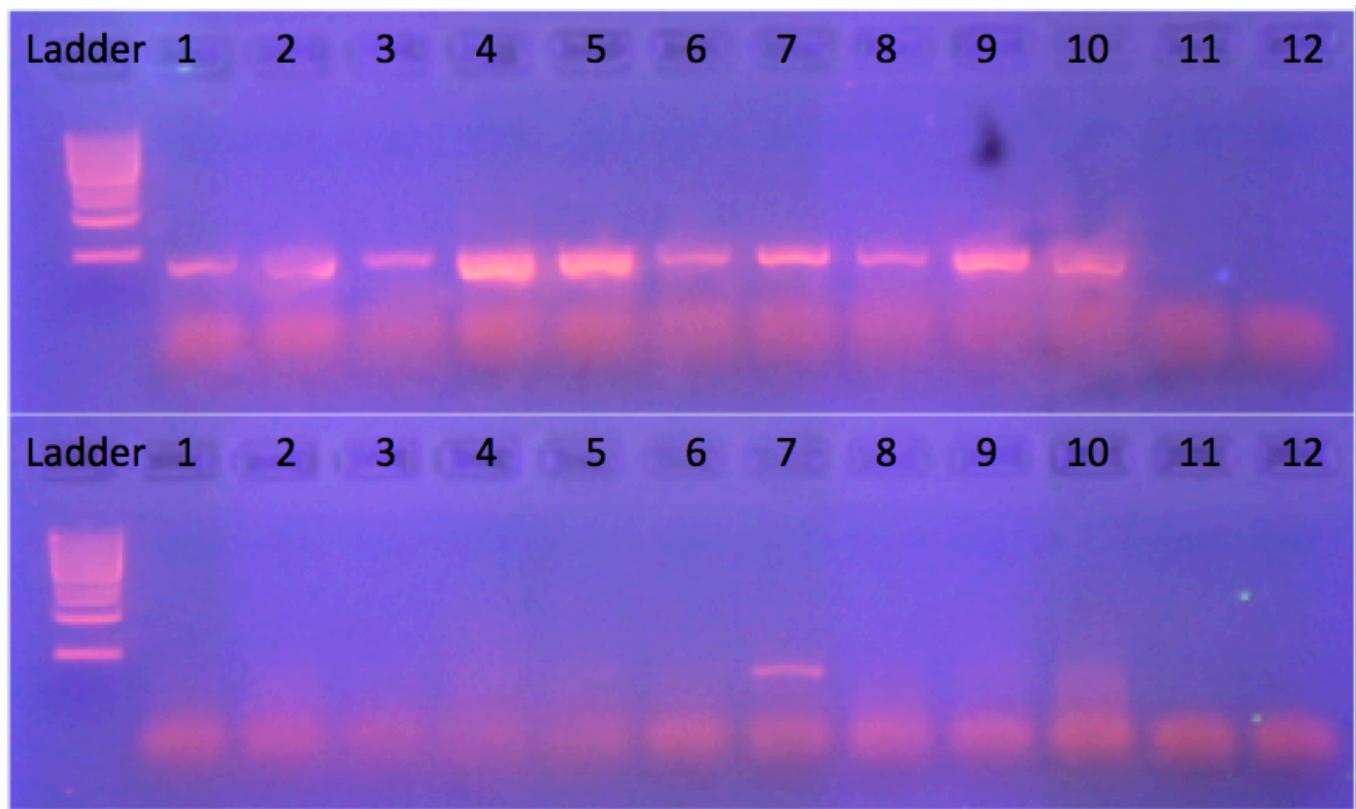


FIG. 4. Example of electrophoresis gel detecting tick DNA (top image) and *E. chaffeensis* DNA (bottom image). Wells 1-10 (in both panels) are PCR products from extracted of 10 different tick collection sites. All are positive for tick DNA, but only the sample in well #7 (from site FP 6_2) of the bottom panel is positive for *E. chaffeensis*. Well #11 of both panels is an extraction control, including all extraction reagents but no ticks. Well #12 is a PCR control without DNA added. No negative controls amplified.

Amplification Plots

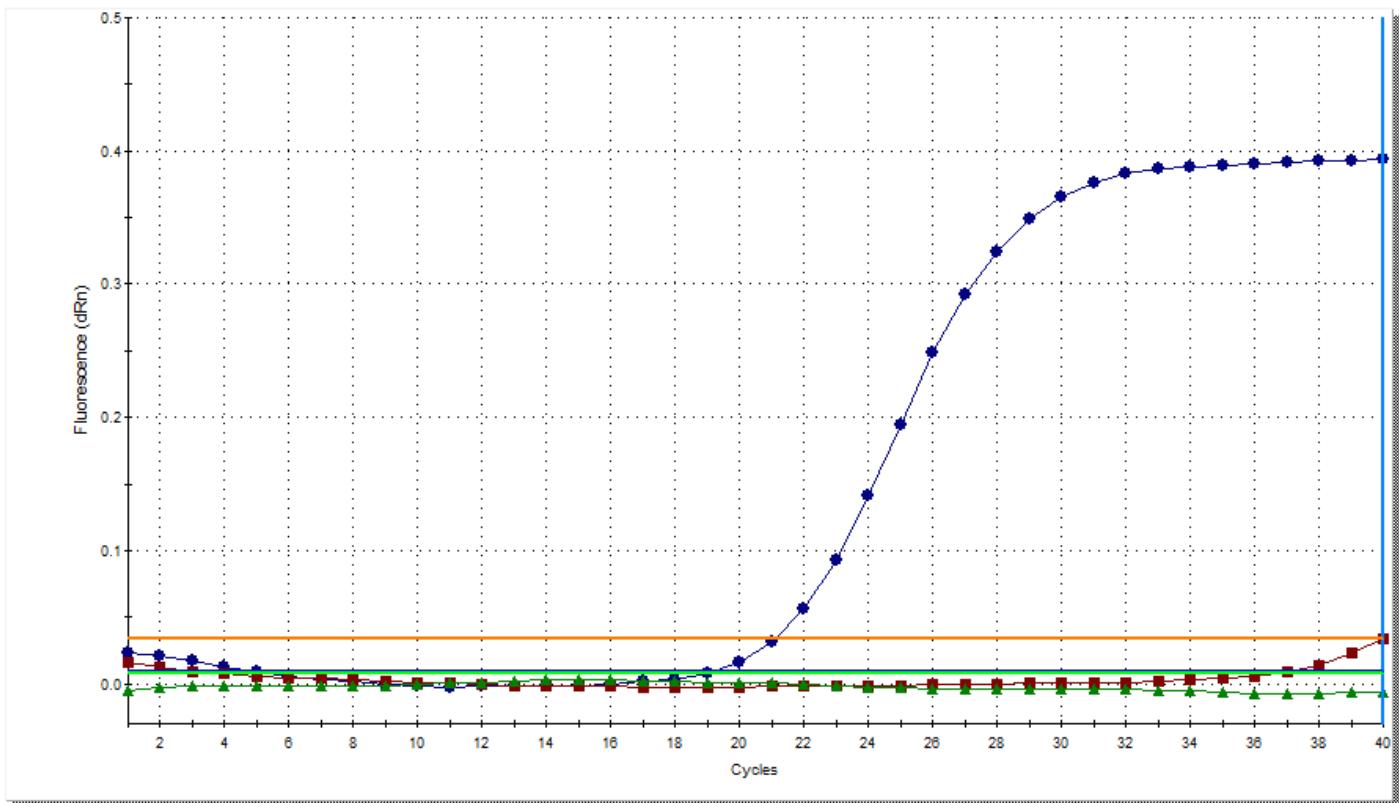


FIG. 5. Example Taqman qPCR Cycle Threshold (Ct) plot from with ticks from site WAM 6_1. Each curve represents the delta Ct of 3 replicates. Blue is tick DNA, red is *E. chaffeensis*, green is *R. parkeri*.

