SEROPREVALENCE OF ANTIBODIES AGAINST *BORRELIA BURGDORFERI* IN DOGS IN GEORGIA

by

LAURA RIDGE

(Under the Direction of Craig Greene)

ABSTRACT

Lyme disease, causative agent *Borrelia burgdorferi*, is a commonly reported tick-borne disease. In the United State it has been reported in forty-nine states. Seroprevalence studies using dogs have been done in some states to assess human exposure risk. This study was undertaken to determine the seroprevalence of *B. burgdorferi* antibodies in dogs in Georgia utilizing three different serologic tests: Whole cell ELISA, C6 peptide ELISA, and Western blot. The state was divided into four physiographic regions and serum from dogs housed in animal shelters in each region were tested with both a whole cell ELISA and a C6 peptide ELISA and, if positive on either test, tested with the Western Blot. Of the 310 serum samples tested, only two results were positive for *B. burgdorferi* exposure. Overall, this study demonstrates that the seroprevalence of antibodies against *B. burgdorferi* in dogs in Georgia is low.

INDEX WORDS: *Borrelia burgdorferi*, Lyme disease, dogs, Georgia, C6 Peptide ELISA

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LAURA RIDGE

B.S., Texas A&M University, 1997

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LAURA RIDGE

Major Professor: Craig Greene

Committee: David Hurley
Dave Stallnecht

Electronic Version Approved:

Maureen Grasso
Dean of the Graduate School
The University of Georgia
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CHAPTER 1

INTRODUCTION

Etiology and Epidemiology of Lyme Disease

Lyme borreliosis is a common worldwide tick-borne disease in humans.[1] First described as a syndrome of chronic progressive arthritis in Lyme, Connecticut in 1976, it has since been identified in all forty-nine states in the United States, with the majority of the cases occurring in southern New England, the eastern parts of the middle Atlantic States, and the upper Midwest.[2] Twelve sates account for 95% of the cases reported: Connecticut, Rhode Island, New Jersey, New York, Delaware, Pennsylvania, Massachusetts, Maryland, Wisconsin, Minnesota, New Hampshire, and Vermont.[3]

Borrelia are small, gram-negative spirochetes. *Borrelia burgdorferi sensu lato* is the causative agent of Lyme disease and includes a number or closely-related organisms isolated from infected people, animals, or ticks in various regions of the world. *Borrelia* spp. are further subgrouped based on outer surface lipoproteins and amino acids sequences. In the United States, *B. burgdorferi sensu stricto* is the most common isolate from people with Lyme disease, though other genospecies have been identified in animals.[1,2] *Borrelia* are transmitted between vertebrate hosts by hematophagous arthropod vectors.[2] The principle vectors for *B. burgdorferi* are *Ixodes scapularis* (black-legged tick) in the Northeast, Midwest, and Southeast and *Ixodes pacificus* in the West.[1] Each stage of the tick's three-stage life cycle can be infected by feeding on a vertebrate reservoir host. Transovarial transmission has not been proven to occur. However, trans-stadial transmission allows the tick to remain infected throughout molts.[3] By this means, infection can be maintained in nature because different stages of the tick feed at different times,

with larvae feeding primarily in the summer, nymphs in the late spring and early summer, and adults in the fall. Infected nymphs are reported to be primarily responsible for the transmission of the infection to people and animals, and to simultaneously feeding uninfected larvae.[2] In order for the tick to transmit the *Borrelia* to a host, the tick must remain attached for approximately 48-hours. In the tick the spirochete lives in the mid-gut and undergoes cell division and changes in surface protein expression while the tick feeds that enables it to leave the midgut and spread to the salivary glands, thus becoming infective to the host.[4,5,6]

Lyme Disease in Humans

Lyme disease in humans is divided into different stages of illness- early localized, early disseminated, and late disease. The early localized form, known as erythema migrans, the classic bull's-eye skin lesion associated with early localized Lyme disease is found. This localized lesion is seen in approximately 90% of people and appears within 3-30 days after the tick bite.[1] This lesion, which has been associated with bacteria from the feeding tick that enter the dermal tissues, is not specific to Lyme disease however and has been associated with bites from *Amblyomma americanum* (Lone Star Tick). A species of *Borrelia* (*B. lonestarii*) has been isolated from these ticks and may be the cause of the localized skin lesion.[7,8] In contrast to signs associated with localized disease, those with early systemic or disseminated Lyme borreliosis include fever, malaise, arthralgia, myalgia, regional lymphadenopathy, and headache. Other manifestations seen with disseminated disease include facial nerve paralysis, meningitis, and uveitis.[1,9] With late or chronic Lyme disease in humans, non-erosive arthritis (most commonly affecting the stifle) and encephalitis, encephalopathy, and polyneuropathy are seen.[1,10] An autoimmune component to chronic arthritis has been proposed.[11,12]

Lyme Disease in Dogs

Lyme disease in dogs causes similar clinical signs as reported in humans. In the acute stages of the disease common clinical signs include fever, lethargy, lymphadenopathy, shifting-leg lameness, and joint discomfort.[2,4] A recurrent, intermittent, non-erosive arthritis (most commonly affecting the carpus) has been attributed to chronic Lyme disease in dogs.[2,4,13] Other manifestations of Lyme disease that have been described include glomerulonephritis, complete heart block, dermatitis, uveitis, and meningoencephalomyelitis.[2,14-17] In experimental models of disease in dogs, these other clinical manifestations of Lyme have not been well documented.[3]

Dogs as Sentinels for Lyme Disease in Humans

A number of serologic studies in dogs have been undertaken to determine the seroprevalence of antibodies against *Borrelia burgdorferi*.[18-27] Because dogs have more frequent environmental exposure to ticks, given their living environments and roaming behaviors, it has been proposed that dogs may act as sentinel animals to assess the risk of *B. burgdorferi* infection in humans.[28] Reports from states with low prevalence of seropositivity in people reflect similar low seropositivity (1.7%) in dogs tested.[20] Likewise, in human Lyme endemic areas higher seroprevalence rates (49.2% and 53%) were seen in correspondingly tested dogs.[19,27] Although the studies did not target clinically affected dogs, the presence of antibodies against the *Borrelia* organism demonstrated a similar and parallel exposure risk for dogs and humans.

Purpose of this study

Despite the initial concern for the prevalence of Lyme disease throughout the United States, the prevalence of human cases of Lyme disease cases in Georgia has been reported to be low. In 2002, only 2 cases of Lyme disease were diagnosed in Georgia based on the Center of Disease Control laboratory criteria for diagnosis.[29,30] A serologic study utilizing a whole-cell antigen enzyme-linked immunosorbent assay (ELISA) in white-tailed deer in Georgia from

1979-1990 was performed and demonstrated an overall reactive antibody prevalence of 19%, with higher prevalence (50%) seen along the Barrier Island region.[31] *Borrelia burgdorferi* has also been found in cotton rats (*Sigmodon hispidus*) in Georgia and the rats have been proposed as a reservoir host in the southeast.[32] *Ixodes scapularis* ticks are present in Georgia, and various studies have also demonstrated the presence of *B. burgdorferi* spirochetes in these ticks.[33-36] Based on this information, there is evidence that *Borrelia burgdorferi* is present in Georgia; however there is no documentation of it causing infection in animals or people. No studies have been performed to determine the exposure rate of dogs in Georgia to *B. burgdorferi,* and to evaluate the dog as a sentinel for the exposure risk in people. The purpose of our study was to evaluate the prevalence of serum antibodies to Borrelia burgdorferi in dogs as defined by a selected C-reactive protein and to determine what, if any, physiographic distribution of exposure might exist.

