Tick Infestations and Their Consequences for Migratory Songbirds During Spring Stopover

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TICK INFESTATIONS AND THEIR CONSEQUENCES FOR MIGRATORY SONGBIRDS DURING SPRING STOPOVER

by

Johnny Michael Sellers, Jr.

A Thesis
Submitted to the Graduate School
of The University of Southern Mississippi
in Partial Fulfillment of the Requirements
for the Degree of Master of Science

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ABSTRACT

TICK INFESTATIONS AND THEIR CONSEQUENCES FOR MIGRATORY SONGBIRDS DURING SPRING STOPOVER

by Johnny Michael Sellers, Jr.

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Migratory birds face a number of challenges during their seasonal movement from tropical/sub-tropical Central and South America to more temperate North America. Maintaining health during migration is of particular concern. This study seeks to understand how haematophagous ectoparasites, such as ticks (Ixodida), impact host body condition as they feed on passerines during migration. We hypothesized that foraging location would impact tick acquisition by migrants and that tick burdens during migration would negatively impact body condition. We surveyed 2,064 birds during spring 2009 and 2010 and found that 2.4% of the surveyed birds were infested with one or more ticks (23 avian species). Ticks are more abundant in low vegetation and on the ground, but species-specific foraging niche did not predict the likelihood of obtaining ticks among migratory birds. Furthermore, birds without ticks were no more likely to be in better body condition than birds with ticks, though body condition tended to decrease with tick burden. Additionally, avian blood and feeding ticks were collected and analyzed via PCR for the presence of *Ehrlichia chaffeensis*, causative agent of the tick-borne disease ehrlichiosis, to determine if ticks and their associated pathogens are capable of being transported to North America by way of migrating birds. We found that 27 (10.2%) of 252 blood samples were positive for *E. chaffeensis* and 109 (97.3%) of the 112 collected ticks were not native to North America.
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CHAPTER I

PREVALENCE OF TICK INFECTIONS AND EFFECTS ON BODY CONDITION IN MIGRATORY BIRDS DURING STOPOVER

Introduction

Literally millions of songbirds engage in long distance, intercontinental movements between temperate breeding areas in North America and more tropical wintering areas in the Caribbean, Mexico, Central, and South America (Rappole et al. 1995). Traveling long distances across areas that vary physiographically is energetically expensive and comes with considerable risks. Indeed, the mortality associated with intercontinental migration may be substantial (e.g., Ketterson and Nolan 1982, 1983, Sillet and Holmes 2002). Arguably the most important constraint during migration is to acquire enough food to meet nutritional demands, and migratory birds are known to become hyperphagic, depositing large energy stores in anticipation of the energetic demands of migration (Blem 1990).

When fat stores are depleted, lean migratory birds are known to expand their foraging repertoire, broaden their use of substrate, and forage at a faster pace than do birds with adequate fat stores remaining following arrival (Loria and Moore 1990, Wang and Moore 2005). As a consequence, by enhanced foraging they achieve a favorable energy balance more quickly than they otherwise would have, decreasing length of stopover and reducing time spent on migration (Wang and Moore 2005). That said, increased foraging activity associated with deposition of fat stores is likely to increase exposure to ectoparasitic ticks seeking a vertebrate host. A tick infestation may have
detrimental consequences for a bird engaged in an energetically demanding, long distance
migration.

Moreover, the spring migration of songbirds coincides with the primary questing period for several tick species (Battaly et al. 1987). Ticks lay their eggs in masses of up to 20,000 on the ground (Sonenshine 1991). Almost immediately upon hatching, the larvae begin seeking a host. During this process, known as questing, the ticks climb on nearby vegetation, lift the forward two appendages and wait to grasp potential vertebrate hosts that pass by the vegetation (Cheng 1967, Oliver 1989). Having just hatched from a cluster of eggs, larval ticks tend to be clumped in distribution, and all engage in questing at the same time. Hence, it is likely that a foraging bird will encounter several immature, questing ticks in the same area (Cheng 1967), and it is not uncommon to find 10 or more ticks on some migratory songbirds (James et al. 2011). Birds such as the Common Yellowthroat (*Geothlypis trichas*), Gray Catbird (*Dumetella carolinensis*) and Northern Waterthrush (*Seiurus noveboracensis*) commonly forage on or near the ground and have been documented carrying a significantly higher tick burden than species that forage further up in the vegetation (Kinsey et al. 2000).