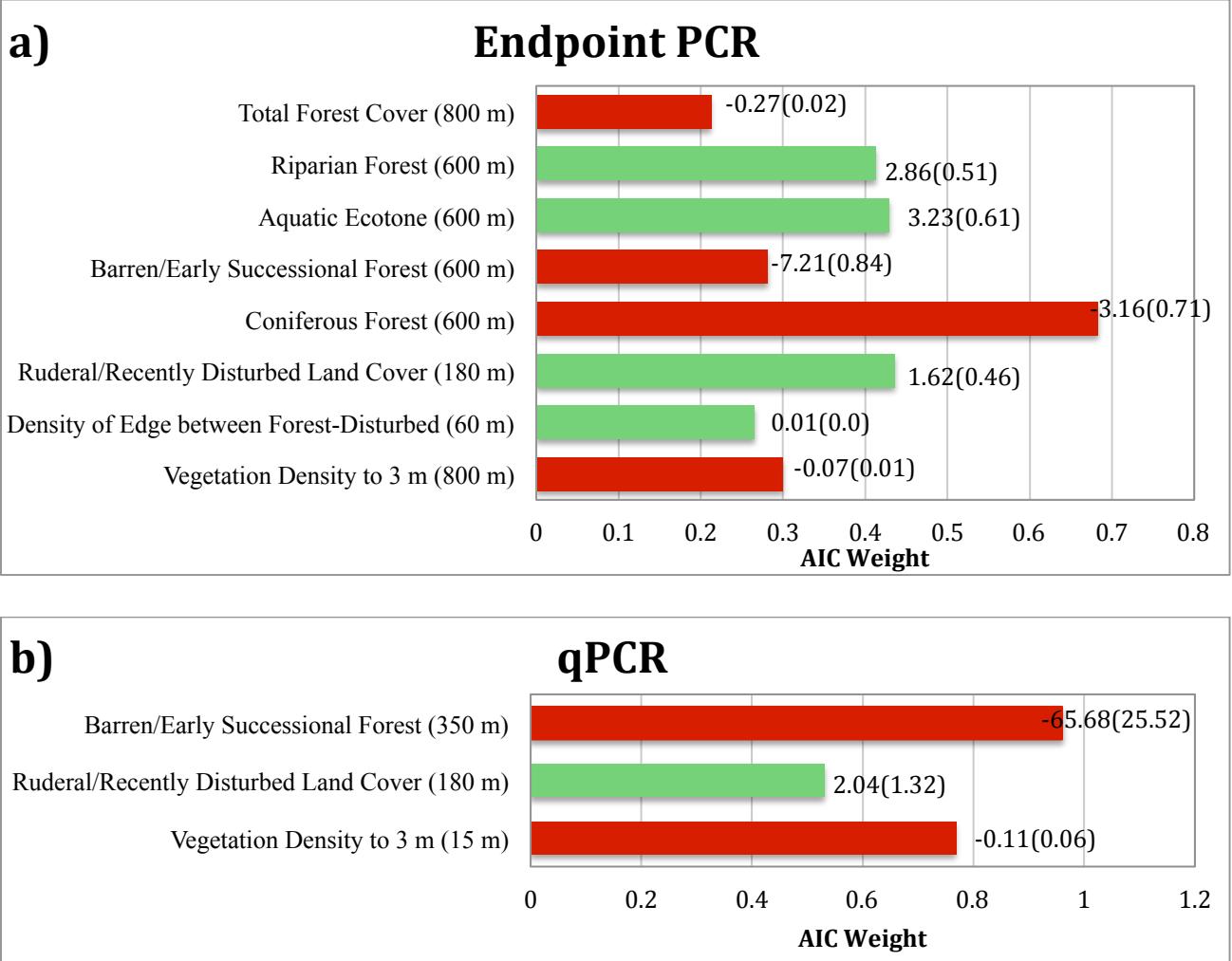


FIG. 6. Evidence of strength (AIC weight) for variables associated with *E. chaffeensis* occurrence: a) endpoint PCR with intercept = 0.02 (0.15) and b) qPCR, with intercept = 0.64 (0.42). Values listed next to each bar represent slopes, plus or minus standard error in parentheses.

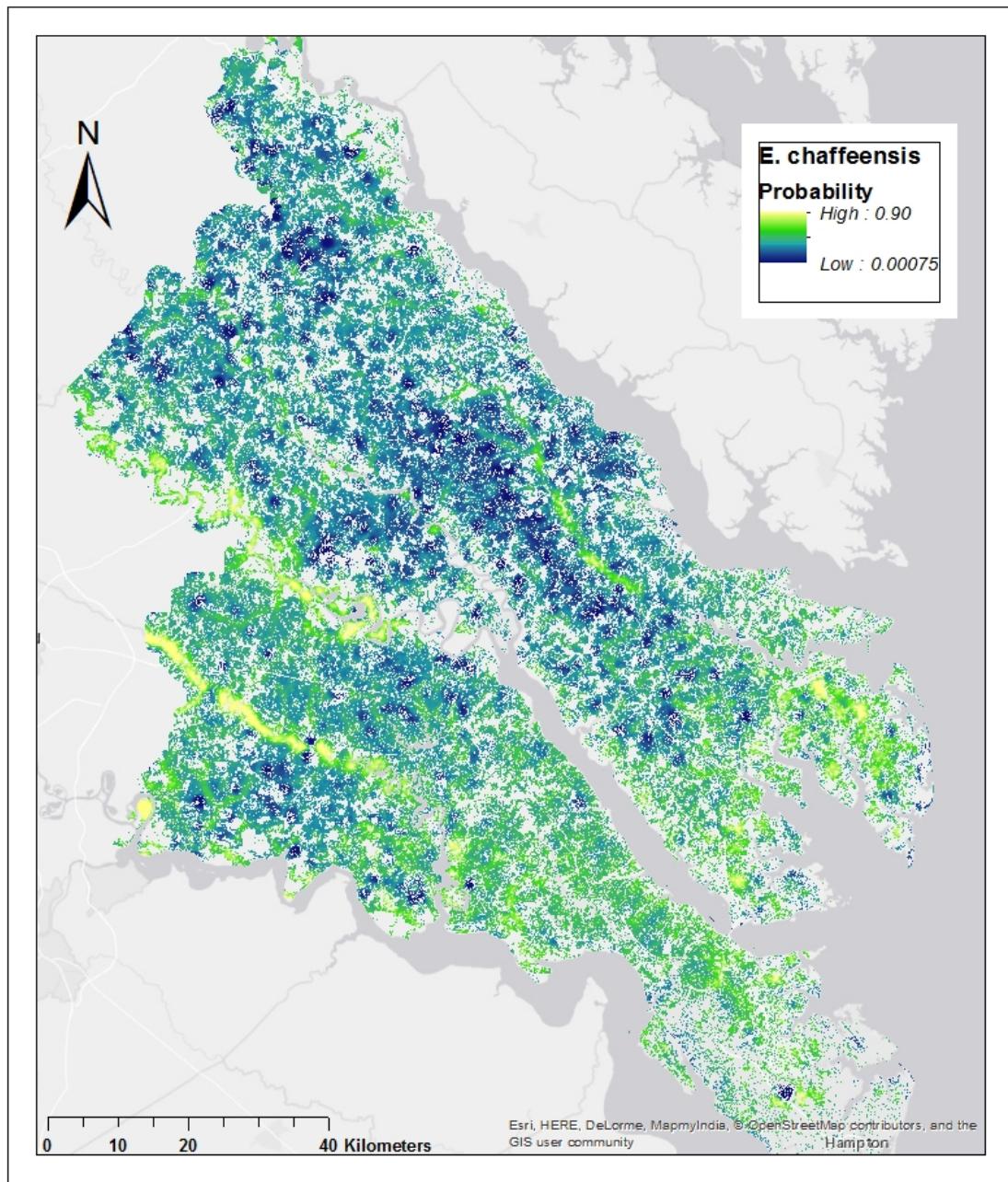


FIG. 7. Spatial model of *E. chaffeensis* probability, based on endpoint PCR, across the forested land cover of the study area.

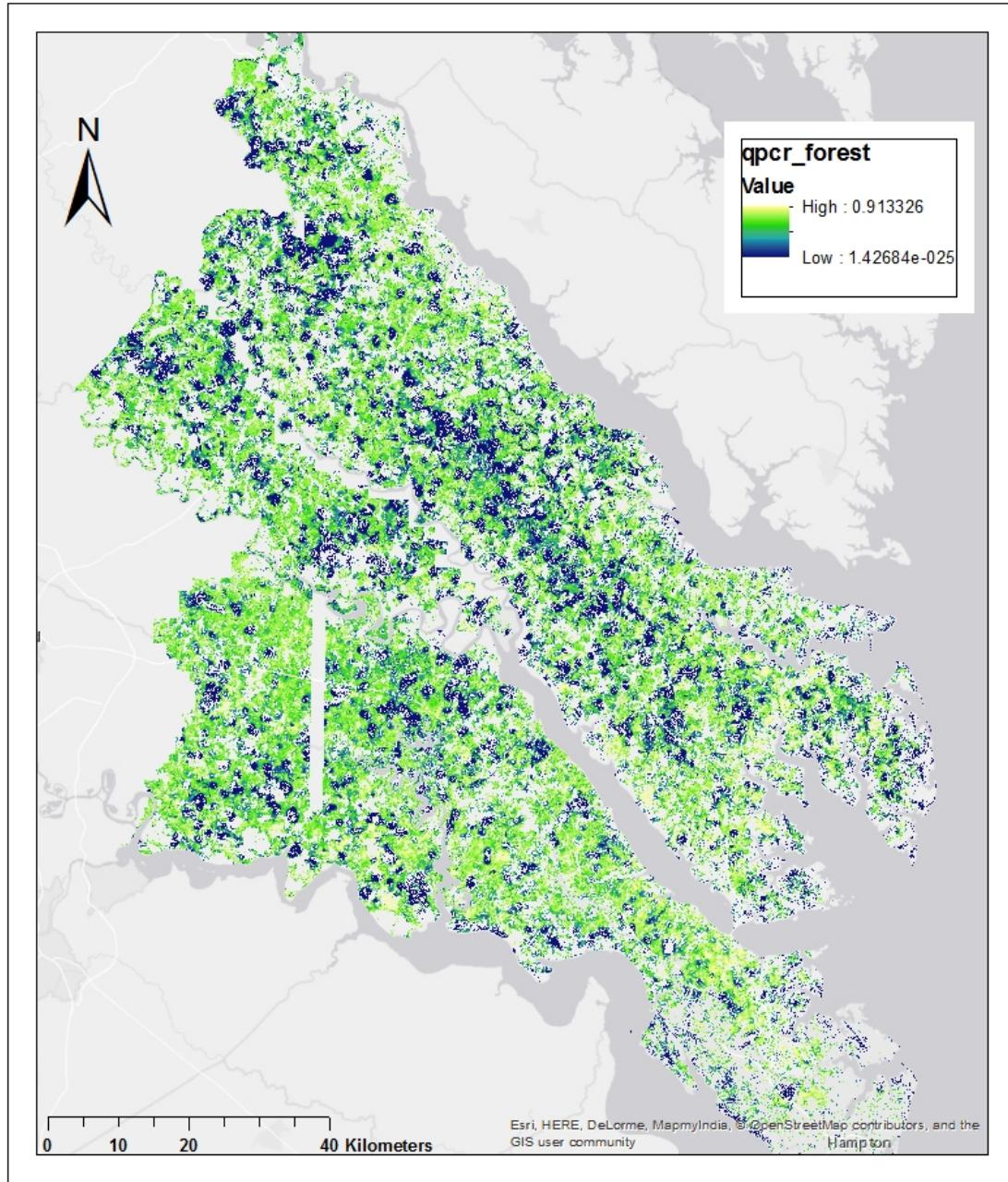


FIG. 8. Spatial model of *E. chaffeensis* probability, based on Taqman qPCR, across the forested land cover of the study area.