CHAPTER 2

LYME DISEASE TESTING

Testing Methods Available for *Borrelia burgdorferi*

There are numerous diagnostic tests available to determine the presence of the *B. burgdorferi* spirochete, or antibodies against the spirochete, in an animal. Methods for direct detection of the organism include dark-field microscopy, or special immunochemical staining of histologic sections of tissue or cerebral spinal fluid. These methods are of limited diagnostic value as the density of spirochetes in clinical samples is usually low.[37] Culturing the organism using modified BSK (Barbour-Stoenner-Kelly) media is considered the gold-standard, but is difficult due to the organisms slow growth and microaerophilic requirements.[3] Success of culture also varies with the type of tissue or fluid cultured, with reports in humans as high as 50-80% for skin culture, compared to 1.2% [with samples] from blood.[37] Qualitative and quantitative polymerase chain reaction (PCR) have been utilized with good success to detect organisms in tissues or body fluids.[37,38] The overall high sensitivity and specificity of PCR makes it a good test. However, the variability in sensitivity from various tissues, the ability to detect DNA but not definitively identify the presence of viable organism, and limited availability have limited its routine use.[4,37] Overall, serologic studies evaluating antibodies against the *B. burgdorferi* spirochete are the most common means of determining exposure, as the body's immune surveillance is considered quite sensitive. The limitations of antibody detection include differentiation of exposure to the *Borrelia burgdorferi* organism from vaccination, cross-reactivity of antibodies against other infectious agents with *B. burgdorferi* antigens, and the

difficulty in correlating titer with clinical disease. Tests available for serologic evaluation include whole-cell ELISA and immunofluorescent assay (IFA), Western Blot, and C6 Peptide ELISA.

Whole-Cell ELISA and IFA

An ELISA or IFA tests utilizing the whole *Borrelia* organism are available for serologic testing of numerous species. The advantage of ELISA testing lies in its ease of standardization and the ability to analyze a large number of sera relatively quickly.[39] [It is also quantitative relative to IFA] Disadvantages of the whole-cell ELISA include the inability to distinguish between antibodies induced after exposure to naturally occurring infection and antibody induced by vaccination.[40,41] The whole-cell ELISA also has the potential for interference by cross-reaction of with antibodies against other infectious agents including *Treponema* species and serovars of *Leptospira interrogans*.[42,43,44] For humans, the Association of Public Health Laboratories and the CDC recommend a 2-tiered approach to serologic testing for Lyme disease with a combination of a whole-cell ELISA or IFA followed by evaluation of all positive or equivocal samples by an immunoblot procedure.[45] In dogs, confirmation of natural exposure via Western Blot testing is also recommended when any whole cell assay is used..[2,3] Another limitation of the ELISA or IFA assessment is that the titer does not necessarily correlate with the presence of clinical signs, as serum IgG titers up to 1:8192 have been found in asymptomatic dogs.[3] Furthermore, because whole-cell ELISAs are not well designed for comparison of IgM to IgG antibodies in serum, the determination of recent exposure is also difficult to interpret. In experimentally infected dogs, IgG titers peak at 3-months and remained elevated for at least one year after exposure. IgM titers rise and remained elevated for only 2 months. In naturally infected dogs, however, IgM titers can persist, especially at low levels, for many months. [2] [Thus, ratio of IgM to IgG is not very useful in staging the duration of infection.]

Western Blot

The Western blot, or immunoblot, procedure has been utilized primarily as a secondary test to confirm a positive serologic titer with whole-cell ELISA or IFA. Different antibody patterns of reactivity on the Western blot can be seen with natural exposure and from vaccination.[2] Western blot analysis of naturally exposed dogs show serum antibody reactivity with the 39-, 29-, and 28-kD bands. Dogs vaccinated with the whole cell bacterins have additional 31- and 34-kd bands while dogs vaccinated with the OspA recombinant vaccines should only have a band at 31-kD. These bands correlate with protein expressed by the *Borrelia* spirochete in the tick and in cell culture.[46,2] The OspA protein, present at the 31-kD site, is necessary for binding of the *Borrelia* organism in the tick gut. However, the expression of *ospA* is downregulated during the blood meal with expression essentially stopped once the organism invades the mammalian host.[5] The *ospB* gene, whose protein is represented by the 34-kD band, has coordinated transcription with *ospA* gene.[47] Although the exact function of the OspB protein are not known, studies with OspA/B-deficient Borrelia have identified the proteins as essential for colonization if the tick midgut.[48] Antibodies against these proteins are induced by vaccination and are ideal proteins to target to differentiate natural exposure from vaccination. A possible drawback may exist, however, as anti-OspA antibodies have been documented in people with chronic Lyme disease arthritis and is speculated to possibly occur in dogs, which may pose a problem in differentiating chronically infected dogs from those with a vaccination history.2, 49 Dogs with dual status (vaccinated and natural exposure) have bands at 80-, 39-, 31-, 34-, 29-, and 28-kD.[46,2]

C6 Peptide ELISA

The C6 peptide ELISA utilizes as the antigen a 26-mer synthetic peptide (C6) based on an invariable region (IR6) of VIsE (Vmp-like sequence, Expressed), a surface antigen of *B. burgdorferi* expressed during natural infections.[50,51] The invariable region 6 (IR6) is highly antigenic and its sequence is conserved among strains of *B. burgdorferi* sensu stricto, making it an ideal peptide to target for an ELISA in many mammalian hosts.[51] In dogs, the C6 ELISA becomes positive as early as 3 weeks post-infection, indicating the assay is both highly specific and capable of detecting early infection.[50] Serum antibodies from dogs vaccinated with Lyme disease vaccine do not cross-react with the C6 peptide, a marked advantage over the whole cell ELISA and IFA.[52] As well, no cross-reactivity has been identified with the C6 ELISA in low number of dogs (< 10 each) with leptospirosis, Rocky Mountain Spotted Fever, dirofilariasis, babesiosis, or ehrlichiosis, or in dog's vaccinated with common DA_2PPL (distemper virus, adenovirus-2, parvovirus, parainfluenza virus, leptospirosis) vaccines.[50] The original test for dogs was a commercially available in-office ELISA test kit, SNAP®, 3Dx (IDEXX Laboratories, Michigan), that simultaneously detected *B. burgdorferi* and *Ehrlichia canis* antibodies and *Dirofilaria immitis* (heartworm) antigen.[53] Quantitative C6 ELISA tests are now available for dogs and humans, allowing a specific titer to be assigned. This quantitative ELISA has been explored for assessing response to antibiotic therapy in dogs and humans [based on the reduction in titer with effective treatement].[54, 55]

CHAPTER 3

PROCEDURES

Study Population

Dogs residing in humane and animal shelters in Georgia were selected as the study population. This population was chosen as they potentially have increased exposure to ticks given their environments and roaming behaviors. Dogs were included that were at least one year of age to allow sufficient time for tick exposure and development of antibodies if exposed to *Borrelia burgdorferi*. Blood samples were collected from July 2003- November 2003 to incorporate times when the various stages of tick feeding occurs. Dogs were included in the study based on the shelter staff's agreement to allow their participation. No dogs were specifically included or excluded based on the presence of any known disease.

The state of Georgia was divided into four physiographic regions similar to those regions used in a prior epidemiologic study of Lyme disease performed in deer in Georgia.[31] These physiographic regions were labeled lower coast, upper coast, piedmont, and mountain (see Figure 3.1). Humane and animal shelters in these physiographic regions were contacted concerning the study and their willingness to participate. Four counties were chosen in each physiographic region based on willingness of the shelters and humane societies to participate (see Figure 3.2). Up to 25 dogs from each shelter or humane society were sampled. Dogs were selected on a random but sequential basis on the day of sampling and were only excluded if they did not meet the criteria outlined above.

