In December 2005 I attended a presentation, "Tick-borne Disease Update," given to state workers by Jeffrey Engel, MD, Head, General Communicable Disease Control Branch and State Epidemiologist. At the presentation he described the Chatham Project as well as NC tick-borne infections in general. Members of the state Vector-Borne Disease Task Force, lab technicians, state entomologists, and others concerned with vector-borne diseases were present. The information about the Chatham Tick Project and the “Update” comes from this presentation as well as input from Drs Engber, Apperson, and Engel. Dr Engber is a state entomologist and Dr Apperson is an entomologist at NCSU.

This paper is written for the benefit of the membership of the Tick-borne Infections Council of North Carolina, Inc (TIC-NC), the public health community, and the public. Our comments on the “Update” and the Chatham Tick Project are included at the end.

– Marcia E. Herman-Giddens PA, MPH, DrPH, February to May, 2006
– President, Tick-borne Infections Council of North Carolina, Inc (TIC-NC)

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Due to rising concern and complaints by citizens of Chatham County, the North Carolina Department of Health and Human Services, Division of Public Health, joined with NC State University, Department of Entomology and the North Carolina Department of Environment and Natural Resources, Public-Health Pest Management Section to conduct an enhanced surveillance project. Chatham County was considered a good place for the study due to the citizen concern, it being a central Piedmont location, and having a willing local health department and practicing physicians. No additional funding was available for the project so all of the work and testing relied on a student volunteer, and efforts of local and state agency staff.

The project took place from June 15 to August 15, 2005. Patients presenting to the participating physicians for suspected tick-borne infections had blood drawn for serologic testing for the agents of Rocky Mountain Spotted Fever (Rickettsia), monocytic and granulocytic Ehrlichiosis (Ehrlichia and Anaplasma), and Lyme Disease (Borrelia burgdorferi). Attempts were made to obtain both acute and convalescent sera but only a portion of the participants were able to give both samples. Sera were tested for Rickettsia and Ehrlichia/Anaplasma at the NC State Laboratory of Public Health. The Centers for Disease Control (CDC) tested the samples for B. burgdorferi by EIA followed by Western Blots if the EIAs were positive. The Southeastern Cooperative Wildlife Disease Study group at the University of Georgia tested the ticks for Ehrlichia, Borrelia, Anaplasma, and Rickettsia. Any positives were further tested to determine species. Tests were not done for other organisms that ticks may carry and sometimes cause co-infections such as Bartonella ssp or Babesia ssp. (These infections are not reportable in NC.) Properties of interested residents (not the patients) identified by the Chatham County agricultural agent, Al Cooke,
were dragged for ticks. In particular, properties were identified when possible where residents had reported tick-borne infections.

Patients.
Seventy-nine patients were screened. Most presented with an acute febrile illness. Only one had an erythema migrans (EM) rash. Twenty-eight were excluded because they did not have appropriate clinical symptoms. Of the 51 patients tested, 16 had probable Rocky Mountain Spotted Fever (RMSF), and seven had probable (1) or confirmed (6) Ehrlichia (all Human Monocytic Ehrlichiosis). The CDC performed EIAs and Western Blots for B burgdorferi only. There were no positives for Lyme disease (some of the Lyme EIAs were positive but these were felt to be false positives according to CDC criteria). There were no positives for arboviruses (EEE, WNV, SLE, and Lacrosse). Not all of the convalescent sera could be obtained.

Ticks.
Over 6000 ticks were collected by dragging. Most were Lone Star ticks (Amblyomma americanum). Other types included 24 male and female American dog ticks (Dermacentor variabilis) and one nymphal black-legged (deer) tick (Ixodes scapularis).

**Tick organism testing:**
For the three species of ticks, 52 (11.9%) of 439 pools were tested for pathogens. The number of ticks in the pools varied. Pools were made from both the Dermacentor variabilis and Amblyomma americanum ticks. The single Ixodes scapularis was tested separately.\(^1\) (Positive test results do not mean that the tick(s)

\(^1\) Details of the testing and results from Dr. Charles Apperson.