Ticks grasp the hair, scales, or feathers of their vertebrate host and move to a location where feeding can begin, often where the host will be less likely to remove them (Stafford 1995). In the case of birds, ticks are often found attached in the head region, presumably because the host cannot reach the tick with its beak (Gregoire et al. 2002). To begin a blood meal, the tick embeds its mouthparts in the skin and secretes a cement-like substance to hold it in place (Oliver 1989, Kinsey 2000). Ticks can remain attached to a host from a few hours to several days.
A tick infestation may have several negative consequences for a migrating bird:

1. A loss of blood to one or more ticks will mean a loss of some nutrients moving through the blood stream (Riek 1957, Alerstam 1990, Gauthier-Clerc et al. 1998). A single tick can consume up to 600 times its own unfed body weight (~ 10mg) in blood per meal (Sonenshine 1991). Birds that have multiple ticks may lose a great deal of blood (Wanless et al. 1997).

2. A heavy infestation may lead to anemia (Kirkwood 1967), and long-term anemia can induce mass loss (Jellison and Kohls 1938, Brown et al. 1995). Severe anemia may hinder the gas exchange process, which would negatively impact flight performance (Riek 1957, Gauthier-Clerc et al. 1998). Impaired flight performance would be especially problematic for long distance migratory birds flying over a large ecological barrier such as the Gulf of Mexico.

3. Female ticks of some species release a neurotoxin while feeding that partially or completely disrupts the nervous system of the host (Rosenstein 1975, Murnaghan 2009). Impaired muscle movement would negatively impact flight performance and foraging behavior.

4. The attachment of a tick causes an immune response (i.e. inflammation) around the parasite’s feeding area which could lead to loss of feathers, disrupting flight capabilities and mate selection (Nelson and Murray 1971, Clayton 1990). Additionally, tick bite sites can leave open wounds, which become susceptible to secondary infections.

5. Ticks carry pathogenic bacteria, including *Borrelia*, *Anaplasma* and *Ehrlichia* (Reed et al. 2002), which may be transmitted to the host during a blood meal via
saliva (Gauthier-Clerc et al. 1998) and cause reduced functionality during migration, not to mention direct mortality (Yoriks and Atkinson 2000, Ricklefs et al. 2005).

This study addressed two questions about the relationship between ticks and migratory songbirds:

1. Do foraging habits of migratory birds contribute to the likelihood of a tick infestation? Because ticks quest for hosts near the ground, I hypothesized that species characterized by foraging niches near or on the ground would be more likely to have ticks and will have more ticks than canopy foraging species. Assignment of individuals to a foraging guild is not straightforward because the foraging behavior of migratory birds may vary with season (Martin and Karr 1990) and with energetic condition (see Loria and Moore 1990; Wang and Moore 2005). Assignment of individuals to a foraging guild was based on our understanding of foraging behavior during migration (see Barrow et al 2000) for two reasons: (a) Birds were sampled for ticks during passage and (b) ticks usually drop off a host within 48 hours, so any ticks observed on a migrating bird were likely acquired while en route.

2. Does tick parasitism negatively impact the condition of migratory bird during stopover? I predicted that parasitized birds would have reduced body mass and reduced fat stores relative to non-parasitized birds when they stopover along the northern coast of the Gulf of Mexico during spring passage. Migrating birds may be especially vulnerable to the negative consequences of tick parasitism at this
time because of the stress imposed by a long-distance, non-stop flight across the Gulf of Mexico.

Methods

Study Site

This study was conducted during the spring 2009 and 2010 migration seasons (March 15 – May 15) at a long term migratory bird banding station near Johnson’s Bayou, Louisiana (29° 45’N, 93° 37’W). Johnson’s Bayou is coastal woodland (chenier) along the northern coast of the Gulf of Mexico, and migratory birds often stopover in chenier habitat to rest and refuel following movement across the Gulf of Mexico. The dominant tree species are live oak (Quercus virginiana Mill), hackberry (genus Celtis), toothache-tree (Aralia spinosa), red mulberry (Morus rubra), and honey locust (Gleditsia triacanthos). Other shrubs include yaupon (Ilex vomitoria), sweet acacia (Acacia farnesiana), palmetto (genus Sabal), honeysuckle (genus Lonicera), poison ivy (Toxicodendron radicans), and greenbrier vines (genus Smilax) (Moore in Able 1999).