Sample Collection

From each dog, approximately 3 to 5-mls of whole blood were collected from the cephalic, lateral saphenous, or jugular veins with standard blood collection techniques using 6-mls syringes and 22-gauge needles. Each sample was placed into a 6-ml serum separator tube labeled with 3 letters correlating to the first three letters of the county name and a number assigned to the dog. Any information about the dog, including whether they were stray or owner abandoned, was recorded if available and shelter identification was recorded along with the assigned study number. The blood in the serum separator tubes was inverted six times to allow activation of the clot enhancer. Each sample was allowed to clot at room temperature for at least 20 minutes before centrifugation. If time to centrifugation was to exceed 30 minutes, the serum separator tubes with blood were stored in a Styrofoam container with ice. Each serum separator tube was centrifuged at 5000 rpm for 5 minutes to allow separation of the serum. After centrifugation, each serum separator tube was stored in a Styrofoam container on ice and transported by automobile to the University of Georgia, College of Veterinary Medicine. At the University of Georgia, each serum sample was harvested from the serum separator tube with a plastic disposable pipette and divided into 1-ml aliquots and placed into 2-ml plastic vials. Each vial was labeled with the appropriate assigned number. The serum aliquots in the plastic storage vials were frozen at -70 degrees Celsius and maintained at that temperature until assayed.

C6 Peptide ELISA

Each serum sample was tested using the SNAP®, 3Dx test kit (IDEXX Laboratories, Michigan) that simultaneously detects antigen against *Dirofilaria immitus* and antibody against *Borrelia burgdorferi* and *Ehrlichia canis*. The test uses a proprietary assay device which

provides reversible chromatographic flow of sample and automatic, sequential flow of wash and enzyme substrate. The C6 synthetic peptide was conjugated to bovine serum albumin (BSA) and to horseradish peroxidase (HRP), using standard methods. The BSA-C6 peptide conjugate was deposited into a polyethylene flow matrix using semiautomatic dispensers to deliver 0.5-0.25 µl of the appropriate spotting solution in a designated orientation. The HRP-C6 peptide conjugate was combined is a diluent with HRP-labeled anti-heartworm antibody, HRP-labeled *E. canis* peptide conjugate, nonspecific protein, and detergent.[53] Three drops of serum were combined with 4 drops of conjugate and added to the sample well as directed by the package insert. When the color first appeared in the activate circle the activator was pushed firmly until flush with the device body. The device was kept in a horizontal position and results read in 8 minutes. The C6 ELISA test was considered positive if a blue-color developed in the area of deposition of the BSA-C6 peptide conjugate (see Figure 3.3). Positive or negative results in relation to *E. canis* antibody and Heartworm antigen were also recorded.

Figure 3.1: Physiographic Regions of Georgia.

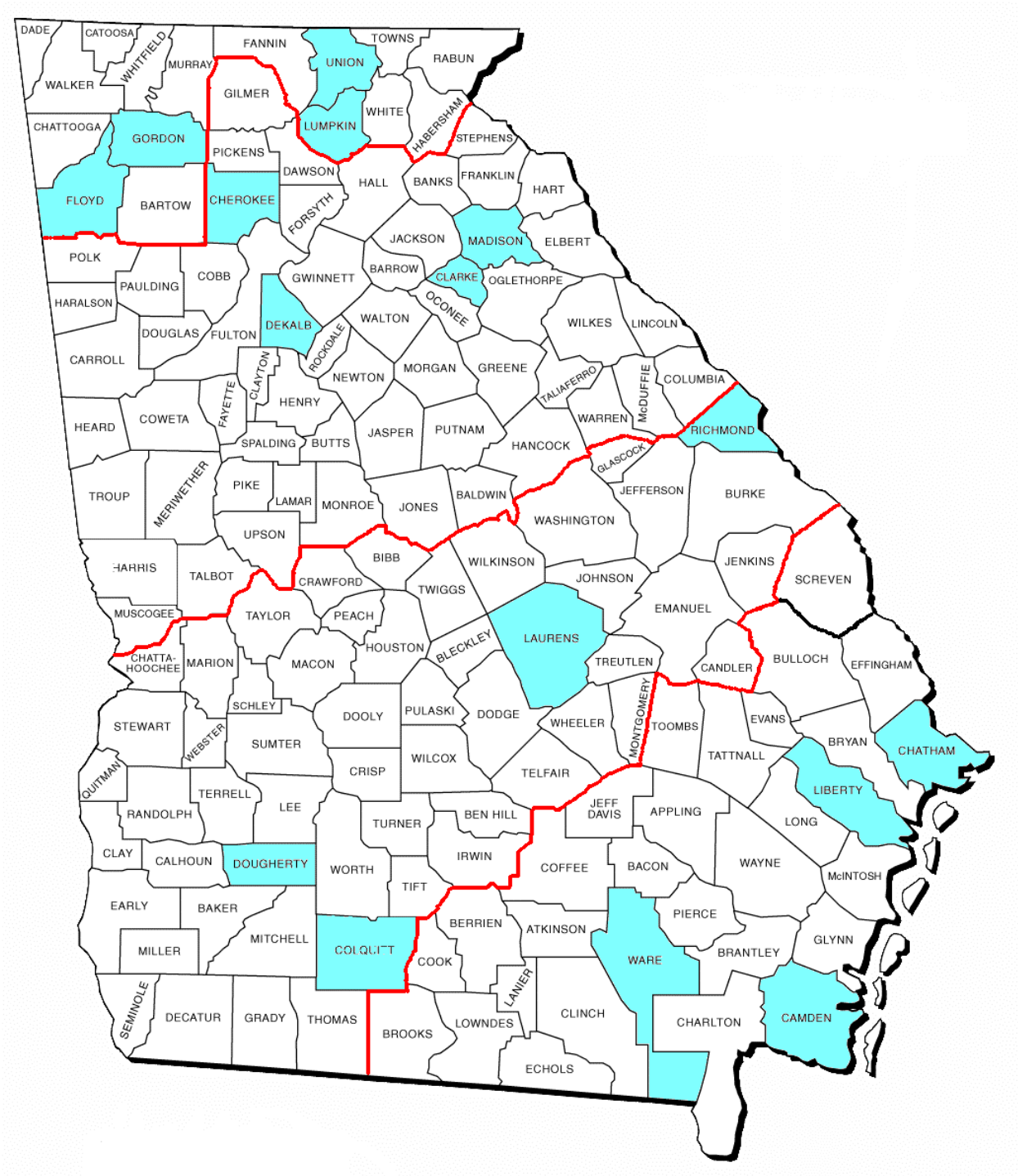

Figure 3.2: Counties in Georgia included in study. The counties with humane/animal shelters participating in the study are shaded in light blue.

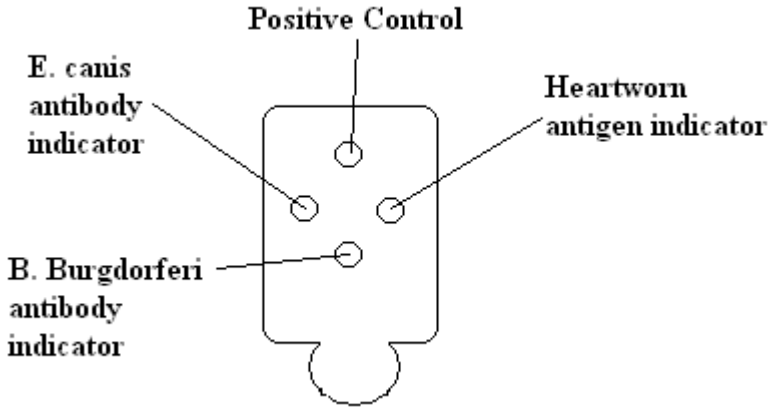

Figure 3.3: Results window for 3Dx SNAP C6 Peptide ELISA. The circles correlate to the location of a color change if the appropriate antigen or antibody is present in the sample tested.

Whole-Cell ELISA

Each serum samples was also evaluated with a whole-cell ELISA. For this procedure, Immulon I flat bottom 96 well microtiter plates (Dynatech Laboratories, Chantilly, VA) were coated with grade 2 *Borrelia burgdorferi* sonicated antigen (Microbix Biosystems Inc., Toronto, Ontario, Canada) The antigen was diluted in carbonate buffer (pH 9.6). Fifty microliters of diluted antigen were added to each row of the plate except for the first row which serves as a control row for the o-phenylenediamine (OPD). The plates were incubated overnight at 4 degrees Celsius. The plates where then brought to room temperature and washed 3 times with PBS/Tween (0.05% Tween 20).