**PCR analyses of ticks.** Ticks were macerated individually or in pools in microcentrifuge tubes containing two copper BBs and sterile PBS (0.5 ml) with a Mixer Mill MM 300 (Qiagen, Inc., Valencia, CA). Total nucleic acids (NA) were extracted from individual samples with a Qiagen Viral RNA kit (Qiagen) according to the manufacturer's instructions. Quality control measures included negative controls (water) that were extracted and amplified in parallel with all specimens. Potential for NA contamination was minimized by using three separate, designated areas for NA extraction and preparation of primary and secondary PCRs. Additionally, two thermal cyclers (PTC-100™, MJ Research, Inc.), designated for either primary or secondary amplification, were used. Furthermore, subsets of the extractions were tested for the presence of tick mitochondrial 16S rDNA, using primers 16S +1 and 16S -1 as described by Norris et al. (1996). Nested PCR assays were used to detect the presence of E. chaffeensis, A. phagocytophilum, Borrelia spp. and Rickettsia spp.

The 16S rDNA gene was targeted for the detection of E. chaffeensis and A. phagocytophilum using 2.5 µl of DNA template in a primary PCR reaction with external primers ECC and ECB, which target all Ehrlichia spp. (Anderson et al. 1992). For secondary PCR amplification, 1 ul of primary product was used in reactions with species-specific primers HE1and HE3 to detect E. chaffeensis (Dawson et al. 1994) and in reactions with primers GA1UR and GE9F to detect A. phagocytophilum (Chen et al. 1994, Little et al. 1998). External primers FLALL and FLARL and internal primers FLALS and FLARS, which amplify a region of the flaB gene of all species in the genus Borrelia, were used in a nested PCR assay as previously described (Barbour et al. 1996, Moore et al. 2003). Primer pairs (17 kDa1/17 kDa2 [external] and 17k Da3/17 kDa4 [internal]) were used to amplify a portion of the 17 kDa gene from Rickettsia spp. (Sekeyova et al. 2001).

Products amplified with internal primer sets GA1UR/GE9F, FLALS/FLARS, and 17k Da3/17k Da4 were purified using a Qiagen Gel Purification Kit (Qiagen Inc., Valencia, CA) and sequenced in both the 3' and 5' directions with a Perkin Elmer ABI Prism 3700 automated DNA sequencer at the Molecular Genetics
is necessarily a transmitter of a particular pathogen only that it harbors live or dead pathogens.)

*Rickettsia* - *Rickettsia rickettsii* was found in 2 American dog tick pools and the single *I. scapularis* nymph. *R. amblyommi* was found in 11 pools of *Amblyomma americanum*. *Rickettsia rickettsii* is the causative agent of RMSF. The American dog tick (*Dermacentor variabilis*) is considered to be the vector of RMSF. It is not known if *R. amblyommi* causes illness in humans, but the organism was found to be very common in these Lone Star ticks and these ticks were very abundant. Since 31% of the ill subjects were positive for RMSF and only about 0.1% of the ticks were the dog tick that carries this *Rickettsia*, this would suggest that the bite of the Lone Star tick (which carries *Rickettsia amblyommi*) may have contributed to the positive *Rickettsia* tests. Further testing about this is being done. It will be very significant if this study shows that *Rickettsia amblyommi* causes human disease.

*Borrelia burgdorferi* - No *B. burgdorferi* was found in any of the 56 pools tested. There is apparently no money to continue this testing.

*Other Borrelia*. The *A. americanum* ticks did not show any other *Borrelia* ssp by PCR testing.

*Ehrlichias*. - *E. chaffeensis* was found in one *Dermacentor variabilis* pool and one pool of *Amblyomma americanum* ticks. Isolation from *D. variabilis* is unusual. *Amblyomma americanum* is considered to be the principal vector of *Ehrlichia chaffeensis* in North Carolina.

*Anaplasma* - *A. phagocytophilum* was found in one pool of *Amblyomma americanum* ticks. *Ixodes scapularis* is considered the principal vector.

Instrumentation Facility at the University of Georgia. The sequences were assembled and edited with the Sequencher software (version 4.0.5, Gene Codes Corp., Ann Arbor, MI). Pathogens were identified by performing a nucleotide–nucleotide BLAST (blastn) search for matching sequences in GenBank sequence database.