Capture of birds occurred under USGS Federal Bird Banding Permit 21221. IACUC approval was under protocol number 09012601. Birds were captured using nylon mist-nets. Captured birds were carefully removed from the nets and taken to the banding station where a USFWS band was applied. Age and sex, when possible, were determined according to Pyle (1997), subcutaneous fat assessed according to Helms and Drury (1960), muscle mass present on keel assessed using a four point scale (Bairlein et al. 1995), and morphometric measurements taken (wing chord, tail length, tarsus length).
**Focal Species**

Although an effort was made to sample all species of migratory birds for ticks, sampling concentrated on nine focal species in order to obtain adequate blood samples from birds with and without ticks. Sampling was based on “foraging guilds” used to describe standard feeding locations (Barrow et al. 2000). The Sub-canopy/Canopy Group consisted of White-eyed Vireos (*Vireo griseus*) (WEVI), Red-eyed Vireos (*Vireo olivaceus*) (REVI) and Scarlet/Summer Tanagers (*Piranga olivacea* and *P. rubra*) (SCTA/SUTA). The Understory Group included the Hooded Warblers (*Wilsonia citrina*) (HOWA), Gray Catbirds (*Dumetella carolinensis*) (GRCA) and Yellow-breasted Chats (*Icteria virens*) (YBCH). The Ground Group consisted of Wood Thrush (*Hylocichla mustelina*) (WOTH), Northern Waterthrush (*Seiurus noveboracensis*) (NOWA) and Swainson’s Thrush (*Catharus ustulatus*) (SWTH).

**Sampling Birds for Ticks and Tick-borne Pathogens**

Birds were sampled for ticks by systematically blowing apart the feathers of the head, neck, breast, and lower ventral surfaces as well as searching the nape, bill, eyes, wings and cloaca (Gregoire et al. 2002). The feathers were pushed apart using forceps and parted with a fine tooth comb when warranted (Kinsey et al. 2000). When a tick was discovered, it was carefully removed using fine-tipped forceps. Ticks were placed in a glass vial containing 70% ethanol. The vial was labeled with a number corresponding to the bird’s band number. The researcher noted the time, date, species, presence/absence of ticks, where the tick(s) were located on the body, whether blood was taken for disease assay (see Chapter II), the bird’s band number, and any additional comments concerning the birds, ticks, or bleeding process. Blood samples were taken from the focal species
regardless of the presence or absence of ticks in order to determine tick-borne disease vectoring capacity (see Chapter II). Ticks collected from the migratory birds were sent to Lorenza Beati-Ziegler at Georgia Southern University in 2009 and to Rich Robin at the Smithsonian Institute in 2010 for expert identification.

Additionally, in 2010, weekly inspections of the study site were performed to determine if ticks were questing in the vegetation. This was accomplished by dragging a 1x1 m flannel cloth across the ground in six locations (see Spielman et al. 1979). The exact sampling locations were determined by using a random numbers table so that the location of sampling sites was unbiased.

**Migratory Bird Condition Index**

Statistical analyses were conducted in JMP 9 (2010 SAS Institute Inc.). Condition indices (CI) were created to compare the energetic condition of individuals within and across species by correcting for variations in size (see Ellegren 1992, Wang and Moore 1997). Individuals within species were grouped by wing chord (increments of 1 mm). A regression of the body mass on the fat score resulted in a b-intercept for each species (i.e. fat score = 0). The b-intercept estimates the lean body mass for a specific wing chord. A regression of the lean body mass was calculated by that equation on the matching wing chords. This second equation was used to calculate size specific fat free mass (SSFFM) for each individual bird. In order to calculate the CI, SSFFM was subtracted from the recorded body mass providing an index corresponding to each bird’s accumulated energy stores.
Results

Over a two year period, 2,064 birds were surveyed out of 6,039 that were banded (34%). Blood samples were taken from 252 birds (12%) to assay for tick-borne pathogens. The researcher observed 293 ticks attached to 49 (2.4%) surveyed birds, and 112 of the ticks were collected for further analysis. Observed ticks were either larval (76%) or nymphal life history stages (24%). No adult ticks were found attached to the migratory birds sampled. Tick attachment sites were near the eyes (24%), the bill (18%), on the nape (16%), crown (12%), cloaca (10%), throat (8%), and the mantle (6%) of the birds, with the remaining 10% distributed throughout the body (Fig 1.1a, b). No ticks were found in the vegetation at the study site.