The controls (IgM positive and negative samples, IgG positive and negative samples) were diluted at 1:128 in PBS/Tween and 50 microliters per well were placed in triplicate in row two Samples were serially diluted from 32 to 4096 on the plate. To do so, 155 microliters of PBS/Tween were placed in the first column, save the first and second rows which serves as

control rows. Fifty microliters were added to columns 2-8. Five microliters of serum samples to be tested were added to the 155 microliters PBS/Tween in column one. For serial dilutions, the contents of the wells in column one were mixed 4 times and then 50 microliters were added to column 2. Column 2 was mixed 4 times and then 50 microliters were transferred to column 3, and so on until reaching column 8. Fifty microliters were discarded from column 8 and from column 1. The plates were then incubated at 37 degree Celsius and washed three times with PBS/Tween.

The antisera, peroxidase labeled goat anti-dog IgM (mu-specific) (Kirkegaard and Perry Laboratories, Inc, Gaithersburg, Maryland) and peroxidase-conjugated rabbit anti-dog IgG (Jackson ImmunoResearch Laboratories, Inc, West Grove, Pennsylvania), were diluted in PBS/Tween. Fifty microliters were added to each well except for the OPD control row. The plates were incubated at 37 degrees Celsius for 30 minutes and then washed 3 times with PBS/Tween.

To make the substrate for each plate, 50 microliters of OPD, 5 microliters of hydrogen peroxide (3%), and 5 milliliters of sodium acetate buffer where combined no sooner than 5 minutes prior to addition to the plate. Fifty microliters were added to all wells including the OPD control row. The plates were incubated at room temperature in the dark for 20-45 minutes. The reaction was stopped by adding 30 microliters of 8N H_2SO_4 to all wells. The plates were read at 490 nm. Titers were considered positive at greater than or equal to 1024 for IgM and greater than or equal to 256 for IgG.

Western Blot

For every sample that was positive on the C6 peptide ELISA and/or the whole cell ELISA (IgG only) were further evaluated by western blot. The QualiCodeTM Canine Lyme

Disease Kit (Immunetics, Inc., Boston, MA) was the test kit used. This kit detects IgG antibodies for *Borrelia burgdorferi*. Prior to starting the procedure, the reagents, wash buffer, working conjugate buffer, and diluted conjugate, were prepared as instructed in the kit manual. Strips used in the kit were manufactured by resolving proteins from the *B. burgdorferi* spirochete with electrophoresis and transferring them by electroblotting onto a nitrocellulose membrane. Each strip was prepared by adding 1 ml of wash buffer to each active channel of the incubation tray and then placing the strips individually into the channels. The strips were incubated for 1 minute on a rocking platform to ensure all the strips became wet. The wash buffer was then aspirated and 1 milliliter of dilution buffer was added to each active channel.

Ten microliters of each test serum sample and 10 microliters of canine Lyme IgG control were added to the appropriate individual active channels. The strips were incubated for 30 minutes at room temperature on the rocking platform. The wells were then aspirated and rinsed with 1 ml of wash buffer three times.

One ml of diluted IgG conjugate was added to each active channel and incubated at room temperature for 15 minutes on the rocking platform. The conjugate was then aspirated and the channels were rinsed twice with 1 ml of wash buffer and twice with 1 ml of distilled water.

One ml of AP substrate solution was added to each active channel and incubated on the rocking platform to initiate the color reaction (approximately 6-8 minutes). The substrate was aspirated from all channels and the channels were rinsed twice with distilled water to stop the color development. The strips were transferred to a paper towel to air dry before interpretation.

CHAPTER 4

RESULTS

A total of 310 dogs were sampled, with 74 dogs from the mountain region, 63 from the piedmont region, 83 from the upper coast region, and 90 from the lower coast region. Of all the serum samples tested, only two results (0.65%) were considered true positives for *Borrelia burgdorferi* exposure based on a positive test results on the C6 peptide ELISA and whole cell ELISA for IgG, and confirmed natural exposure with the western blot. For positive results, one dog was located in the upper coast physiographic region and one dog was located in the lower coast physiographic region. Reactions from three dogs were considered false positives. Two of these dog's sera had positive reactions only on the whole cell ELISA for IgG antibody but these were considered to be due to vaccination with further analysis by the Western blot. The third dog's result was positive only for IgG on the whole cell ELISA but was negative for all exposure and vaccination on the Western blot. Two dogs from the mountain region had positive results for IgM antibody on the whole cell ELISA. Because the conjugate used in the Western blot was only specific for IgG antibodies, for which these dog's sera were not reactive, further confirmation of the seroreactivity with the Western blot was not performed. The results for all dog's sera tested are available in appendix A. A summary of the results is found in Table 4.1 and illustrated in Figure 4.1.

Table 4.1: Summary of serologic test results for *Borrelia burgdorferi* antibodies in dogs in Georgia.

Physiographic Region	County	Number of Dogs Sampled	C6 Peptide ELISA Positive	Whole Cell ELISA IgG Positive	Whole Cell ELISA IgM Positive	Western Blot Results
Mountain	Floyd	16	0	0	0	Not Done
	Lumpkin	23	0	1	0	Vaccinate
	Town and Union	15	0	0	0	Not Done
	Gordon	20	0	0	0	Not Done
Piedmont	Cherokee	11	0	0	0	Not Done
	Madison	20	0	0	0	Not Done
	Dekalb	11	0	0	0	Not Done
	Clarke	21	0	0	0	Not Done
Upper Coast	Richmond	21	0	0	2	Not Done
	Dougherty	25	0	0	0	Not Done
	Colquitt	17	0	0	0	Not Done
	Laurens	20	1	1	0	Natural Exposure
Lower Coast	Chatham	25	1	1	0	Natural Exposure
	Ware	24	0	1	0	Vaccinate
	Cambden	20	0	0	0	Not Done
	Liberty	21	0	1	0	Negative for Exposure