Table 1: *E. chaffeensis* occurrence in relation to number of DNA extractions per site.

Site	Extraction 1			Extraction 2			Extraction 3			Extraction 4			Extraction 5		
	Ticks Pooled	Endpoint PCR	Taqman qPCR												
YRSP 7_2	20	No	No												
NQP 2_2	20	Yes	No	12	No	No	-	N/A	N/A	-	N/A	N/A	-	N/A	N/A
COLW 2_2	20	No	No												
WAM 9_2	20	Yes	No	20	No	No									
NNP 15_1	20	No	No	20	No	Yes	17	No	No	-	N/A	N/A	-	N/A	N/A
CNPS															
13_2	20	No	No	-	N/A	N/A									
FP 6_2	20	Yes	Yes	8	Yes	Yes	-	N/A	N/A	-	N/A	N/A	-	N/A	N/A
WAM 5_1	21	Yes	No	20	No	No	-	N/A	N/A	-	N/A	N/A	-	N/A	N/A

Spaces marked by hyphens indicate that an extraction of that number was not performed for that site.

Table 2: Occurrence of *E. chaffeensis* in sites sampled multiple times during field season based on both endpoint PCR and qPCR.

Site	Sampling 1					Sampling 2				
	Date Collected	Ticks Collected	Extraction Number	Endpoint PCR	qPCR	Date Collected	Ticks Collected	Extraction Number	Endpoint PCR	qPCR
WAM 3_2	5/31/13	3	1	Yes	N/A	7/22/13	1	1	No	No
WAM 13_1	6/10/13	2	1	N/A	N/A	7/22/13	9	1	No	Yes
WAM 12_1	6/9/13	3	1	Yes	Yes	7/22/13	4	1	No	No
WAM 1_2	5/30/13	7	1	No	Yes	7/22/13	1	1	No	No
WAM 14_1	6/9/13	5	1	No	No	7/22/13	7	1	No	No
WAM 10_2	5/31/13	6	1	No	No	7/19/13	18	1	No	No
WAM 6_1	5/31/13	6	1	Yes	Yes	7/19/13	3	1	Yes	No
WAM 5_1	5/31/13	43	1	Yes	No	7/19/13	2	1	Yes	No
			2	No	No					
WAM 15_2	5/30/13	8	1	Yes	Yes	7/19/13	4	1	Yes	No
WAM 8_1	5/30/13	3	1	Yes	No	7/19/13	1	1	Yes	No
WAM 9_2	5/31/13	147	1	Yes	No	7/19/13	6	1	No	No
			2	No	No					
			3	No	No					
			4	No	No					
			5	No	No					

WAM 13_1 did not successfully amplify tick DNA, and so no *E. chaffeensis* results from this site were included in analyses. There was not enough extracted DNA left from WAM 3_2 to perform qPCR.

Table 3: Top four candidate models based on endpoint PCR.

Model Number	Intercept	Vegetation Density to 3 m _{800m}	Density of Edge between Forest-Disturbed _{60m}	Ruderal/Recently Disturbed Land Cover _{180m}	Coniferous Forest _{600m}	Barren/Early Successional Forest _{600m}	Aquatic Ecotone _{600m}	Riparian Forest _{600m}	Total Forest Cover _{800m}	df	logLik	AICc	ΔAICc	weight	Cumulative AIC Weight
45	-0.592153436	NA	NA	4.505460415	-4.914788521	NA	7.474123678	NA	NA	4	-59.33468472	127.1089299	0	0.039682447	0.039682447
13	-0.280087056	NA	NA	3.990622377	-5.685323543	NA	NA	NA	NA	3	-60.54255409	127.3459777	0.237047862	0.035247162	0.07492961
77	-0.504197848	NA	NA	3.440451717	-5.270797114	NA	NA	5.884939368	NA	4	-59.73466169	127.9088838	0.799953938	0.026600553	0.101530162
109	-0.807007608	NA	NA	4.040635295	-4.584908254	NA	7.211511216	5.702290553	NA	5	-58.6260264	127.9187195	0.809789583	0.026470057	0.128000219

The full list of all 131 candidate models included to reach a cumulative AIC weight of .95 can be found in the appendix.

Table 4: Candidate Models for qPCR.

Model Number	Intercept	Vegetation Density to 3 m (15m)	Ruderal/Recently Disturbed Land Cover (180 m)	Barren/Early Successional Forest (350 m)	df	logLik	AICc	ΔAICc	weight
8	0.488425008	0.140725902	3.837058977	-66.51644029	4	-50.629658	109.7531432	0	0.418836244
6	1.10206209	-0.14026442	NA	-72.61671645	3	-51.91212561	110.1169342	0.363790992	0.349178943
7	0.002470445	NA	3.873573045	-62.11427022	3	-53.05100111	112.3946851	2.641541987	0.111799716
5	0.622629838	NA	NA	-68.77859255	2	-54.45691749	113.0584133	3.305270135	0.080225783

Appendix

Appendix Table 1: LANDFIRE EVT cell values and counts of 30-m cells within the study area.

Value	Count	LANDFIRE Existing Vegetation Type (EVT) Classification
11	1233873	Open Water
13	2286	Developed-Upland Deciduous Forest
14	58596	Developed-Upland Evergreen Forest
15	4756	Developed-Upland Mixed Forest
16	234871	Developed-Upland Herbaceous
17	31368	Developed-Upland Shrubland
23	73268	Developed-Medium Intensity
24	31757	Developed-High Intensity
25	193696	Developed-Roads
31	175224	Barren
32	8511	Quarries-Strip Mines-Gravel Pits
61	73	NASS-Vineyard
62	101	NASS-Bush fruit and berries
63	313151	NASS-Row Crop-Close Grown Crop
64	811547	NASS-Row Crop
65	176136	NASS-Close Grown Crop
67	234986	NASS-Pasture and Hayland
75	4721	Herbaceous Semi-dry
76	2403	Herbaceous Semi-wet
81	335565	Agriculture-Pasture and Hay
82	245324	Agriculture-Cultivated Crops and Irrigated Agriculture
95	261877	Herbaceous Wetlands
2181	1095	Introduced Upland Vegetation-Annual Grassland

2185	1694	Introduced Wetland Vegetation-Mixed
2191	18576	Recently Logged-Herb and Grass Cover
2303	29	Northeastern Interior Dry-Mesic Oak Forest
2316	10089	Southern Piedmont Mesic Forest
2324	228097	Northern Atlantic Coastal Plain Hardwood Forest
2335	37	Southern Atlantic Coastal Plain Dry and Dry-Mesic Oak Forest
2343	2557603	Southern Atlantic Coastal Plain Mesic Hardwood Forest
2347	111810	Atlantic Coastal Plain Upland Longleaf Pine Woodland
2361	35	Central Atlantic Coastal Plain Maritime Forest
2368	47644	Southern Piedmont Dry Oak(-Pine) Forest
2370	25747	Appalachian (Hemlock-)Northern Hardwood Forest
2379	35210	Northern Atlantic Coastal Plain Maritime Forest
2400	8964	Central Appalachian Alkaline Glade and Woodland
2436	26791	Northern Atlantic Coastal Plain Dune and Swale
2452	973	Atlantic Coastal Plain Peatland Pocosin and Canebrake
2456	4	Northern Atlantic Coastal Plain Pitch Pine Lowland
2468	2337	Atlantic Coastal Plain Streamhead Seepage Swamp-Pocosin-Baygall
2471	24	Central Interior and Appalachian Floodplain Systems
2472	26	Central Interior and Appalachian Riparian Systems
2473	108353	Gulf and Atlantic Coastal Plain Floodplain Systems
2474	161284	Gulf and Atlantic Coastal Plain Small Stream Riparian Systems
2479	6740	Central Interior and Appalachian Swamp Systems
2480	129281	Gulf and Atlantic Coastal Plain Swamp Systems
2490	6013	Gulf and Atlantic Coastal Plain Tidal Marsh Systems
2498	30	Gulf and Atlantic Coastal Plain Sparsely Vegetated Systems
2501	72761	Southern Atlantic Coastal Plain Nonriverine Swamp and Wet Hardwood Forest
2531	7	Ruderal Upland-Old Field
2532	877192	Ruderal Forest-Northern and Central Hardwood and Conifer

2533	97515	Ruderal Forest-Southeast Hardwood and Conifer
2534	1153	Managed Tree Plantation-Northern and Central Hardwood and Conifer Plantation Group
2535	924769	Managed Tree Plantation-Southeast Conifer and Hardwood Plantation Group
2541	2	Recently Disturbed Developed Upland Deciduous Forest
2542	1	Recently Disturbed Developed Upland Evergreen Forest
2543	2	Recently Disturbed Developed Upland Mixed Forest
2545	1	Recently Disturbed Developed Upland Shrubland

58 LANDFIRE EVT types included in the study area before reclassification. Count indicates the number of cells designated as each classification type in the study area.

Appendix Table 2: LANDFIRE EVT reclassification.