Figure 1. Pie chart showing instances of tick infestation in relation to attachment site on birds captured during spring migration in Johnson Bayou, LA, 2009 – 2010.
Parasitized birds were distributed across 23 species and three different foraging guilds (Appendix A). Most ticks were attached to individuals in the ground foraging guild (184 observed ticks among 22 individuals), followed by the canopy (34 ticks among
17 individuals) and the understory (54 ticks among 10 individuals) (Fig. 3). Of the birds with ticks, the ground guild had the lowest mean tick burden (1.2) while the canopy guild had the highest (2.0). The understory birds had a mean of 1.7 ticks/bird. The likelihood of being parasitized, however, was not related to foraging location (Fig. 4) according to a One-way ANOVA of number of ticks by foraging guild ($F_{2,43} = 1.80, p = 0.18$).

Figure 4. ANOVA results of passerine birds by foraging guild. Compares condition index between categories with ticks and without during spring migration in Johnson Bayou, LA, 2009 – 2010. Warblers (BAWW, HOWA, KEWA, TEWA, OVEN); Thrushes (GCBT, SWTH, WOTH); Indigo Buntings (INBU). Connected bars indicate means and standard error. Outer bars, +/- one standard deviation.

Phylogenetically similar species were combined (Catharus thrushes: n = 12 and Parulidae warblers: n = 12) to attain a larger sample of migrants with ticks to analyze in relation to body/energetic condition. Birds with ticks and birds without were treated as separate categories. According to a One-way ANOVA of CI by category, no difference was detected in CI between warblers with and without ticks ($F_{1,25} = 0.021, p = 0.89$) nor thrushes ($F_{1,31} = 1.82, p = 0.19$) (Fig. 5).
Figure 5. ANOVA results comparing CI of birds without and with ticks in Johnson Bayou, LA, 2009 – 2010. Compares condition index between categories with ticks and without during spring migration in Johnson Bayou, LA, 2009 – 2010. Warblers (BAWW, HOWA, KEWA, TEWA, OVEN); Thrushes (GCBT, SWTH, WOTH); Indigo Buntings (INBU). Connected bars indicate means and standard error. Outer bars, +/- one standard deviation.
Figure 6. Condition index as a function of number of ticks on *Parulidae* Warblers (BAWW, HOWA, KEWA, TEWA, OVEN) at Johnson Bayou, LA, 2009-2010.

Figure 7. Condition index as a function of number of ticks on *Catharus* Thrushes (GCBT, SWTH, WOTH) at Johnson Bayou, LA, 2009-2010.

Figure 8. Condition index as a function of number of ticks on all infested burds at Johnson Bayou, LA, 2009 – 2010.
A linear regression performed on the number of ticks and CI of their avian host revealed a relationship between body condition and tick burden on warblers \((F_{1,9} = 6.44, p = 0.032)\) (Fig. 6), but not on thrushes \((F_{1,8} = 2.36, p = 0.16)\) (Fig. 7). On the other hand, there was a difference between Indigo Buntings \((Passerina cyanea)\) with ticks and without ticks (Fig. 5) according to a One-way ANOVA of CI by category \((F_{1,14} = 9.73, p = 0.0075, n = 6)\). A one-tailed t-test revealed that INBU with ticks had a higher condition index value than birds without ticks \((\alpha = 0.05, t = -4.5631, DF = 9, p = 0.9993)\).

Additionally, a linear regression on the number of ticks and CI of all birds parasitized by ticks revealed a negative relationship between tick burden and body condition \((F_{1,31} = 11.13, p = 0.0022)\) (Fig. 8).