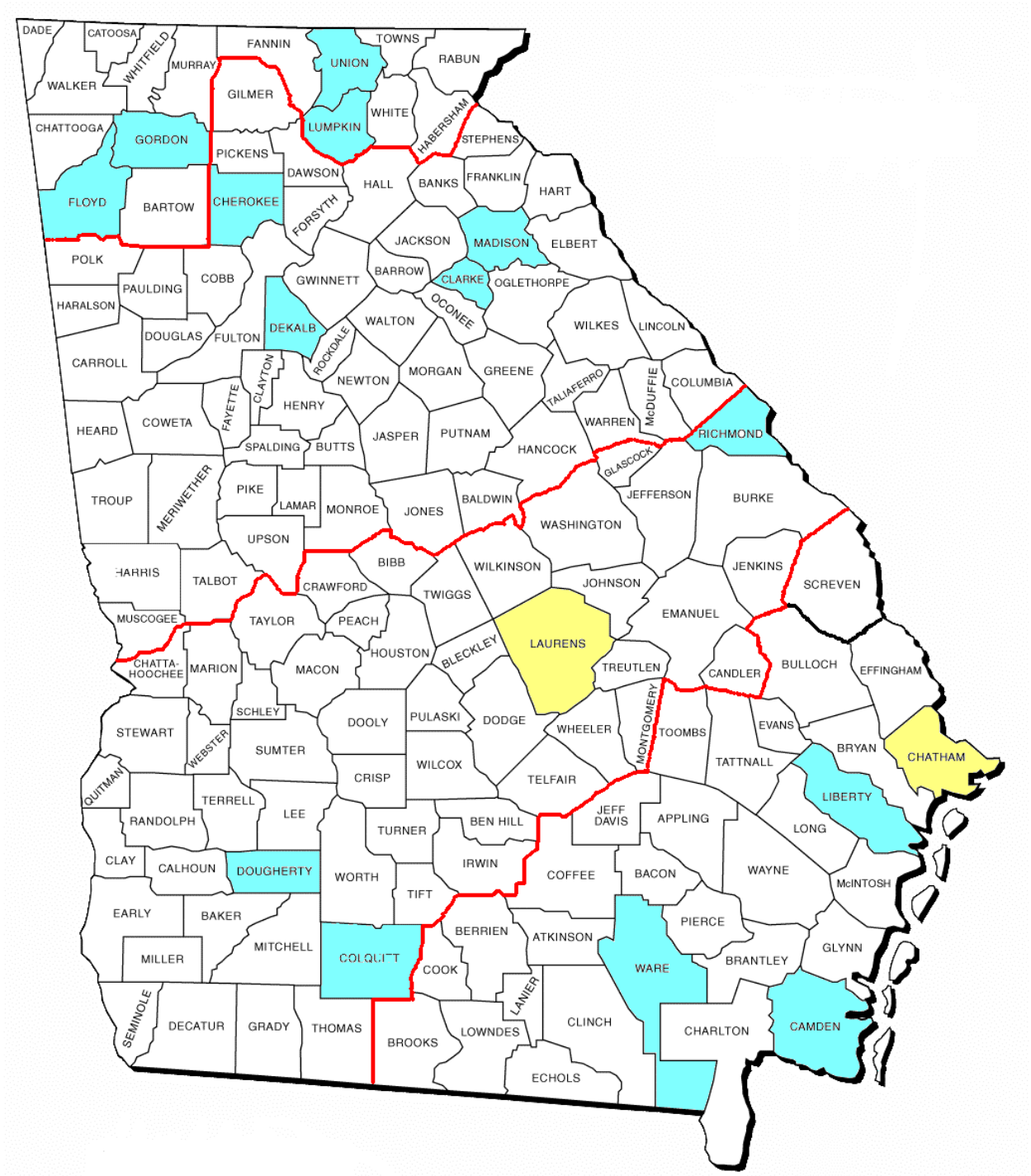

Figure 4.1: Results of serologic testing for antibodies against *Borrelia burgdorferi* in dogs in Georgia. The counties shaded in yellow correspond to the counties were dogs positive for antibodies against natural exposure to *B. burgdorferi* were found. The other counties tested are shaded in blue.

CHAPTER 5

DISCUSSION

Seroprevalence in Georgia

The prevalence of serum antibodies against *Borrelia burgdorferi* in young adult dogs from humane shelters throughout Georgia is low. Out of a total of 308 dog sampled, only 2 dog's sera were found to have positive results on the C6 peptide ELISA, whole cell ELISA, and confirmed to be naturally exposed with results from the Western blot. Two additional dogs were found to have positive test results on the whole cell ELISA for IgM antibody, but because the Western blot used was specific only for IgG, the determination as to whether these positive results represent true exposure, vaccination, or false positives is not known. These two samples could represent early exposure before IgG levels began to arise or could be false positives from vaccination or cross reactivity of antibodies (against other infectious agents) with the *Borrelia* proteins. With the tests used in this study, repeat convalescent titers in 2 to 4 weeks after the original samples were collected would have been ideal to fully evaluate the significance of the positive IgM titer results and to determine if a rise is IgG occurred that could be evaluated with the Western blot or detected with the C6 ELISA. Unfortunately, given the nature of the study and the animals included in the study, the dogs were no longer housed at the original shelter at the time of testing and further blood sampling was not possible. Given the small number of positive sample results obtained, no statistical analysis could be performed to determine any physiographic distribution of positive samples nor could comparison be made between the sensitivity and specificity of the C6 ELISA and the whole cell ELISA.

Proposed Reasons for Low Seroprevalence

The low seroprevalence found in the study population correlate with the low number of positive Lyme disease cases reported in Georgia by the Centers for Disease Control. There are a number of proposed explanations for the low seroprevalence in found in this study despite documentation that the Lyme spirochete and the tick that has the ability to transmit the organism are both simultaneously present in geographic regions within Georgia, and that higher seroprevalence rates occurred in a study of white-tailed deer in Georgia.[31-36] Because the whole-cell ELISA used in the white-tailed deer study was not confirmed with Western Blot, the true antibody response could not be confirmed to be specifically for *Borrelia burgdorferi*. Whole-cell tests notoriously cause a reactivity to a wide degree of antigens.[42-44] Therefore, the prevalence rates may be falsely elevated. Tick affinity for mammalian hosts may also affect the prevalence rate. A phenomenon seen in Georgia that is not seen in the hyper-endemic northeast and mid-western states is a high infestation of *Ixodes scapularis* in lizards. With skinks and lizards found to be important hosts of the immature stages of *I. scapularis* in Georgia it has been proposed, based on results from some studies that have shown some lizard species to be refractory to infection with the *B. burgdorferi* spirochete, that they serve to reduce the number of ticks that become infected with the spirochete.[56,57] It is also possible that our study population of shelter dogs may not represent the best sentinel animals for Lyme disease in Georgia. A recent study investigating ticks parasitizing domestic dogs in southeastern Georgia found that unlike family-owned dogs who were most frequently infested with *Ixodes scapularis* (54% of all ticks from this group), shelter dogs were most common infested with *Rhipicephalus sanguineus* (73.6%).[58] This study could suggest that the dogs included in this study were not highly exposed to the tick necessary to confer exposure to *B. burgdorferi*. Despite this reported higher infestation with

Rhipicephalus sanguineus in shelter dogs, there was a relatively low prevalence of antibodies against *Ehrlichia canis* in shelter dogs in this study, and the primary tick transmitting that organism is *Rhipicephalus sanguineus*, (see Table 5.1).

Table 5.1 Seroprevalence of antibodies against Ehrlichia canis in dogs in Georgia.

Physiographic Region	Number of Dogs Samples	*E. canis* ELISA positive
Mountain	74	0
Piedmont	63	3
Upper Coast	83	2
Lower Coast	90	1

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APPENDIX A

RESULTS

County	Dog #	IgG Titer	IgM Titer	Lyme	Heartworm	Snap	Phys Region	Western Blot IgG
Richmond	RIC1	0	256	Neg	Neg	Neg	Upper Coast	
Richmond	RIC2	0	256	Neg	Neg	Neg	Upper Coast	
Richmond	RIC3	0	256	Neg	Neg	Neg	Upper Coast	
Richmond	RIC4*	0	256	Neg	Neg	Neg	Upper Coast	
Richmond	RIC5	0	256	Neg	Pos	Neg	Upper Coast	
Richmond	RIC6	0	256	Neg	Neg	Neg	Upper Coast	
Richmond	RIC 7	0	128	Neg	Neg	Neg	Upper Coast	
Richmond	RIC8	0	512	Neg	Neg	Neg	Upper Coast	
Richmond	RIC9*	0	256	Neg	Neg	Neg	Upper Coast	
Richmond	RIC10*	0	256	Neg	Neg	Neg	Upper Coast	
Richmond	RIC11*	0	512	Neg	Neg	Neg	Upper Coast	
Richmond	RIC12*	0	128	Neg	Neg	Neg	Upper Coast	
Richmond	RIC13*	0	128	Neg	Neg	Neg	Upper Coast	
Richmond	RIC14*	0	256	Neg	Neg	Neg	Upper Coast	
Richmond	RIC15	0	256	Neg	Neg	Neg	Upper Coast	
Richmond	RIC16*	0	1024	Neg	Neg	Neg	Upper Coast	
Richmond	RIC17*	0	1024	Neg	Neg	Neg	Upper Coast	
Richmond	RIC18*	0	512	Neg	Neg	Neg	Upper Coast	
Richmond	RIC19	0	64	Neg	Neg	Neg	Upper Coast	
Richmond	RIC20*	0	512	Neg	Pos	Neg	Upper Coast	
Richmond	RIC21*	0	64	Neg	Pos	Neg	Upper Coast	
Dougherty	DOU1*	0	32	Neg	Neg	Neg	Upper Coast	
Dougherty	DOU2*	0	64	Neg	Neg	Neg	Upper Coast	
Dougherty	DOU3*	0	32	Neg	Neg	Neg	Upper Coast	
Dougherty	DOU4*	0	32	Neg	Neg	Neg	Upper Coast	
Dougherty	DOU5	0	64	Neg	Neg	Neg	Upper Coast	
Dougherty	DOU6*	0	32	Neg	Neg	Neg	Upper Coast	
Dougherty	DOU7	0	0	Neg	Neg	Neg	Upper Coast	
Dougherty	DOU8*	0	128	Neg	Neg	Neg	Upper Coast	
Dougherty	DOU9*	0	0	Neg	Neg	Neg	Upper Coast	
Dougherty	DOU10*	0	64	Neg	Neg	Neg	Upper Coast	
Dougherty	DOU11	0	64	Neg	Neg	Neg	Upper Coast	
Dougherty	DOU12*	0	32	Neg	Pos	Neg	Upper Coast	
Dougherty	DOU13*	0	32	Neg	Neg	Neg	Upper Coast	
Dougherty	DOU14*	0	32	Neg	Pos	Neg	Upper Coast	
Dougherty	DOU15*	0	0	Neg	Neg	Neg	Upper Coast	