For the three species of ticks, 52 (11.9%) of 439 pools were tested for pathogens. Of the pools tested for *E. chaffeensis*, *A. phagocytophilum*, and *Borrelia* ssp., *E. chaffeensis* was detected in 1 pool of 10 male *A. americanum* and in a single *D. variabilis* female (Table 2). *Anaplasma phagocytophilum* was detected in a pool containing 3 male *A. americanum*. *Borrelia* ssp. were not detected in the 52 samples tested. Tick pools tested for the presence of *Rickettsia* ssp. yielded 21 PCR-positive samples. PCR products of 17 samples were sequenced to identify the *Rickettsia* species. *Rickettsia amblyommi* was identified in 11 *A. americanum* pools (104 ticks) and in 2 single-tick pools of *D. variabilis*. Specific identification based on sequenced segments of the 17 kDa gene amplicons could not be made for 4 pools of *A. americanum*. Differentiation between *R. rickettsii* and *R. montana* could not be made in 2 *D. variabilis* pools (4 ticks) and in the single *I. scapularis* tested. In another sample containing a single *D. variabilis* female, we could not differentiate between *R. felis* and *R. rickettsii*. Analysis of a segment of the RompB gene for these samples is ongoing.
**Other items of interest discussed at the meeting.**

Several items were noted in the discussion following the presentation:

a. The large increase in the prevalence of the Lone Star tick.

b. The fact that it is not known whether the *Rickettsia amblyommii* organism that the Lone Star tick carries causes human disease.

c. Some attendees questioned whether the EM rash from STARI is really a rash from an infection or simply an allergic reaction.

d. Reasons for proposing that Lyme disease is uncommon in NC included reports suggesting an EM rash may be STARI rather than LD, 20 skin biopsies over a period of years sent to the CDC have been negative for *Bb*, a C6 study on dogs found little evidence for Lyme disease, and there have been no outbreaks of arthritis such as occurred in Connecticut.

e. The group wished they could do other tests but there is no funding available.

f. The group hopes to finish up the Chatham study soon and to put out a summary report.

The above description of the Chatham Project is as presented by state professionals. The comments and concerns about the findings, any implied findings, and other points presented below are written by the Tick-borne Infections Council of North Carolina, Inc.

**DISCUSSION AND CONCERNS ABOUT THE COMMENTS AT THE DECEMBER MEETING AND THE INTERPRETATIONS OF THE FINDINGS OF THE CHATHAM PROJECT**

When people read about the Chatham Project or the statements made by public health officials as above, we are concerned that some inaccurate assumptions could be made, especially by those who know very little about ticks and the infections they carry. Therefore, we present the comments below to help with the interpretation and use of the information.

**The prevalence of the various kinds of ticks of interest.**

It cannot be concluded that the results of the tick drag present an accurate picture of the prevalence of the various kinds of ticks of interest. The methods used were not intended to produce accurate sampling for establishing prevalences of types of ticks in Chatham County that bite humans or the prevalences of any disease-causing organisms they may carry. The season of the year, time of day, location, and methods used (such as dragging, CO₂ traps, collection from small and large animals, etc) affect the type and stage of tick that will be collected.²,³ As stated by Schulze, et al, “Failure to recognize the biases in

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these commonly used sampling techniques can potentially lead to incorrect conclusions that can have significant public health consequences (our emphasis).³

Because of ongoing concern with the risk of acquiring Lyme disease in NC, it is important to discuss the *Ixodes scapularis* tick. This is the tick that can carry the organism that causes Lyme disease. [Note about nomenclature: Many people in North Carolina call the nymph of the Lone Star tick (*Amblyomma americanum*) a ‘deer tick’ since they have heard that deer ticks were small and the Lone Star is often found on deer. The better common name for the *Ixodes scapularis* tick is the black-legged tick.] Although only one nymphal black-legged tick was collected in the 2005 project, it cannot be assumed that the black-legged (*Ixodes scapularis*) tick is uncommon in our location because neither the season or the technique used was appropriate for optimal collection of the nymphal stage of this tick.³,⁵ In fact, it has already been shown that *Ixodes scapularis* ticks are established in many counties in NC including Chatham County⁶ and are associated with human cases of Lyme disease in this county and a number of other places in the state.⁷,⁸,⁹ In addition, adult *Ixodes scapularis* ticks were found on pets in a central Chatham location during the winter of 2005-2006, the first time noted by these pet owners.¹⁰ Research has shown that the organism that causes Lyme disease, *Borrelia burgdorferi*, is widely distributed across the south.¹¹,¹²

For Chatham County, since the tick dragging methods were not geared toward collecting black-legged ticks we do not know:

- what their prevalence is in Chatham County.
- the proportion that carry the Lyme disease organism or any other pathogens.