New Classification	LANDFIRE EVT Values	New Value
developed herbaceous/shrubland	16, 17	1
developed forest	13-15	2
ruderal/recently disturbed	2531-2533, 2541-2545	3
aquatic ecotone	11, 2452	4
developed (high, medium, roads)	23-25	5
riparian forest	2456, 2468, 2471-2474, 2479, 2480, 2501	6
open habitat (grasslands, fields)	75, 2181, 2191, 2498	7
agriculture	61-65, 67, 2452, 81, 82	8
wetland/marsh	76, 95, 2181, 2185, 2436, 2490	9
mesic forest	2316, 2343	10
coniferous forest	2347, 2534, 2535	11
deciduous forest	2303, 2324, 2335	12
mixed evergreen deciduous forest	2368, 2370, 2400	13
barren/early successional forest	31, 32	14
NoData	2361, 2379	15

LANDFIRE EVT values included in each new land classification value.

Appendix Table 3: Number of ticks collected.

	Site	Ticks Collected
Sampling 1	CNPS_1_2	6
	CNPS_10_2	13
	CNPS_11_1	2
	CNPS_12_1	3
	CNPS_13_2	81
	CNPS_14_1	22
	CNPS_15_1	2
	CNPS_16_2	11
	CNPS_17_2	3
	CNPS_18_2	1
	CNPS_19_1	6
	CNPS_21_1	3
	CNPS_22_2	3
	CNPS_23_2	18
	CNPS_24_1	3
	CNPS_25_1	7
	CNPS_28_1	1
	CNPS_29_1	4
	CNPS_3_1	22
	CNPS_38_1	0
	CNPS_39_1	0
	CNPS_4_1	1
	CNPS_40_2	7
	CNPS_41_2	7
	CNPS_5_2	6
	CNPS_6_1	1
	CNPS_7_1	1
	CNPS_8_1	0
	CNPS_9_1	13
	COLW_1_2	18
	COLW_2_2	177
	COLW_3_1	2
	COLW_4_1	7
	COLW_5_2	20
	DRSF_1_2	1
	DRSF_10_2	3
	DRSF_11_1	1
	DRSF_12_1	0
	DRSF_13_1	0

DRSF_2_2	0
DRSF_3_1	0
DRSF_4_1	0
DRSF_6_2	2
DRSF_8_2	0
DRSF_9_1	0
FP_1_1	2
FP_2_2	0
FP_3_1	1
FP_4_2	2
FP_5_1	9
FP_6_2	29
FP_7_1	5
FTE_1	0
FTE_2	0
FTE_4	0
FTE_5	0
FTE_6	1
FTE_7	0
FTE_8	1
FTE_9	0
GT_1_1	1
GT_2_1	6
GT_3_2	9
GT_4_2	4
GT_5	0
NNP_1_2	0
NNP_10_2	1
NNP_12_1	1
NNP_13_1	3
NNP_15_1	63
NNP_17_1	0
NNP_18_2	3
NNP_20_1	0
NNP_22_2	2
NNP_23_1	15
NNP_29_2	2
NNP_3_1	10
NNP_30_2	9
NNP_31_2	5
NNP_8_2	1
NNP_9_2	1
NQP_2_2	34

NQP_3_3	7
NQP_4_1	12
NQP_5_2	4
NQP_6_2	4
SB_1_1	11
SB_2_2	2
SB_3_2	4
SPSF_1_1	3
SPSF_2_1	14
SPSF_3_2	2
SPSF_4_1	1
SPSF_6_2	0
WAM_1_2	7
WAM_10_2	6
WAM_12_1	3
WAM_13_1	2
WAM_14_1	5
WAM_15_2	8
WAM_2_2	3
WAM_3_2	3
WAM_4_2	3
WAM_5_1	43
WAM_6_1	6
WAM_7_2	1
WAM_8_1	3
WAM_9_2	147
WM_1_2	0
WM_2_1	10
WM_3_2	2
WM_4_2	9
WM_5_1	7
WM_6_2	17
WM_7_1	3
YRSP_1_1	5
YRSP_2_2	14
YRSP_3_1	14
YRSP_4_1	9
YRSP_5_1	6
YRSP_6_1	7
YRSP_7_2	115
Sampling 2	
CNPS_19_1	8
WAM_1_2	1
WAM_10_2	18

WAM_12_1	4
WAM_13_1	9
WAM_14_1	7
WAM_15_2	4
WAM_2_2	0
WAM_3_2	1
WAM_4_2	0
WAM_5_1	2
WAM_6_1	3
WAM_8_1	1
WAM_9_2	6
Total	1284

Appendix Table 4: Concentration of extracted DNA samples.

	Site	Extraction Number	Ticks Extracted	[DNA] (ng/uL)
Sampling 1	CNPS 1_2	1	6	18.60
	CNPS 10_2	1	12	5.10
	CNPS 11_1	1	2	2.14
	CNPS 12_1	1	3	5.84
	CNPS 13_2	1	20	10.50
	CNPS 13_2	2	20	5.74
	CNPS 13_2	3	20	7.72
	CNPS 13_2	4	20	9.48
	CNPS 14_1	1	19	45.80
	CNPS 15_1	1	2	0.63
	CNPS 16_2	1	11	4.68
	CNPS 17_2	1	3	1.34
	CNPS 18_2	1	1	0.38
	CNPS 21_1	1	5	0.51
	CNPS 22_2	1	2	0.32
	CNPS 23_2	1	19	10.30
	CNPS 24_1	1	2	0.12
	CNPS 25_1	1	7	1.97
	CNPS 28_1	1	1	0.25
	CNPS 29_1	1	4	TOO LOW
	CNPS 3_1	1	20	77.00
	CNPS 4_1	1	1	0.25
	CNPS 40_2	1	8	14.60
	CNPS 41_2	1	7	8.98
	CNPS 5_2	1	6	8.22
	CNPS 6_1	1	1	0.16
	CNPS 7_1	1	1	0.27
	CNPS 9_1	1	14	27.20
	COLW 1_2	1	20	1.46
	COLW 2_2	1	20	9.26
	COLW 2_2	2	20	39.40
	COLW 2_2	3	20	4.32
	COLW 2_2	4	20	8.30
	COLW 2_2	5	20	9.48
	COLW 3_1	1	2	2.80
	COLW 4_1	1	9	2.18
	COLW 5_2	1	18	63.40
	DRSF 1_2	1	1	0.38

DRSF 10_2	1	4	3.72
DRSF 11_1	1	1	0.27
DRSF 6_2	1	2	0.66
FP 1_1	1	2	TOO LOW
FP 3_1	1	1	0.76
FP 4_2	1	3	0.13
FP 5_1	1	10	1.97
FP 6_2	1	20	36.40
FP 6_2	2	8	4.48
FP 7_1	1	7	9.16
FTE_6	1	1	TOO LOW
FTE_8	1	1	0.48
GT 1_1	1	1	0.37
GT 2_1	1	5	5.02
GT 3_2	1	8	0.51
GT 4_2	1	2	0.99
NNP 10_2	1	1	0.11
NNP 12_1	1	1	0.41
NNP 13_1	1	2	0.15
NNP 15_1	1	20	14.70
NNP 15_1	2	20	4.84
NNP 15_1	3	17	11.20
NNP 18_2	1	3	5.44
NNP 22_2	1	1	TOO LOW
NNP 23_1	1	15	7.76
NNP 29_2	1	1	0.35
NNP 3_1	1	10	21.20
NNP 30_2	1	9	13.70
NNP 31_2	1	4	0.23
NNP 8_2	1	1	1.10
NNP 9_2	1	1	TOO LOW
NQP 2_2	1	20	33.60
NQP 2_2	2	12	12.60
NQP 3_3	1	7	9.44
NQP 4_1	1	12	26.40
NQP 5_2	1	4	0.61
NQP 6_2	1	4	0.21
SB 1_1	1	10	1.80
SB 2_2	1	2	0.13
SB 3_2	1	3	0.23
SPSF 1_1	1	2	1.86
SPSF 2_1	1	11	6.72
SPSF 3_2	1	2	2.12

	WAM 1_2	1	7	4.70
	WAM 10_2	1	6	5.06
	WAM 12_1	1	3	1.80
	WAM 13_1	1	2	TOO LOW
	WAM 14_1	1	5	2.06
	WAM 15_2	1	8	7.64
	WAM 2_2	1	1	0.35
	WAM 3_2	1	3	0.12
	WAM 4_2	1	3	0.49
	WAM 5_1	1	21	19.50
	WAM 5_2	2	20	20.00
	WAM 6_1	1	6	0.27
	WAM 7_2	1	1	0.18
	WAM 8_1	1	3	1.56
	WAM 9_2	1	20	16.20
	WAM 9_2	2	20	6.86
	WAM 9_2	3	20	22.00
	WAM 9_2	4	20	19.70
	WAM 9_2	5	20	29.80
	WM 2_1	1	11	22.80
	WM 3_2	1	2	1.80
	WM 4_2	1	13	18.60
	WM 5_1	1	7	4.10
	WM 6_2	1	18	14.60
	WM 7_1	1	3	2.02
	YRSP 1_1	1	6	1.40
	YRSP 2_2	1	15	7.16
	YRSP 3_1	1	13	4.00
	YRSP 4_1	1	7	2.95
	YRSP 5_1	1	6	0.18
	YRSP 6_1	1	7	0.92
	YRSP 7_2	1	20	47.00
	YRSP 7_2	2	20	4.66
	YRSP 7_2	3	20	13.40
	YRSP 7_2	4	20	12.40
	YRSP 7_2	5	20	16.00
Sampling 2	WAM 1_2	1	1	0.41
	WAM 10_2	1	17	33.80
	WAM 12_1	1	3	2.76
	WAM 13_1	1	8	4.60
	WAM 14_1	1	7	2.62
	WAM 15_2	1	3	3.47
	WAM 3_2	1	1	1.60

WAM 5_1	1	2	0.33
WAM 6_1	1	3	2.24
WAM 8_1	1	1	0.13
WAM 9_2	1	5	3.78

Samples marked “TOO LOW” had a concentration below the QuBit Fluorometer’s threshold of measurement.