Dougherty	DOU16	0	32	Neg	Neg	Neg	Upper Coast	
Dougherty	DOU17	0	32	Neg	Neg	Neg	Upper Coast	
Dougherty	DOU18	128	64	Neg	Neg	Neg	Upper Coast	
Dougherty	DOU19	0	512	Neg	Neg	Neg	Upper Coast	
Dougherty	DOU20*	0	128	Neg	Neg	Neg	Upper Coast	
Dougherty	DOU21*	0	32	Neg	Neg	Neg	Upper Coast	
Dougherty	DOU22*	0	256	Neg	Neg	Neg	Upper Coast	
Dougherty	DOU23	0	32	Neg	Neg	Neg	Upper Coast	
Dougherty	DOU24	64	128	Neg	Neg	Neg	Upper Coast	
Dougherty	DOU25*	0	32	Neg	Neg	Neg	Upper Coast	
Colquitt	COL1*	0	32	Neg	Neg	Neg	Upper Coast	
Colquitt	COL2*	0	128	Neg	Neg	Neg	Upper Coast	
Colquitt	COL3*	0	32	Neg	Lite Pos	Neg	Upper Coast	
Colquitt	COL4	0	32	Neg	Neg	Neg	Upper Coast	
Colquitt	COL5	0	0	Neg	Neg	Neg	Upper Coast	
Colquitt	COL6*	0	32	Neg	Pos	Neg	Upper Coast	
Colquitt	COL7*	0	256	Neg	Neg	Neg	Upper Coast	
Colquitt	COL8*	0	32	Neg	Neg	Neg	Upper Coast	
Colquitt	COL9*	0	64	Neg	Pos	Neg	Upper Coast	
Colquitt	COL10*	0	128	Neg	Neg	Neg	Upper Coast	
Colquitt	COL11*	0	32	Neg	Neg	Neg	Upper Coast	
Colquitt	COL12*	0	256	Neg	Pos	Neg	Upper Coast	
Colquitt	COL13	0	0	Neg	Neg	Neg	Upper Coast	
Colquitt	COL14*	0	0	Neg	Neg	Neg	Upper Coast	
Colquitt	COL15*	0	0	Neg	Neg	Neg	Upper Coast	
Colquitt	COL16*	0	64	Neg	Neg	Neg	Upper Coast	
Colquitt	COL17*	0	0	Neg	Neg	Neg	Upper Coast	
Laurens	LAU1*	0	128	Neg	Neg	Neg	Upper Coast	
Laurens	LAU2*	0	32	Neg	Neg	Neg	Upper Coast	
Laurens	LAU3*	0	256	Neg	Neg	Neg	Upper Coast	
Laurens	LAU4*	0	256	Neg	Lt Pos	Neg	Upper Coast	
Laurens	LAU5*	0	256	Neg	Pos	Neg	Upper Coast	
Laurens	LAU6*	0	256	Neg	Neg	Neg	Upper Coast	
Laurens	LAU7*	0	256	Neg	Neg	Neg	Upper Coast	
Laurens	LAU8*	0	256	Neg	Neg	Neg	Upper Coast	
Laurens	LAU9*	0	256	Neg	Neg	Neg	Upper Coast	
Laurens	LAU10*	0	256	Neg	Neg	Neg	Upper Coast	
Laurens	LAU11*	0	256	Neg	Neg	Neg	Upper Coast	
Laurens	LAU12*	0	64	Neg	Neg	Neg	Upper Coast	
Laurens	LAU13*	0	512	Neg	Pos	Neg	Upper Coast	
Laurens	LAU14*	0	512	Neg	Neg	Neg	Upper Coast	
Laurens	LAU15**	0	256	Neg	Pos	Neg	Upper Coast	
Laurens	LAU16*	0	64	Neg	Neg	Neg	Upper Coast	
Laurens	LAU17*	2048	128	Pos	Neg	Pos	Upper Coast	Natural Exposure
Laurens	LAU18*	0	256	Neg	Neg	Neg	Upper Coast	
Laurens	LAU19*	0	256	Neg	Neg	Pos	Upper Coast	
Laurens	LAU20*	0	256	Neg	Neg	Neg	Upper Coast	
Chatham	CHA1	0	32	Neg	Neg	Neg	Lower Coast	

Chatham	CHA2	0	64	Neg	Neg	Neg	Lower Coast	
Chatham	CHA3*	0	256	Neg	Neg	Neg	Lower Coast	
Chatham	CHA4	0	64	Neg	Neg	Neg	Lower Coast	
Chatham	CHA5*	0	512	Neg	Neg	Neg	Lower Coast	
Chatham	CHA6	0	128	Neg	Neg	Neg	Lower Coast	
Chatham	CHA7*	0	128	Neg	Neg	Neg	Lower Coast	
Chatham	CHA8	0	128	Neg	Neg	Neg	Lower Coast	
Chatham	CHA9*	0	128	Neg	Neg	Neg	Lower Coast	
Chatham	CHA10*	0	32	Neg	Neg	Neg	Lower Coast	
Chatham	CHA11*	0	64	Neg	Neg	Neg	Lower Coast	
Chatham	CHA12*	0	64	Neg	Neg	Neg	Lower Coast	
Chatham	CHA13*	0	64	Neg	Pos	Neg	Lower Coast	
Chatham	CHA14*	0	0	Neg	Neg	Neg	Lower Coast	
Chatham	CHA15*	1024	0	Pos	Pos	Neg	Lower Coast	Natural Exposure
Chatham	CHA16*	0	32	Neg	Neg	Neg	Lower Coast	
Chatham	CHA17*	0	32	Neg	Neg	Neg	Lower Coast	
Chatham	CHA18*	9	32	Neg	Neg	Neg	Lower Coast	
Chatham	CHA19	0	32	Neg	Neg	Neg	Lower Coast	
Chatham	CHA20	0	256	Neg	Neg	Neg	Lower Coast	
Chatham	CHA21*	128	256	Neg	Neg	Neg	Lower Coast	
Chatham	CHA22*	0	128	Neg	Pos	Neg	Lower Coast	
Chatham	CHA23*	0	256	Neg	Neg	Neg	Lower Coast	
Chatham	CHA24*	0	256	Neg	Neg	Neg	Lower Coast	
Chatham	CHA25	0	256	Neg	Neg	Neg	Lower Coastt	
Ware	WAR1	0	512	Neg	Neg	Neg	Lower Coast	
Ware	WAR2	0	256	Neg	Pos	Neg	Lower Coast	
Ware	WAR3*	0	512	Neg	Neg	Neg	Lower Coast	
Ware	WAR4*	256	0	Neg	Neg	Neg	Lower Coast	Vaccine
Ware	WAR5	0	512	Neg	Pos	Neg	Lower Coast	
Ware	WAR6	0	128	Neg	Neg	Neg	Lower Coast	
Ware	WAR7*	0	128	Neg	Pos	Neg	Lower Coast	
Ware	WAR8*	0	128	Neg	Neg	Neg	Lower Coast	
Ware	WAR9*	0	32	Neg	Lite Pos	Neg	Lower Coast	
Ware	WAR10*	0	128	Neg	Pos	Neg	Lower Coast	
Ware	WAR11*	0	64	Neg	Neg	Neg	Lower Coast	
Ware	WAR12*	0	256	Neg	Neg	Neg	Lower Coast	
Ware	WAR13*	0	256	Neg	Neg	Neg	Lower Coast	
Ware	WAR14	0	128	Neg	Neg	Neg	Lower Coast	
Ware	WAR15*	0	128	Neg	Neg	Neg	Lower Coast	
Ware	WAR16*	0	0	Neg	Lite Pos	Neg	Lower Coast	
Ware	WAR17*	0	128	Neg	Neg	Neg	Lower Coast	
Ware	WAR18*	0	0	Neg	Pos	Neg	Lower Coast	
Ware	WAR19*	0	32	Neg	Neg	Neg	Lower Coast	
Ware	WAR20	0	32	Neg	Neg	Neg	Lower Coast	
Ware	WAR21	0	0	Neg	Lite Pos	Neg	Lower Coast	
Ware	WAR22	0	0	Neg	Neg	Neg	Lower Coast	