It also cannot be concluded that the ratio of American dog ticks to the other two types of ticks collected reflects the actual situation since dragging may not be the optimal way to collect the American dog tick. We already know that the Lone Star tick is very prevalent in this area of North Carolina and is an aggressive human biter in every developmental stage. It also has a long season of activity. The Chatham Project confirms this and reminds us that because of the characteristics of the *A americanum* tick, citizens of NC are

at increased risk of diseases that this tick carries as compared to the other ticks. The public health implications need to be kept in mind.

The diseases.

**Rickettsia ssp.** Rocky Mountain Spotted Fever. North Carolina leads the nation in the number of reported cases of RMSF with over 500 cases reported in 2004. RMSF is a serious disease with up to a 20% mortality rate if not treated. There is a 5% risk of death even with treatment. It is of interest that almost 1/3 of the tested patients in the Chatham Project 2005 had probable rickettsial infections since so few American dog ticks were collected (as discussed in the project description). It could be that *D varabilis* proportions are not accurately reflected by the collection methods and/or that the *Rickettsia* in the Lone Star ticks is also making people sick.

Other rickettsial diseases include human monocytic ehrlichiosis (HME) caused by *Ehrlichia chaffeensis*, human granulocytotropic ehrlichiosis (HGE) caused by *Anaplasma phagocytophilum, Ehrlichia ewingii*, and other emerging rickettsial diseases. Two recent studies in southeast and south central states including North Carolina found that 22% of children tested had serologic evidence of *E chaffeensis* and *R rickettsii* infections indicating exposure is widespread and that many cases may be subclinical. The CDC suggests that only 1/3 of fatal RMSF cases are reported. Given this figure and the subclinical occurrence of some infections, actual numbers of rickettsial diseases in humans are likely to be many times higher in NC than the number reported to the state. Under-reporting also occurs because many cases are treated without the serological testing required for reporting.

**Lyme disease (LD).** Lyme disease (or Lyme borreliosis) is present in NC but is not as likely an infection as in the highly endemic states. There are differences in risk of Lyme borreliosis between the north and south. It is likely that people are bitten less often by black-legged ticks in North Carolina even when these ticks are present and may be less likely to get infected for a number of reasons. So far studies find a lower proportion of *I scapularis* ticks are infected with human pathogens than in the highly endemic areas, although more research needs to be done. The nymphs, which are most likely to transmit disease due to their small size delaying discovery and removal, stay closer to the ground than in the north and tend to use lizards and small mammals as hosts. (Lizards are not as common in the north.) In addition, lizards may not be competent vectors for *B burgdorferi*, though research is still going on regarding this question.

Lyme borreliosis should not be characterized as rare or uncommon in North Carolina. Five-hundred and three cases have been reported in NC from 2000 through 2004. Mortality from Lyme disease does occur but is infrequent, although it is likely that it occurs more often than is recognized. There has been only one reported death in NC due to LD in the period from 1999-2002 (and 20 nationally).
Some have argued that reports are largely due to incorrect diagnoses (i.e. rash and illness from *Borrelia lonestari* rather than *Bb*, see below). While this may happen in some cases, no North Carolina studies have examined this possibility further. A number of studies have verified the presence of the vector, *Ixodes scapularis*, and that a portion carry the bacteria that causes Lyme disease (*Borrelia burgdorferi*). Some have argued that reports are largely due to incorrect diagnoses (i.e. rash and illness from *Borrelia lonestari* rather than *Bb*, see below). While this may happen in some cases, no North Carolina studies have examined this possibility further. A number of studies have verified the presence of the vector, *Ixodes scapularis*, and that a portion carry the bacteria that causes Lyme disease (*Borrelia burgdorferi*). \(^5\) \(^9\) *Borrelia burgdorferi* is present and is cycling enzootically in NC. Serum antibodies to *B. borrelia* have been found in animals in NC. \(^6\) \(^17\) \(^18\) Lyme disease has been a considerable problem on the military bases in NC, especially Camp Lejeune, and Fort Bragg has been declared a moderate risk area. \(^7\) \(^19\) We recognize that some NC residents may have obtained LD during travels to highly endemic areas and may then be diagnosed here. Data are not available to TIC-NC about the origin of the infection in reported cases. Nonetheless, it is important for NC medical providers to be aware that NC citizens may have LD regardless of the geographic origin of the disease.