Appendix Table 5: Endpoint PCR-based *E. chaffeensis* detection.

	Site	Extraction Number	<i>E. chaffeensis</i> Positive
Sampling 1	CNPS 1_2	1	No
	CNPS 10_2	1	Yes
	CNPS 11_1	1	Yes
	CNPS 12_1	1	No
	CNPS 13_2	1	No
	CNPS 13_2	2	No
	CNPS 13_2	3	No
	CNPS 13_2	4	No
	CNPS 14_1	1	No
	CNPS 15_1	1	No
	CNPS 16_2	1	Yes
	CNPS 17_2	1	No
	CNPS 18_2	1	Yes
	CNPS 21_1	1	Yes
	CNPS 22_2	1	Yes
	CNPS 23_2	1	No
	CNPS 24_1	1	No
	CNPS 25_1	1	Yes
	CNPS 28_1	1	No
	CNPS 29_1	1	No
	CNPS 3_1	1	No
	CNPS 4_1	1	No
	CNPS 40_2	1	No
	CNPS 41_2	1	No
	CNPS 5_2	1	No
	CNPS 6_1	1	No
	CNPS 7_1	1	No
	CNPS 9_1	1	Yes
	COLW 1_2	1	No
	COLW 2_2	1	No
	COLW 2_2	2	No
	COLW 2_2	3	No
	COLW 2_2	4	No
	COLW 2_2	5	No
	COLW 3_1	1	No
	COLW 4_1	1	Yes
	COLW 5_2	1	No
	DRSF 1_2	1	No
	DRSF 10_2	1	No
	DRSF 11_1	1	No
	DRSF 6_2	1	No
	FP 1_1	1	N/A
	FP 3_1	1	Yes
	FP 4_2	1	Yes

FP 5_1	1	Yes
FP 6_2	1	Yes
FP 6_2	2	Yes
FP 7_1	1	No
FTE_6	1	Yes
FTE_8	1	No
GT 1_1	1	No
GT 2_1	1	Yes
GT 3_2	1	Yes
GT 4_2	1	Yes
NNP 10_2	1	Yes
NNP 12_1	1	No
NNP 13_1	1	Yes
NNP 15_1	1	No
NNP 15_1	2	No
NNP 15_1	3	No
NNP 18_2	1	Yes
NNP 22_2	1	Yes
NNP 23_1	1	No
NNP 29_2	1	No
NNP 3_1	1	No
NNP 30_2	1	No
NNP 31_2	1	Yes
NNP 8_2	1	No
NNP 9_2	1	Yes
NQP 2_2	1	Yes
NQP 2_2	2	No
NQP 3_3	1	No
NQP 4_1	1	No
NQP 5_2	1	Yes
NQP 6_2	1	Yes
SB 1_1	1	Yes
SB 2_2	1	Yes
SB 3_2	1	Yes
SPSF 1_1	1	No
SPSF 2_1	1	No
SPSF 3_2	1	No
WAM 1_2	1	No
WAM 10_2	1	No
WAM 12_1	1	Yes
WAM 13_1	1	N/A
WAM 14_1	1	No
WAM 15_2	1	Yes
WAM 2_2	1	No
WAM 3_2	1	Yes
WAM 4_2	1	Yes
WAM 5_1	1	Yes
WAM 5_1	2	No
WAM 6_1	1	Yes

	WAM 7_2	1	No
	WAM 8_1	1	No
	WAM 9_2	1	Yes
	WAM 9_2	2	No
	WAM 9_2	3	No
	WAM 9_2	4	No
	WAM 9_2	5	No
	WM 2_1	1	No
	WM 3_2	1	Yes
	WM 4_2	1	No
	WM 5_1	1	Yes
	WM 6_2	1	Yes
	WM 7_1	1	Yes
	YRPS 5_1	1	Yes
	YRSP 1_1	1	Yes
	YRSP 2_2	1	No
	YRSP 3_1	1	Yes
	YRSP 4_1	1	Yes
	YRSP 6_1	1	Yes
	YRSP 7_2	1	No
	YRSP 7_2	2	No
	YRSP 7_2	3	No
	YRSP 7_2	4	No
	YRSP 7_2	5	No
<hr/>			
Sampling 2	WAM 1_2	1	No
	WAM 10_2	1	No
	WAM 12_1	1	No
	WAM 13_1	1	No
	WAM 14_1	1	No
	WAM 15_2	1	Yes
	WAM 3_2	1	No
	WAM 5_1	1	Yes
	WAM 6_1	1	Yes
	WAM 8_1	1	Yes
	WAM 9_2	1	No

The two sites marked N/A, FP 1_1 and WAM 13_1 did not amplify tick DNA and so *E. chaffeensis* results from these sites was not included in the analyses.

Appendix Table 6: qPCR-based *E. chaffeensis* detection.

	Site	Extraction Number	Tick Replicates			<i>E. chaffeensis</i> Replicates			<i>E. chaffeensis</i> Positive
			1	2	3	1	2	3	
Sampling 1	CNPS 1_2	1	22.47	22.44	23.02	No Ct	No Ct	No Ct	No
	CNPS 10_2	1	19.33	19.07	26.27	33.85	32.71	34.07	Yes
	CNPS 11_1	1	19.32	19.50	19.30	34.82	34.13	35.35	Yes
	CNPS 12_1	1	22.76	23.07	22.63	37.85	38.95	38.93	No
	CNPS 13_2	1	20.36	19.97	19.37	No Ct	No Ct	No Ct	No
	CNPS 13_2	2	21.45	21.95	20.40	No Ct	No Ct	36.81	No
	CNPS 13_2	3	20.86	20.23	19.94	37.95	38.51	37.68	No
	CNPS 13_2	4	20.70	19.52	19.87	No Ct	38.01	No Ct	No
	CNPS 14_1	1	24.44	22.37	21.85	No Ct	38.74	No Ct	No
	CNPS 15_1	1	23.23	20.81	21.08	34.45	35.90	35.42	Yes
	CNPS 16_2	1	20.32	20.02	21.30	37.29	38.82	38.93	No
	CNPS 17_2	1	27.56	23.59	23.33	37.63	37.40	38.32	No
	CNPS 18_2	1	23.17	22.86	No Ct	31.62	31.98	31.40	Yes
	CNPS 21_1	1	21.40	22.02	21.79	37.50	37.69	No Ct	No
	CNPS 22_2	1	22.04	23.49	No Ct	24.08	23.48	22.29	Yes
	CNPS 23_2	1	21.75	21.95	21.74	39.51	No Ct	37.30	No
	CNPS 25_1	1	19.93	20.00	19.86	25.89	25.99	25.87	Yes
	CNPS 28_1	1	20.84	20.18	19.77	30.71	32.10	31.23	Yes
	CNPS 3_1	1	25.94	24.01	23.92	No Ct	No Ct	No Ct	No
	CNPS 4_1	1	29.49	20.55	20.77	33.60	37.42	No Ct	No
	CNPS 40_2	1	28.65	27.87	24.72	No Ct	35.11	34.67	Yes
	CNPS 41_2	1	21.34	20.27	No Ct	No Ct	38.07	No Ct	No
	CNPS 5_2	1	24.67	19.98	19.83	No Ct	No Ct	No Ct	No
	CNPS 6_1	1	23.01	23.00	22.95	36.88	36.96	36.34	No
	CNPS 9_1	1	20.22	19.78	19.83	No Ct	35.46	35.46	Yes
	COLW 1_2	1	32.63	17.38	16.99	38.26	38.90	36.91	No
	COLW 2_2	1	23.20	24.13	23.36	No Ct	38.92	39.04	No
	COLW 2_2	2	23.11	23.48	24.14	No Ct	No Ct	No Ct	No
	COLW 2_2	4	19.03	18.92	19.00	37.93	38.73	No Ct	No
	COLW 2_2	3	20.66	20.87	20.97	39.85	No Ct	No Ct	No
	COLW 2_2	5	19.88	20.23	20.10	No Ct	38.70	No Ct	No
	COLW 3_1	1	23.15	23.11	24.00	37.92	38.75	No Ct	No
	COLW 4_1	1	20.78	21.62	18.85	26.07	25.97	25.81	Yes
	COLW 5_2	1	23.66	23.49	23.62	No Ct	No Ct	No Ct	No
	DRSF 1_2	1	23.01	22.4	22.24	34.79	34.83	35.01	Yes
	DRSF 10_2	1	23.14	22.69	21.93	37.96	37.35	38.07	No
	DRSF 11_1	1	21.65	21.46	21.51	No Ct	37.70	38.98	No
	DRSF 6_2	1	20.88	19.81	23.71	No Ct	No Ct	No Ct	No
	FP 3_1	1	22.28	20.99	29.00	31.54	32.29	32.25	Yes
	FP 4_2	1	19.51	21.88	22.98	29.57	29.38	28.06	Yes