Ware	WAR23*	0	128	Neg	Lite Pos	Neg	Lower Coast	
Ware	WAR24	0	32	Neg	Pos	Neg	Lower Coast	
Cambden	CAM1*	0	128	Neg	Neg	Neg	Lower Coast	
Cambden	CAM2*	32	32	Neg	Neg	Neg	Lower Coast	
Cambden	CAM3*	32	32	Neg	Neg	Neg	Lower Coast	
Cambden	CAM4	0	128	Neg	Neg	Neg	Lower Coast	
Cambden	CAM5	0	32	Neg	Neg	Neg	Lower Coast	
Cambden	CAM6*	128	128	Neg	Neg	Neg	Lower Coast	
Cambden	CAM7*	0	128	Neg	Neg	Neg	Lower Coast	
Cambden	CAM8*	0	32	Neg	Neg	Neg	Lower Coast	
Cambden	CAM9	0	128	Neg	Neg	Neg	Lower Coast	
Cambden	CAM10*	0	128	Neg	Neg	Neg	Lower Coast	
Cambden	CAM11*	0	32	Neg	Neg	Neg	Lower Coast	
Cambden	CAM12*	0	32	Neg	Neg	Neg	Lower Coast	
Cambden	CAM13*	0	32	Neg	Pos	Pos	Lower Coast	
Cambden	CAM14*	0	32	Neg	Neg	Neg	Lower Coast	
Cambden	CAM15*	0	64	Neg	Neg	Neg	Lower Coast	
Cambden	CAM16	0	32	Neg	Neg	Neg	Lower Coast	
Cambden	CAM17	0	32	Neg	Neg	Neg	Lower Coast	
Cambden	CAM18*	0	32	Neg	Neg	Neg	Lower Coast	
Cambden	CAM19	0	32	Neg	Neg	Neg	Lower Coast	
Cambden	CAM20	0	128	Neg	Neg	Neg	Lower Coast	
Liberty	LIB1*	0	32	Neg	Pos	Neg	Lower Coast	
Liberty	LIB2*	0	32	Neg	Neg	Neg	Lower Coast	
Liberty	LIB3*	0	0	Neg	Neg	Neg	Lower Coast	
Liberty	LIB4*	0	0	Neg	Neg	Neg	Lower Coast	
Liberty	LIB5*	0	0	Neg	Neg	Neg	Lower Coast	
Liberty	LIB6*	0	32	Neg	Neg	Neg	Lower Coast	
Liberty	LIB7*	0	32	Neg	Neg	Neg	Lower Coast	
Liberty	LIB8	0	32	Neg	Neg	Neg	Lower Coast	
Liberty	LIB9	0	32	Neg	Neg	Neg	Lower Coast	
Liberty	LIB10*	0	128	Neg	Neg	Neg	Lower Coast	
Liberty	LIB11*	0	32	Neg	Lite Pos	Neg	Lower Coast	
Liberty	LIB12*	0	0	Neg	Neg	Neg	Lower Coast	
Liberty	LIB13**	0	0	Neg	Neg	Neg	Lower Coast	
Liberty	LIB14	0	32	Neg	Neg	Neg	Lower Coast	
Liberty	LIB15*	256	64	Neg	Neg	Neg	Lower Coast	Neg for exposure
Liberty	LIB16*	0	32	Neg	Neg	Neg	Lower Coast	
Liberty	LIB17*	32	32	Neg	Neg	Neg	Lower Coast	
Liberty	LIB18*	0	512	Neg	Pos	Neg	Lower Coast	
Liberty	LIB19	0	0	Neg	Neg	Neg	Lower Coast	
Liberty	LIB20*	0	32	Neg	Pos	Neg	Lower Coast	
Liberty	LIB21*	0	32	Neg	Neg	Neg	Lower Coast	
Cherokee	CHE1	0	32	Neg	Neg	Neg	Piedmont	
Cherokee	CHE2	0	256	Neg	Neg	Neg	Piedmont	
Cherokee	CHE3	0	256	Neg	Neg	Neg	Piedmont	
Cherokee	CHE4	0	512	Neg	Neg	Neg	Piedmont	
Cherokee	CHE5	0	128	Neg	Neg	Neg	Piedmont	

Cherokee	CHE6	0	256	Neg	Neg	Neg	Piedmont
Cherokee	CHE7	0	256	Neg	Neg	Neg	Piedmont
Cherokee	CHE8	0	256	Neg	Neg	Neg	Piedmont
Cherokee	CHE9	0	512	Neg	Neg	Neg	Piedmont
Cherokee	CHE10	0	64	Neg	Neg	Neg	Piedmont
Cherokee	CHE12	64	256	Neg	Neg	Neg	Piedmont
Madison	MAD1	0	256	Neg	Lite Pos	Neg	Piedmont
Madison	MAD3	0	128	Neg	Pos	Lite Pos	Piedmont
Madison	MAD4	0	32	Neg	Neg	Neg	Piedmont
Madison	MAD5	0	256	Neg	Neg	Neg	Piedmont
Madison	MAD6	0	256	Neg	Neg	Neg	Piedmont
Madison	MAD7	0	256	Neg	Neg	Neg	Piedmont
Madison	MAD8	0	256	Neg	Neg	Neg	Piedmont
Madison	MAD10	0	256	Neg	Neg	Pos	Piedmont
Madison	MAD11	0	512	Neg	Neg	Neg	Piedmont
Madison	MAD12	0	128	Neg	Neg	Neg	Piedmont
Madison	MAD13	0	256	Neg	Neg	Neg	Piedmont
Madison	MAD14	0	64	Neg	Neg	Neg	Piedmont
Madison	MAD15	0	32	Neg	Neg	Neg	Piedmont
Madison	MAD16	0	32	Neg	Neg	Neg	Piedmont
Madison	MAD17	0	256	Neg	Neg	Neg	Piedmont
Madison	MAD18*	0	0	Neg	Neg	Neg	Piedmont
Madison	MAD19	0	256	Neg	Neg	Neg	Piedmont
Madison	MAD20	0	32	Neg	Neg	Neg	Piedmont
Madison	MAD21	0	128	Neg	Neg	Neg	Piedmont
Madison	MAD22**	0	256	Neg	Neg	Neg	Piedmont
Dekalb	DEK1	0	128	Neg	Lite Pos	Neg	Piedmont
Dekalb	DEK3	0	0	Neg	Neg	Neg	Piedmont
Dekalb	DEK4	0	32	Neg	Neg	Neg	Piedmont
Dekalb	DEK5	0	32	Neg	Neg	Neg	Piedmont
Dekalb	DEK6	0	32	Neg	Neg	Neg	Piedmont
Dekalb	DEK7	0	64	Neg	Neg	Neg	Piedmont
Dekalb	DEK8	0	32	Neg	Neg	Neg	Piedmont
Dekalb	DEK9	0	128	Neg	Neg	Neg	Piedmont
Dekalb	DEK10	0	0	Neg	Neg	Neg	Piedmont
Dekalb	DEK11	0	64	Neg	Neg	Neg	Piedmont
Dekalb	DEK12	0	64	Neg	Neg	Neg	Piedmont
Clarke	CLA1	0	256	Neg	Neg	Neg	Piedmont
Clarke	CLA2	0	32	Neg	Neg	Neg	Piedmont
Clarke	CLA4	0	32	Neg	Neg	Neg	Piedmont
Clarke	CLA5	0	256	Neg	Neg	Neg	Piedmont
Clarke	CLA6	0	128	Neg	Neg	Neg	Piedmont
Clarke	CLA7	0	32	Neg	Neg	Neg	Piedmont
Clarke	CLA8	0	0	Neg	Neg	Neg	Piedmont
Clarke	CLA9	0	32	Neg	Neg	Neg	Piedmont
Clarke	CLA10	0	0	Neg	Neg	Neg	Piedmont
Clarke	CLA11	0	64	Neg	Neg	Neg	Piedmont