In contrast to the 500+ cases reported in NC in 4 years, in 1993 only 8 cases were reported in Iowa, increasing to 42 cases in 2002 (compared to 137 in NC). Yet, Iowa began a statewide surveillance program in 1990 due to growing public concern. The project monitors tick populations and infection rates of the ticks. \(^20\) The authors stated, “…Iowa’s mean annual incidence of human infection with *B burgdorferi* was 0.67 per 100,000. As interest and concern about Lyme disease increased, it was important to monitor tick populations across the state.” In contrast, in NC, where Lyme disease has not been widely recognized, the average annual incidence rate from 2000 to 2004 was about 1.53 per 100,000. \(^21\)

The CDC has stated that the actual number of Lyme disease cases are likely 6 to 12 times the number reported. \(^22\) If that is true for NC, this state could have had from 3,000 to 6,000 cases occur in the first 4 years following the millennium. Putting forth a public health message that LD is uncommon in NC may be interpreted as ‘almost never occurring.’ The effect of thinking it seldom occurs in NC misinforms the public so they are not aware of their risk. It also misleads medical providers into thinking they do not have to consider this diagnosis when confronted with a patient with a history and findings that could be indicative of Lyme borreliosis (or Lyme-like disease). Because this disease can become chronic with serious, debilitating symptoms leading to total disability in some individuals,

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21. Calculations based on an average population of 8,130,000 and an average of 125 cases per year. The US rate in 2004 was 6.7 cases per 100,000. CDC data.
it is essential to have a high index of suspicion and follow CDC guidelines for a clinical diagnosis and prompt treatment of acute cases. Lastly, considering the disease as uncommon prevents the appropriate level of attention to LD risk in NC as part of an overall public health response to our growing problems with several human-biting ticks and infections they are known to carry.

The lack of laboratory evidence as manifested by the 20 negative skin biopsies sent to the CDC over an unknown number of years has been cited as evidence of the lack of Lyme borreliosis in NC. First, skin biopsy cultures have been shown in a recent paper to be positive in only 22% of probable cases (defined by histories and laboratory tests indicative of LD). Similarly, for skin biopsy PCRs on patients with proven Lyme borreliosis, 44% were negative. It has been found that some strains of *Borellia burgdorferi* senso stricto (*Bb*ss) will not grow in culture, a fact possibly related to the low proportion of positive cultures in the study above. Some researchers feel the CDC interpretation standards for the Western Blot are too restrictive. Therefore, there are many reasons why having 20 negative skin biopsies over an unknown number of years does not provide evidence for a low prevalence of Lyme disease in North Carolina.

A recently published study of dogs in North Carolina found only 0.4% positive for *Borrelia burgdorferi* by the C6 peptide-based enzyme-linked immunosorbent assay. From 8.7% to 25% of dogs were C6 positive from the other states in the study—Virginia, Maryland, and Pennsylvania. The study used the SNAP® 3Dx® test to screen for the C6 antigen. C6 is a synthetic peptide derived from VlsE, an outer-surface immunodominant portion of *Bb* (*IDEXX Laboratories 2004*). The assay is based on dogs with arthritic symptoms after exposure to *Bb*. Therefore, while dogs in NC are not mounting antibodies to the genomic region used for the test, presumably made from a *Bb* strain that causes arthritic symptoms, the lack of response may be due to the fact that we have a wider variety of strains in NC and arthritis does not appear to be as prominent a feature of clinical symptoms in NC as in the Northeast. Studies are lacking on this point, however. Since *Bb* has been shown to be present in NC some dogs would be exposed. Different tests may be necessary for detection.

The lack of “outbreaks of arthritis” such as occurred in Lyme, Connecticut has been proposed as further evidence of the infrequency of LD in NC. We cannot be certain there have been no “outbreaks of arthritis” among North Carolina children because we do not know if any epidemiological studies have been conducted to identify such outbreaks. In

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our larger state (than Connecticut) and communities, clusters would be easy to miss by lay people (mothers first identified the clusters in Lyme, Connecticut). In addition, different strains of the Lyme disease organism cause different kinds of symptoms.26, 27 If Lyme or Lyme-like disease exists here, we cannot assume that it behaves exactly like Lyme disease in the Northeast. In addition, the outbreaks in Lyme, Connecticut occurred before the disease had been recognized; therefore those children were not treated with antibiotics. Now if a parent had an ill child with an EM rash, the child would likely be taken for medical care and, presumably treated for a tick-related illness. This would usually prevent the development of arthritis.