FP 5_1	1	19.07	18.80	23.41	32.40	32.44	31.11	Yes
FP 6_2	1	21.04	21.82	20.32	29.60	28.94	27.43	Yes
FP 6_2	2	21.11	22.64	22.19	31.96	32.12	31.79	Yes
FP 7_1	1	23.58	22.44	23.44	35.75	35.33	35.25	Yes
FTE_6	1	25.77	26.05	25.40	27.95	27.81	27.99	Yes
FTE_8	1	21.36	21.18	21.4	34.66	35.75	35.98	Yes
GT 1_1	1	22.52	22.94	22.04	38.18	37.62	No Ct	No
GT 2_1	1	No Ct	23.00	21.34	34.36	34.40	No Ct	Yes
GT 3_2	1	20.63	20.78	21.50	29.07	28.53	27.59	Yes
GT 4_2	1	20.17	20.14	20.97	36.32	No Ct	36.19	Yes
NNP 12_1	1	23.04	22.17	21.64	34.71	35.09	35.05	Yes
NNP 15_1	1	24.78	22.50	22.55	No Ct	No Ct	38.59	No
NNP 15_1	2	19.74	19.12	19.98	31.75	31.59	31.87	Yes
NNP 15_1	3	19.15	18.79	19.39	No Ct	38.77	No Ct	No
NNP 18_2	1	19.88	20.13	19.70	35.35	33.93	35.13	Yes
NNP 23_1	1	21.07	20.16	19.74	31.82	31.93	31.78	Yes
NNP 29_2	1	20.64	20.42	21.69	38.42	37.58	37.77	No
NNP 3_1	1	25.86	28.36	20.13	37.59	No Ct	No Ct	No
NNP 30_2	1	21.84	26.07	21.77	37.85	No Ct	No Ct	No
NNP 31_2	1	25.30	24.98	24.59	30.49	30.49	30.93	Yes
NNP 8_2	1	23.55	23.46	23.46	No Ct	No Ct	38.69	No
NQP 2_2	1	19.75	19.81	19.67	37.71	37.89	35.36	No
NQP 2_2	2	24.30	20.96	21.09	No Ct	39.98	No Ct	No
NQP 3_3	1	19.87	19.59	19.11	No Ct	No Ct	39.38	No
NQP 4_1	1	22.23	22.74	22.26	37.15	35.77	35.00	Yes
NQP 5_2	1	27.71	22.53	25.57	32.84	33.04	33.50	Yes
NQP 6_2	1	22.16	20.72	21.09	30.24	30.22	30.13	Yes
SB 1_1	1	21.78	21.67	21.99	31.22	31.19	30.50	Yes
SB 2_2	1	26.74	20.86	21.59	32.83	33.00	32.71	Yes
SB 3_2	1	23.52	24.62	25.18	23.66	23.57	23.87	Yes
SPSF 1_1	1	25.49	24.24	22.08	37.89	No Ct	38.17	No
SPSF 2_1	1	22.96	19.92	19.20	No Ct	No Ct	No Ct	No
SPSF 3_2	1	25.33	22.95	30.68	37.91	37.89	No Ct	No
WAM 1_2	1	26.58	24.55	23.58	33.66	34.23	35.75	Yes
WAM 10_2	1	No Ct	22.91	23.84	38.48	No Ct	No Ct	No
WAM 12_1	1	20.02	19.49	19.91	33.00	32.09	31.71	Yes
WAM 14_1	1	22.62	22.85	22.91	38.34	No Ct	No Ct	No
WAM 15_2	1	20.84	19.72	20.16	33.11	34.01	32.12	Yes
WAM 4_2	1	18.68	18.77	19.61	31.32	31.88	31.31	Yes
WAM 5_1	1	21.51	21.04	22.03	No Ct	36.90	36.23	No
WAM 5_1	2	21.32	21.00	22.41	No Ct	39.20	No Ct	No
WAM 6_1	1	21.44	21.13	22.00	26.69	26.26	27.00	Yes
WAM 7_2	1	21.64	21.60	21.72	No Ct	No Ct	No Ct	No
WAM 8_1	1	25.06	27.27	24.06	No Ct	No Ct	40.00	No
WAM 9_2	1	20.56	20.22	20.84	39.27	34.96	No Ct	No
WAM 9_2	2	21.62	21.47	21.83	38.30	38.12	38.31	No

	WAM 9_2	4	19.16	19.29	20.55	No Ct	No Ct	No Ct	No
	WAM 9_2	3	21.20	21.16	22.29	No Ct	No Ct	No Ct	No
	WAM 9_2	5	19.30	19.06	19.22	No Ct	No Ct	39.32	No
	WM 2_1	1	20.6	20.68	20.8	35.26	No Ct	35.29	Yes
	WM 3_2	1	No Ct	20.11	19.76	34.91	34.87	34.99	Yes
	WM 4_2	1	20.94	21.01	20.63	39.94	No Ct	No Ct	No
	WM 5_1	1	23.83	20.06	19.92	34.15	35.54	35.31	Yes
	WM 6_2	1	22.14	22.60	No Ct	38.76	No Ct	No Ct	No
	WM 7_1	1	21.91	21.65	21.99	34.47	34.13	34.23	Yes
	YRSP 1_1	1	24.91	24.98	No Ct	32.21	31.24	33.09	Yes
	YRSP 2_2	1	20.58	21.36	21.99	34.13	35.45	35.70	Yes
	YRSP 3_1	1	19.91	19.34	22.40	24.64	24.72	24.72	Yes
	YRSP 4_1	1	21.05	20.58	20.87	26.31	26.48	26.34	Yes
	YRPS 5_1	1	24.48	23.54	22.79	29.60	29.35	29.14	Yes
	YRSP 6_1	1	21.51	21.72	21.83	27.90	26.61	26.84	Yes
	YRSP 7_2	1	21.54	20.39	21.18	37.87	No Ct	37.73	No
	YRSP 7_2	2	19.95	20.41	20.37	37.75	No Ct	No Ct	No
	YRSP 7_2	3	21.20	21.87	22.36	39.36	No Ct	No Ct	No
	YRSP 7_2	4	21.75	21.80	21.99	39.31	No Ct	No Ct	No
	YRSP 7_2	5	21.14	20.98	21.64	No Ct	37.37	No Ct	No
Sampling 2	WAM 1_2	1	22.03	20.96	22.71	No Ct	38.23	No Ct	No
	WAM 10_2	1	24.00	20.78	20.93	No Ct	No Ct	No Ct	No
	WAM 12_1	1	21.31	20.73	23.28	37.75	37.97	37.15	No
	WAM 13_1	1	22.36	21.42	21.95	33.42	33.44	33.41	Yes
	WAM 14_1	1	22.40	20.60	20.45	36.91	37.22	36.58	No
	WAM 15_2	1	26.25	26.93	26.23	No Ct	No Ct	No Ct	No
	WAM 3_2	1	24.85	24.61	27.62	37.91	38.58	36.42	No
	WAM 5_1	1	33.33	23.23	22.87	39.59	37.45	37.29	No
	WAM 6_1	1	18.96	18.92	20.54	37.83	No Ct	No Ct	No
	WAM 8_1	1	24.02	24.94	23.55	38.33	37.60	37.76	No
	WAM 9_2	1	20.74	22.52	23.99	No Ct	No Ct	38.16	No

Positive sites were those with at least two of three replicates having a Ct score of ≤36.48. All tick DNA samples amplified.

Appendix Table 7: Candidate endpoint PCR-based models. Shown are slopes for each candidate variable, and AIC_c, ΔAIC_c, and AIC weights for each model.

Model Number	Intercept	Vegetation Density to 3 m _{800m}	Density of Edge between Forest-Disturbed _{60m}	Ruderal/Recently Disturbed Land Cover _{180m}	Coniferous Forest _{600m}	Barren/Early Successional Forest _{600m}	Aquatic Ecotone _{600m}	Riparian Forest _{600m}	Total Forest Cover _{800m}	df	logLik	AICc	ΔAICc	AIC weight	Cumulative AIC Weight
45	-0.59215	NA	NA	4.50546	-4.91479	NA	7.47412	NA	NA	4	-59.3346	127.10893	0.00000	0.03968	0.03968
13	-0.28009	NA	NA	3.99062	-5.68532	NA	NA	NA	NA	3	-60.5425	127.34598	0.23705	0.03525	0.07493
77	-0.50420	NA	NA	3.44045	-5.27080	NA	NA	5.88494	NA	4	-59.7346	127.90888	0.79995	0.02660	0.10153
109	-0.80701	NA	NA	4.04064	-4.58491	NA	7.21151	5.70229	NA	5	-58.6260	127.91872	0.80979	0.02647	0.12800
73	-0.10492	NA	NA	NA	-4.55348	NA	NA	7.22427	NA	3	-61.0414	128.34384	1.23491	0.02140	0.14940
61	-0.45033	NA	NA	4.24017	-4.44900	-20.73096	7.93865	NA	NA	5	-58.9488	128.56441	1.45548	0.01917	0.16857
46	0.03139	-0.18536	NA	4.36477	-4.78683	NA	7.37069	NA	NA	5	-58.9688	128.60429	1.49536	0.01879	0.18736
14	0.37046	-0.19371	NA	3.85918	-5.59382	NA	NA	NA	NA	4	-60.1585	128.75656	1.64763	0.01741	0.20477
11	-0.12241	NA	0.03163	NA	-4.88825	NA	NA	NA	NA	3	-61.3323	128.92547	1.81654	0.01600	0.22077
29	-0.15008	NA	NA	3.74321	-5.28639	-17.46558	NA	NA	NA	4	-60.2585	128.95660	1.84767	0.01575	0.23652
9	0.26538	NA	NA	NA	-4.92899	NA	NA	NA	NA	2	-62.4151	128.95926	1.85033	0.01573	0.25225
75	-0.40031	NA	0.02719	NA	-4.52950	NA	NA	6.45519	NA	4	-60.2658	128.97128	1.86235	0.01564	0.26789
105	-0.27630	NA	NA	NA	-3.96749	NA	5.63320	7.09890	NA	4	-60.3406	129.12077	2.01184	0.01451	0.28241
141	0.30342	NA	NA	3.93373	-5.29891	NA	NA	NA	-0.98171	4	-60.3596	129.15884	2.04991	0.01424	0.29664
89	0.01729	NA	NA	NA	-4.00881	-24.89333	NA	7.51609	NA	4	-60.3923	129.22420	2.11527	0.01378	0.31042
173	-0.26409	NA	NA	4.45360	-4.73519	NA	7.17080	NA	-0.53108	5	-59.2841	129.23486	2.12593	0.01371	0.32413
47	-0.62045	NA	0.00731	4.07082	-4.83469	NA	7.40891	NA	NA	5	-59.3000	129.26674	2.15781	0.01349	0.33762
78	0.15598	-0.19818	NA	3.27886	-5.11873	NA	NA	6.02004	NA	5	-59.3197	129.30615	2.19722	0.01323	0.35085
43	-0.33974	NA	0.03300	NA	-4.19898	NA	6.30000	NA	NA	4	-60.4524	129.34444	2.23551	0.01298	0.36383
74	0.57305	-0.21330	NA	NA	-4.21032	NA	NA	7.31819	NA	4	-60.4734	129.38644	2.27751	0.01271	0.37653
93	-0.36808	NA	NA	3.11320	-4.78671	-20.04575	NA	6.23386	NA	5	-59.3638	129.39428	2.28535	0.01266	0.38919