**Discussion**

Intercontinental migratory birds arrived along the northern coast of the Gulf of Mexico carrying immature ticks during spring migration. The rate of tick infestation (3%) was similar to other efforts to sample tick parasitism in songbird migrants (Kinsey et al. 2000) and varied among species. The rate of infestation that I observed probably underestimates the level of parasitism in the population of migratory birds stopping over following trans-gulf migration because birds observed without ticks may have had a tick burden at the time of departure only to have the engorged tick(s) fall off prior to examination. As all of the ticks were partially engorged, and the bird hosts were usually examined shortly after their 18–24 hour, non-stop flight crossing the Gulf of Mexico, I concluded that the ticks had been acquired while foraging in tropical locations shortly before departure on a trans-gulf flight. Moreover, no ticks were found associated with the vegetation at the Johnson Bayou study site.
**Foraging Habits and Ticks**

Ticks were found on 23 different passerine species, 12 of which were assigned to the Canopy/Sub-canopy group, four to the Understory Group, and seven to the Ground Group. Because ticks quest for hosts near the ground, individuals that spend much of their time near or on the ground are more likely to be parasitized (e.g., Comstedt 2006, James et al. 2011). Although the heaviest tick burdens observed during my two year study were among members of the ground foraging guild, ground foraging birds were no more likely to be parasitized than birds in the canopy or subcanopy foraging guilds. Keeping in mind that the Comstedt (2006) and the James (2011) studies were performed outside of migration during the breeding season, the lack of a clear difference in parasitism among foraging guilds may reflect increased variation in foraging location among birds about to cross the Gulf of Mexico. When migratory birds are (re)depositing fat stores necessary to fuel long distance flights, they increased their rate of feeding (hyperhagia), increased time spent foraging, and often broaden both their foraging repertoire and foraging substrate (Loria and Moore 1990, Wang and Moore 1993, 2005), which would necessarily increase exposure to questing ticks.

That said, ground foraging birds may exhibit behaviors selected to avoid questing ticks. As ticks often lay eggs in leaf litter (Sonenshine 1991), populations of birds that characteristically forage on the ground and within the leaf litter may have experienced selective pressure to avoid potential questing areas during the height of questing. This type of behavior has been noted in oystercatchers (*Haematopus palliatus*) who avoided feeding on large cockles as they had more parasitic helminths than smaller cockles (Norris 1999). Typical canopy foraging birds that opportunistically change their behavior
to forage on the ground during migration may lack tick avoidance mechanisms.

Broadening the foraging niche when depositing fat stores necessary for migration may explain some of the observed variation in tick burden among foraging guilds during stopover. If this line of reasoning has merit, one would expect lean birds that are more likely to increase their rate of foraging and to broaden their foraging niche (e.g., Loria and Moore 1990) to be more susceptible to tick parasitism than fat birds.

*Impact on Body Condition*

Intercontinental migratory birds are exposed to a diverse array of pathogens by virtue of their travels (Ricklefs et al. 2005), and migration is no time to be fighting an infection or coping with the negative consequences of haematophageous ectoparasites. For example, the prevalence and intensity of infection of blood parasites varies widely within and among species, and the parasite infections pose a physiological cost for migratory birds (Yorinks and Atkinson 2000, Garvin et al. 2006). Moreover, when migratory birds arrive at a stopover site in poor condition, they are more likely to be immunocompromised and to experience increased susceptibility to disease or parasite infection (Owen and Moore 2008a, 2008b). Nevertheless, I found that a burden of one to three ticks did not appear to negatively impact body condition of migratory birds that stopover along the northern coast of the Gulf of Mexico following trans-gulf flight. Likewise, Heylan and Metthysen (2008) found tick infestation did not affect the body condition of captive Great Tits (*Parus major*) during the breeding season though tick infested individuals did show a reduced hematocrit and increased sedimentation rate. I did observe that the few birds with hyperinfestations – two Wood Thrushes (*Hylocichla mustelina*) with over 100 and 60 ticks, respectively, and a Worm-eating Warbler
(Helmitheros vermivorum) with 39 ticks – were underweight and had much lower than average condition indices.