**Southern tick associated rash disease** (STARI).

STARI, sometimes referred to as Master’s disease, is a phenomenon that has been reported from Georgia, South Carolina, North Carolina, and Missouri and may be common in other parts of the Southeast according to the CDC. It is a Lyme-like disease that may occur following exposure to Lone Star ticks characterized by an expanding rash that looks like the erythema migrans of Lyme disease. It is often followed by symptoms associated with Lyme disease that respond to the same antibiotics used to treat LD. It has been reported that it may be persistent and debilitating (Masters). Most patients with this illness have negative serologic assays for antibodies to *B. burgdorferi*. The putative agent, *Borrelia lonestari*, was recently cultured28 but could not be implicated in a recent study.29 It remains a mystery as to what causes STARI. The inability to identify the causative organism of STARI could be because the numbers of organisms are too low to be detected in individuals that have been studied, or what is being called STARI could be due to another Borrelial or non-Borrelial organism.

The 1997 North Carolina study on a Chatham girls’ camp16 cannot be used to suggest that the Lyme-like illness from Lone Star ticks is due to *Borrelia lonestari*. The lead author, Dr. Kirkland, never stated that the children’s’ illnesses were due to *B. lonestari*. It was only suggested that they might be. Therefore, this study cannot be used as evidence of *B. lonestari* infections in NC or to help with the understanding of the epidemiology of the rash and illness that sometimes follows the bites of Lone Star ticks. The children in the 1997 study were not followed long-term16 so it cannot be assumed that they had no long-term problems from the illness from their tick bites. (Since they were treated promptly as though they had Lyme disease long-term effects may have been absent anyway.) In addition, some of the serologies from these children indicated exposure to Borrelias. We do not know the specifics on the testing done by the CDC for this study. If the CDC used only the primers designed to detect the B31 strain of *Borrelia burgdorferi* in the samples from these children, such a technique could miss other strains of *Bbss* and, certainly, other Borrelias.30 Over 300 strains are known, 250 from the south alone.31

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It is premature and without any supportive research to suggest that an EM rash following the bite of local ticks (probably the Lone Star tick) is simply due to an allergic reaction. Many people have become ill with Lyme-like symptoms following these bites.\textsuperscript{16, 29, 32} There are some differences between the EM rashes of STARI and LD but often they are indistinguishable.\textsuperscript{29} The Wormser, et al. study only followed patients for 3 months, so, again, although systemic symptoms were milder in the Missouri patients than the New York patients, long-term data are lacking. In addition, these patients were identified and treated in the acute phase of their illness. No data are available on the long-term sequelae of the untreated or late-treated illness termed ‘STARI.’

The CDC has designed a study to look further at STARI and is calling for physicians to enroll patients. It seeks to ascertain if a \textit{Borrelia} organism other than \textit{B. burgdorferi} is causing the Lyme-like disease, called (to date) Southern Tick-Associated Rash Illness (STARI), in southern states. The document links below are the CDC letters of explanation to physicians and their patients interested in participating in this study. As of Spring 2006, North Carolina has not promoted participation in this study to state physicians.

Information needed for the CDC STARI study is contained in the links below. We urge the NC Department of Health and Human Services to mount a campaign to enlist physicians and to inform the public. The CDC STARI link is: \url{http://www.cdc.gov/ncidod/dvbid/stari/index.htm}

**Conclusion**

Chatham County is fortunate to have the state’s interest with regard to our tick-borne infections problem. Many residents have been sick from bites of local ticks and a number are chronically ill and disabled from the sequelae. We encourage the state to continue the Chatham Tick Project. We know from the data from the Chatham Tick Project and from previous studies that tick-borne illnesses are a considerable problem in North Carolina and warrant an improved and extensive public health response. Rickettsial diseases are a particular problem. Lyme disease is not uncommon and a Lyme-like disease apparently associated with the bite of the Lone Star tick may be a serious as Lyme disease. This disease, in particular, needs further study since the tick vector is an aggressive biter of humans and the habitat for these ticks is increasing with suburbanization and the deer population. Statewide studies are lacking on the prevalence of TBIs in people, proportions of ticks by type in different geographic areas, and the infection prevalence of the ticks. Information from state public health officials needs to be as accurate as possible given this rapidly changing field.

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\textsuperscript{31} Personal communication, Dr. James Oliver, Jr, March 22, 2006. The laboratory at the Institute of Arthropodology and Parasitology, Georgia Southern University has isolated approximately 250 strains of \textit{Borrelia burgdorferi} sensu lato from the Southern US, most unpublished to date.

\textsuperscript{32} Case registry files from Tick-borne Infections Council of North Carolina, Inc.