15	-0.31955	NA	0.00947	3.42755	-5.56817	NA	NA	NA	NA	4	-60.4838	129.40720	2.29827	0.01258	0.40177
41	0.07519	NA	NA	NA	-4.28811	NA	6.07209	NA	NA	3	-61.6079	129.47681	2.36788	0.01215	0.41391
107	-0.60443	NA	0.02893	NA	-3.89562	NA	5.95692	6.32993	NA	5	-59.4783	129.62342	2.51449	0.01129	0.42520
121	-0.15416	NA	NA	NA	-3.34467	-27.61842	6.29431	7.37947	NA	5	-59.5592	129.78519	2.67626	0.01041	0.43561
205	-0.03940	NA	NA	3.42070	-4.98826	NA	NA	5.61949	-0.76379	5	-59.6267	129.92018	2.81125	0.00973	0.44534
25	0.39146	NA	NA	NA	-4.41203	-23.08240	NA	NA	NA	3	-61.8474	129.95581	2.84688	0.00956	0.45490
10	0.93669	-0.20941	NA	NA	-4.62247	NA	NA	NA	NA	3	-61.8670	129.99498	2.88605	0.00937	0.46427
79	-0.53934	NA	0.00889	2.91092	-5.15270	NA	NA	5.83202	NA	5	-59.6836	130.03400	2.92507	0.00919	0.47347
12	0.57015	-0.21562	0.03159	NA	-4.63496	NA	NA	NA	NA	4	-60.7983	130.03629	2.92736	0.00918	0.48265
91	-0.26513	NA	0.02558	NA	-4.05397	-23.86466	NA	6.78167	NA	5	-59.7155	130.09777	2.98884	0.00890	0.49155
76	0.29392	-0.21711	0.02701	NA	-4.24391	NA	NA	6.53630	NA	5	-59.7165	130.09976	2.99083	0.00890	0.50045
27	0.01529	NA	0.03027	NA	-4.46573	-21.76024	NA	NA	NA	4	-60.8670	130.17373	3.06480	0.00857	0.50902
106	0.38724	-0.21077	NA	NA	-3.54995	NA	5.63988	7.18605	NA	5	-59.7645	130.19575	3.08682	0.00848	0.51750
57	0.20166	NA	NA	NA	-3.66955	-26.38097	6.75270	NA	NA	4	-60.8964	130.23253	3.12360	0.00832	0.52582
201	0.40249	NA	NA	NA	-4.23703	NA	NA	6.90678	-0.83815	4	-60.9075	130.25461	3.14568	0.00823	0.53405
113	-0.49495	NA	NA	NA	NA	-34.34361	8.29196	8.19761	NA	4	-60.9237	130.28710	3.17817	0.00810	0.54215
38	-0.21867	-0.21895	NA	3.74778	NA	NA	9.45161	NA	NA	4	-60.9491	130.33781	3.22888	0.00790	0.55005
142	1.31886	-0.24023	NA	3.80736	-5.17390	NA	NA	NA	-1.33376	5	-59.8393	130.34532	3.23639	0.00787	0.55792
59	-0.19649	NA	0.03157	NA	-3.69519	-25.11202	6.95516	NA	NA	5	-59.8502	130.36706	3.25813	0.00778	0.56570
98	0.13356	-0.24974	NA	NA	NA	NA	7.41297	7.88908	NA	4	-60.9709	130.38144	3.27251	0.00773	0.57343
102	-0.43216	-0.22257	NA	3.21256	NA	NA	8.89199	6.90801	NA	5	-59.8666	130.39995	3.29102	0.00766	0.58108
117	-0.97752	NA	NA	3.11860	NA	-31.00615	9.68789	7.25775	NA	5	-59.8895	130.44569	3.33676	0.00748	0.58856
44	0.33259	-0.21289	0.03318	NA	-3.84933	NA	6.34290	NA	NA	5	-59.8976	130.46190	3.35297	0.00742	0.59598
42	0.73045	-0.20639	NA	NA	-3.90562	NA	6.07467	NA	NA	4	-61.0503	130.54019	3.43126	0.00714	0.60312
101	-1.28756	NA	NA	3.58999	NA	NA	9.62181	7.15102	NA	4	-61.0608	130.56127	3.45234	0.00706	0.61018
137	0.94126	NA	NA	NA	-4.48067	NA	NA	NA	-1.15233	3	-62.1539	130.56871	3.45978	0.00704	0.61722
53	-0.74808	NA	NA	3.67772	NA	-30.51787	10.39498	NA	NA	4	-61.0656	130.57077	3.46184	0.00703	0.62425
30	0.39579	-0.17013	NA	3.71251	-5.35850	-14.30175	NA	NA	NA	5	-59.9923	130.65135	3.54242	0.00675	0.63100

157	0.58178	NA	NA	3.64331	-4.79529	-20.30749	NA	NA	-1.19436	5	-59.9963	130.65937	3.55044	0.00672	0.63772
37	-1.05421	NA	NA	4.12049	NA	NA	10.21489	NA	NA	3	-62.2203	130.70155	3.59262	0.00658	0.64431
139	0.48143	NA	0.03076	NA	-4.50017	NA	NA	NA	-1.01152	4	-61.1360	130.71173	3.60280	0.00655	0.65086
100	-0.22575	-0.24099	0.02974	NA	NA	NA	7.68615	7.09447	NA	5	-60.0453	130.75742	3.64849	0.00640	0.65726
66	0.31395	-0.25801	NA	NA	NA	NA	NA	8.17783	NA	3	-62.2664	130.79380	3.68487	0.00629	0.66355
16	0.34261	-0.19947	0.01104	3.19910	-5.44332	NA	NA	NA	NA	5	-60.0794	130.82553	3.71660	0.00619	0.66973
90	0.53978	-0.16879	NA	NA	-3.90910	-21.08187	NA	7.51891	NA	5	-60.0862	130.83909	3.73016	0.00615	0.67588
203	0.05868	NA	0.02672	NA	-4.25314	NA	NA	6.18973	-0.74959	5	-60.1605	130.98772	3.87879	0.00571	0.68159
115	-0.80624	NA	0.02683	NA	NA	-32.94647	8.50421	7.57186	NA	5	-60.1610	130.98872	3.87979	0.00570	0.68729
36	0.03976	-0.24189	0.03437	NA	NA	NA	8.25032	NA	NA	4	-61.2769	130.99348	3.88455	0.00569	0.69298
217	0.68258	NA	NA	NA	-3.60971	-27.01991	NA	7.12036	-1.07682	5	-60.1786	131.02389	3.91496	0.00560	0.69858
31	-0.19019	NA	0.01058	3.11076	-5.15501	-18.01902	NA	NA	NA	5	-60.1852	131.03714	3.92821	0.00557	0.70415
81	-0.34302	NA	NA	NA	NA	-33.43349	NA	8.61950	NA	3	-62.4257	131.11233	4.00340	0.00536	0.70951
202	1.36519	-0.24244	NA	NA	-3.82306	NA	NA	6.88900	-1.14652	5	-60.2346	131.13591	4.02698	0.00530	0.71481
114	0.11507	-0.18744	NA	NA	NA	-27.79969	7.83188	7.96851	NA	5	-60.2396	131.14588	4.03695	0.00527	0.72008
97	-0.76197	NA	NA	NA	NA	NA	8.01293	8.22055	NA	3	-62.4818	131.22459	4.11566	0.00507	0.72515
233	0.05007	NA	NA	NA	-3.79640	NA	5.36514	6.89679	-0.52488	5	-60.2904	131.24765	4.13872	0.00501	0.73016
143	0.25568	NA	0.00887	3.40408	-5.19587	NA	NA	NA	-0.96299	5	-60.3083	131.28345	4.17452	0.00492	0.73508
68	-0.01408	-0.24881	0.02751	NA	NA	NA	NA	7.41879	NA	4	-61.4461	131.33190	4.22297	0.00480	0.73989
138	1.92801	-0.24893	NA	NA	-4.11801	NA	NA	NA	-1.46363	4	-61.4634	131.36638	4.25745	0.00472	0.74461
34	0.48696	-0.24925	NA	NA	NA	NA	8.07904	NA	NA	3	-62.5567	131.37438	4.26545	0.00470	0.74931
169	0.57532	NA	NA	NA	-4.00982	NA	5.64297	NA	-0.82971	4	-61.4772	131.39410	4.28517	0.00466	0.75397
153	1.23243	NA	NA	NA	-3.87227	-26.01893	NA	NA	-1.40050	4	-61.4778	131.39530	4.28637	0.00465	0.75862
99	-1.09625	NA	0.03013	NA	NA	NA	8.25659	7.49258	NA	4	-61.4887	131.41704	4.30811	0.00460	0.76323
171	0.05657	NA	0.03231	NA	-3.98864	NA	5.94502	NA	-0.64274	5	-60.3765	131.41966	4.31073	0.00460	0.76782
49	-0.13045	NA	NA	NA	NA	-34.63736	9.12899	NA	NA	3	-62.5981	131.45721	4.34828	0.00451	0.77234
54	-0.17268	-0.17277	NA	3.55424	NA	-24.38831	9.82729	NA	NA	5	-60.4059	131.47864	4.36971	0.00446	0.77680
51	-0.52594	NA	0.03112	NA	NA	-32.72857	9.25719	NA	NA	4	-61.5358	131.51131	4.40238	0.00439	0.78119