Clarke	CLA12*	0	0	Neg	Pos	Neg	Piedmont
Clarke	CLA13*	0	64	Neg	Neg	Pos	Piedmont
Clarke	CLA14*	0	32	Neg	Pos	Neg	Piedmont
Clarke	CLA15	0	128	Neg	Neg	Neg	Piedmont
Clarke	CLA16*	0	32	Neg	Lite Pos	Neg	Piedmont
Clarke	CLA17*	0	0	Neg	Neg	Neg	Piedmont
Clarke	CLA18	0	0	Neg	Neg	Neg	Piedmont
Clarke	CLA19	0	32	Neg	Neg	Neg	Piedmont
Clarke	CLA20*	0	32	Neg	Neg	Neg	Piedmont
Clarke	CLA21*	0	0	Neg	Neg	Neg	Piedmont
Clarke	CLA22*	0	128	Neg	Very Lite Pos	Neg	Piedmont
Floyd	FLO1	0	512	Neg	Neg	Neg	Mountain
Floyd	FLO2	32	512	Neg	Neg	Neg	Mountain
Floyd	FLO3	0	32	Neg	Lite Pos	Neg	Mountain
Floyd	FLO4*	0	256	Neg	Neg	Neg	Mountain
Floyd	FLO5	0	256	Neg	Neg	Neg	Mountain
Floyd	FLO6	0	256	Neg	Neg	Neg	Mountain
Floyd	FLO7	0	256	Neg	Neg	Neg	Mountain
Floyd	FLO8	32	32	Neg	Neg	Neg	Mountain
Floyd	FLO9	0	32	Neg	Neg	Neg	Mountain
Floyd	FLO10	0	64	Neg	Neg	Neg	Mountain
Floyd	FLO11	0	32	Neg	Pos	Neg	Mountain
Floyd	FLO12	0	128	Neg	Neg	Neg	Mountain
Floyd	FLO13	0	512	Neg	Neg	Neg	Mountain
Floyd	FLO14	0	128	Neg	Neg	Neg	Mountain
Floyd	FLO15	0	256	Neg	Neg	Neg	Mountain
Floyd	FLO16	0	128	Neg	Lite Pos	Neg	Mountain
Lumpkin	LUM1	0	128	Neg	Neg	Neg	Mountain
Lumpkin	LUM2*	0	128	Neg	Neg	Neg	Mountain
Lumpkin	LUM3	0	0	Neg	Neg	Neg	Mountain
Lumpkin	LUM4	0	128	Neg	Neg	Neg	Mountain
Lumpkin	LUM5	0	32	Neg	Neg	Neg	Mountain
Lumpkin	LUM6	0	128	Neg	Neg	Neg	Mountain
Lumpkin	LUM7*	0	32	Neg	Neg	Neg	Mountain
Lumpkin	LUM8	0	64	Neg	Neg	Neg	Mountain
Lumpkin	LUM9	0	0	Neg	Neg	Neg	Mountain
Lumpkin	LUM10	128	512	Neg	Neg	Neg	Mountain
Lumpkin	LUM11	0	128	Neg	Neg	Neg	Mountain
Lumpkin	LUM12	0	512	Neg	Neg	Neg	Mountain
Lumpkin	LUM13	0	256	Neg	Neg	Neg	Mountain
Lumpkin	LUM14	0	32	Neg	Neg	Neg	Mountain
Lumpkin	LUM15	0	128	Neg	Neg	Neg	Mountain
Lumpkin	LUM16	0	64	Neg	Neg	Neg	Mountain
Lumpkin	LUM17	0	64	Neg	Neg	Neg	Mountain
Lumpkin	LUM18	0	32	Neg	Neg	Neg	Mountain
Lumpkin	LUM19	0	64	Neg	Pos	Neg	Mountain
Lumpkin	LUM20	0	64	Neg	Neg	Neg	Mountain

Lumpkin	LUM21	256	32	Neg	Neg	Neg	Mountain	Vaccine
Lumpkin	LUM22	0	64	Neg	Neg	Neg	Mountain	
Lumpkin	LUM23	0	64	Neg	Neg	Neg	Mountain	
Town and Union	TOW1	32	128	Neg	Neg	Neg	Mountain	
Town and Union	TOW2	0	0	Neg	Neg	Neg	Mountain	
Town and Union	TOW3	0	32	Neg	Neg	Neg	Mountain	
Town and Union	TOW4	0	128	Neg	Neg	Neg	Mountain	
Town and Union	TOW6	0	128	Neg	Neg	Neg	Mountain	
Town and Union	TOW7	0	256	Neg	Neg	Neg	Mountain	
Town and Union	TOW8	0	32	Neg	Neg	Neg	Mountain	
Town and Union	TOW9	0	32	Neg	Neg	Neg	Mountain	
Town and Union	TOW10	0	64	Neg	Neg	Neg	Mountain	
Town and Union	TOW11	0	64	Neg	Pos	Neg	Mountain	
Town and Union	TOW12	0	0	Neg	Neg	Neg	Mountain	
Town and Union	TOW13	0	0	Neg	Neg	Neg	Mountain	
Town and Union	TOW14	0	32	Neg	Neg	Neg	Mountain	
Town and Union	TOW15	0	64	Neg	Neg	Neg	Mountain	
Town and Union	TOW16	0	64	Neg	Neg	Neg	Mountain	
Gordon	GOR1	0	32	Neg	Neg	Neg	Mountain	
Gordon	GOR2	0	128	Neg	Neg	Neg	Mountain	
Gordon	GOR3	0	128	Neg	Neg	Neg	Mountain	
Gordon	GOR5	0	256	Neg	Neg	Neg	Mountain	
Gordon	GOR6	0	256	Neg	Neg	Neg	Mountain	
Gordon	GOR7	0	512	Neg	Neg	Neg	Mountain	
Gordon	GOR8	0	128	Neg	Neg	Neg	Mountain	
Gordon	GOR9	0	256	Neg	Pos	Neg	Mountain	
Gordon	GOR10	0	256	Neg	Neg	Neg	Mountain	
Gordon	GOR11	0	128	Neg	Neg	Neg	Mountain	
Gordon	GOR12	0	32	Neg	Neg	Neg	Mountain	
Gordon	GOR13	0	32	Neg	Neg	Neg	Mountain	
Gordon	GOR14	0	0	Neg	Pos	Neg	Mountain	
Gordon	GOR15*	0	64	Neg	Neg	Neg	Mountain	
Gordon	GOR16	0	32	Neg	Neg	Neg	Mountain	
Gordon	GOR17	0	0	Neg	Neg	Neg	Mountain	
Gordon	GOR18	0	256	Neg	Neg	Neg	Mountain	
Gordon	GOR19*	0	128	Neg	Neg	Neg	Mountain	

Gordon	GOR20* 0	256	Neg	Neg	Neg	Mountain
Gordon	GOR21* 0	32	Neg	Neg	Neg	Mountain