26	0.91562	-0.16916	NA	NA	-4.32709	-19.35742	NA	NA	NA	4	-61.5371	131.51383	4.40490	0.00439	0.78558	
194	1.56584	-0.27168	NA	NA	NA	NA	NA	NA	7.32882	-1.87982	4	-61.5493	131.53832	4.42939	0.00433	0.78991
140	1.52608	-0.25883	0.03082	NA	-4.19877	NA	NA	NA	-1.36359	5	-60.4594	131.58548	4.47655	0.00423	0.79414	
166	0.85199	-0.23287	NA	3.63746	NA	NA	8.26767	NA	-1.58112	5	-60.4764	131.61956	4.51063	0.00416	0.79830	
82	0.28950	-0.19710	NA	NA	NA	-25.61543	NA	8.32239	NA	4	-61.6192	131.67797	4.56904	0.00404	0.80234	
28	0.58480	-0.18514	0.03105	NA	-4.39349	-18.44487	NA	NA	NA	5	-60.5139	131.69452	4.58559	0.00401	0.80635	
209	0.92590	NA	NA	NA	NA	-34.28938	NA	7.65923	-1.95423	4	-61.6308	131.70131	4.59238	0.00399	0.81034	
70	-0.06731	-0.23847	NA	2.32933	NA	NA	NA	7.37137	NA	4	-61.6415	131.72266	4.61373	0.00395	0.81429	
241	0.45009	NA	NA	NA	NA	-35.19621	7.25337	7.54734	-1.42511	5	-60.5282	131.72313	4.61420	0.00395	0.81825	
181	0.32466	NA	NA	3.55781	NA	-31.63265	9.07842	NA	-1.63712	5	-60.5347	131.73609	4.62716	0.00392	0.82217	
165	0.02691	NA	NA	3.97530	NA	NA	8.87900	NA	-1.64725	4	-61.6653	131.77020	4.66127	0.00386	0.82603	
155	0.79094	NA	0.02917	NA	-3.98059	-24.66585	NA	NA	-1.26625	5	-60.5722	131.81117	4.70224	0.00378	0.82981	
58	0.69577	-0.16018	NA	NA	-3.52042	-23.07532	6.63679	NA	NA	5	-60.6024	131.87148	4.76255	0.00367	0.83348	
226	1.08300	-0.26081	NA	NA	NA	NA	6.46374	7.26309	-1.38856	5	-60.6118	131.89027	4.78134	0.00363	0.83711	
83	-0.62823	NA	0.02502	NA	NA	-32.37184	NA	8.03185	NA	4	-61.7405	131.92065	4.81172	0.00358	0.84069	
52	0.08760	-0.19775	0.03312	NA	NA	-26.36406	8.73256	NA	NA	5	-60.6506	131.96800	4.85907	0.00350	0.84418	
35	-0.81727	NA	0.03451	NA	NA	NA	8.89769	NA	NA	3	-62.8778	132.01657	4.90764	0.00341	0.84760	
185	0.84748	NA	NA	NA	-3.32451	-28.33830	6.21208	NA	-1.05077	5	-60.6942	132.05517	4.94624	0.00335	0.85094	
4	0.27789	-0.25085	0.03256	NA	NA	NA	NA	NA	NA	3	-62.9016	132.06419	4.95526	0.00333	0.85427	
50	0.47743	-0.18957	NA	NA	NA	-27.78789	8.56645	NA	NA	4	-61.8226	132.08494	4.97601	0.00330	0.85757	
65	-0.60393	NA	NA	NA	NA	NA	NA	8.55859	NA	2	-63.9804	132.08987	4.98094	0.00329	0.86086	
229	-0.38085	NA	NA	3.49955	NA	NA	8.56671	6.49250	-1.34287	5	-60.7132	132.09308	4.98415	0.00328	0.86414	
85	-0.66633	NA	NA	2.24716	NA	-31.15066	NA	7.87156	NA	4	-61.8506	132.14077	5.03184	0.00321	0.86735	
40	-0.24977	-0.22403	0.01729	2.72387	NA	NA	9.18513	NA	NA	5	-60.7485	132.16379	5.05486	0.00317	0.87052	
130	2.14989	-0.27463	NA	NA	NA	NA	NA	NA	-2.25344	3	-63.0067	132.27444	5.16551	0.00300	0.87351	
6	0.16797	-0.23565	NA	2.97638	NA	NA	NA	NA	NA	3	-63.0074	132.27569	5.16676	0.00300	0.87651	
196	1.20013	-0.26521	0.02633	NA	NA	NA	NA	6.63302	-1.78714	5	-60.8153	132.29741	5.18848	0.00296	0.87948	
170	1.51296	-0.23562	NA	NA	-3.57438	NA	5.48276	NA	-1.13411	5	-60.8178	132.30241	5.19348	0.00296	0.88243	

164	1.09445	-0.25695	0.03270	NA	NA	NA	7.11919	NA	-1.54683	5	-60.8222	132.31121	5.20228	0.00294	0.88538
2	0.69983	-0.25926	NA	NA	NA	NA	NA	NA	NA	2	-64.0932	132.31560	5.20667	0.00294	0.88831
177	1.02461	NA	NA	NA	NA	-35.50927	7.72388	NA	-1.79556	4	-61.9428	132.32526	5.21633	0.00292	0.89124
210	1.65620	-0.21926	NA	NA	NA	-28.11501	NA	7.43423	-1.99235	5	-60.8349	132.33648	5.22755	0.00291	0.89415
162	1.64160	-0.26324	NA	NA	NA	NA	6.85441	NA	-1.74079	4	-61.9558	132.35130	5.24237	0.00289	0.89703
132	1.67450	-0.26937	0.03080	NA	NA	NA	NA	NA	-2.11778	4	-61.9748	132.38930	5.28037	0.00283	0.89986
134	1.60907	-0.25237	NA	2.94773	NA	NA	NA	NA	-2.22506	4	-61.9757	132.39112	5.28219	0.00283	0.90269
84	0.00213	-0.19943	0.02653	NA	NA	-24.98574	NA	7.63629	NA	5	-60.8680	132.40268	5.29375	0.00281	0.90550
67	-0.90836	NA	0.02807	NA	NA	NA	NA	7.85038	NA	3	-63.0889	132.43879	5.32986	0.00276	0.90827
193	0.69250	NA	NA	NA	NA	NA	NA	7.55585	-1.99573	3	-63.1018	132.46461	5.35568	0.00273	0.91099
69	-0.96758	NA	NA	2.69419	NA	NA	NA	7.64841	NA	3	-63.1194	132.49970	5.39077	0.00268	0.91367
225	0.21849	NA	NA	NA	NA	NA	6.93161	7.49586	-1.47532	4	62.03873	132.51702	5.40809	0.00266	0.91633
198	1.19472	-0.25231	NA	2.35191	NA	NA	NA	6.48559	-1.89691	5	-60.9296	132.52596	5.41703	0.00264	0.91897
103	-1.32029	NA	0.01326	2.80164	NA	NA	9.39220	7.07366	NA	5	-60.9400	132.54674	5.43781	0.00262	0.92159
55	-0.78508	NA	0.01342	2.88022	NA	-30.61000	10.19078	NA	NA	5	-60.9422	132.55117	5.44224	0.00261	0.92420
33	-0.39725	NA	NA	NA	NA	NA	8.77954	NA	NA	2	-64.2256	132.58031	5.47138	0.00257	0.92677
39	-1.09210	NA	0.01395	3.28723	NA	NA	9.99843	NA	NA	4	-62.0848	132.60930	5.50037	0.00254	0.92931
145	1.55067	NA	NA	NA	NA	-34.11449	NA	NA	-2.38481	3	-63.1844	132.62974	5.52081	0.00251	0.93182
179	0.55635	NA	0.02949	NA	NA	-33.92053	7.95058	NA	-1.64839	5	-60.9987	132.66423	5.55530	0.00247	0.93429
211	0.60502	NA	0.02364	NA	NA	-33.35548	NA	7.10458	-1.87128	5	-61.0273	132.72144	5.61251	0.00240	0.93669
154	2.09031	-0.21910	NA	NA	-3.77531	-23.07754	NA	NA	-1.67422	5	-61.0319	132.73060	5.62167	0.00239	0.93907
213	0.60436	NA	NA	2.24489	NA	-31.91929	NA	6.88645	-1.95484	5	-61.0682	132.80324	5.69431	0.00230	0.94137
86	-0.04962	-0.18648	NA	2.13189	NA	-23.72744	NA	7.59659	NA	5	-61.1057	132.87816	5.76923	0.00222	0.94359
149	1.05859	NA	NA	2.85458	NA	-31.05302	NA	NA	-2.32975	4	-62.2241	132.88776	5.77883	0.00221	0.94580
227	-0.20384	NA	0.02880	NA	NA	NA	7.26156	6.86758	-1.32115	5	-61.1426	132.95192	5.84299	0.00214	0.94794
17	0.05331	NA	NA	NA	NA	-33.16159	NA	NA	NA	2	-64.4142	132.95746	5.84853	0.00213	0.95007