Several possible explanations come to mind when considering the lack of a difference in condition between parasitized and non-parasitized individuals. Aside from the unlikely possibility that an ectoparasitic infestation has no negative effects, migratory birds that have prepared themselves to cross an ecological barrier like the Gulf of Mexico may be more resistant to negative impacts on body condition. Migratory birds deposit very large fat stores and increase muscle mass in anticipation of the physiological demands of long-distance flights, which may obscure an effect, especially if subtle. Second, migratory birds are known to increase their flight speed when crossing ecological barriers (see Yohannes et al. 2009), which may allow migratory birds to finish crossing the Gulf of Mexico before problems associated with a tick infestation manifest themselves. Alternatively, the cost of a tick infestation may result in increased likelihood of direct mortality during migration, so many of the birds that arrived on the northern coast of the Gulf of Mexico with moderate tick infestations were survivors able to offset negative effects (cf. Latta 2003). Clearly, the stress of migration as a selective agent of disease resistance is poorly understood and warrants more attention (see Sheldon and Verhulst 1996, Raberg et al. 1998, Owen et al. 2010).
CHAPTER II
PRESENCE OF THE PATHOGEN EHRLICHIA CHAFFEENSIS IN
HEMATOPHAGEOUS TICKS AND MIGRATORY BIRDS

Introduction

Millions of songbirds engage in long distance, intercontinental movements between temperate breeding areas in North America and subtropical-tropical wintering areas in the Caribbean, Mexico, Central, and South America (Rappole et al. 1995), and each migrant carries parasites and pathogens, many of them potentially harmful to other species, including humans (see Ricklefs 2005). For example, migrating birds have been implicated in the rapid spread of West Nile virus across North America (Rappole et al. 2000; Rappole and Hubalek 2003), which infects a variety of birds and mammals. Recently, Owen et al. (2006) experimentally demonstrated that Swainson’s Thrush (Catharus ustulatus) and Gray Catbird (Dumetella carolinensis), both Nearctic-Neotropical migratory songbirds, are potential dispersal vehicles for WNV. Migrants have also been observed to carry ixodid ticks, many of which are infected with Ehrlichia pathogens and the spirochete bacterium Borelia, which is the causal agent of Lyme disease (see Smith et al. 1996, Alekseev et al. 2001). This research focused on the host–parasite relationship between migratory birds and ticks and the role of host migration in the movement of parasite organisms and associated pathogens.

The spring migration of songbirds coincides with the primary questing period for several tick species (Battaly et al. 1987). Ticks lay their eggs in masses of up to 20,000 on the ground (Sonenshine 1991). Almost immediately upon hatching, the larvae begin seeking a host. During this process, known as questing, the ticks climb on nearby
vegetation, lift two sets of appendages and wait to grasp passing, potential vertebrate hosts (Cheng 1967, Oliver 1989). Having just hatched from a cluster of eggs, larval ticks tend to be clumped in distribution, and all engage in questing at the same time. Hence, it is not unlikely that a bird host will encounter several immature, questing ticks in the same area (Cheng 1967), and it is not uncommon to find 10 or more ticks on some migratory songbirds (James et al. 2011; see Chapter I). Once attached, migrating songbirds provide a means for tick dispersal that would normally not be possible.

Ticks are known vectors for a variety of diseases. *Amblyomma* ticks are commonly found on Cattle Egrets (*Bubulcus ibis*) and harbor the rickettsia *Cowdria ruminatum*, causative agent of Heartwater disease. These ticks will drop from their avian hosts after migration and seek out mammals. Once attached to nearby cattle, the ticks can transmit the pathogen. Heartwater has been known to cause high mortality and heavy economic losses in domesticated animals in both the Caribbean and Africa (Burridge et al. 2002). Should this disease be moved via ticks attached to Cattle Egrets into the United States, it could cause problems within the beef, pork, and poultry industries.

Lyme disease is caused by the bacterium *Borrelia burgdorferi* and transmitted by several species of tick. Birds are known to aid in the dispersal of Lyme carrying ticks (Anderson et al. 1986, Weisbrod and Johnson 1989, Poupon et al. 2005, Jordan et al. 2009), sometimes up to thousands of kilometers (Morshed et al. 2005). After the blood meal is complete, ticks will either reproduce or molt and seek a new host, potentially spreading the infection to local wildlife, domestic animals, and even humans.

Ticks that feed on individuals infected with West Nile virus can act as a suitable reservoir for this pathogen under experimental conditions (Abbassy et al. 1993). If the
virus adapts to be a more effective agent of infection and if more ticks become exposed to the pathogen, the vectoring capacity for this pathogen increases substantially (Abbassy et al. 1993).

*Ehrlichia chaffeensis*, the causative agent of human monocytic ehrlichiosis, is commonly transmitted by *Amblyomma* ticks (Demma et al. 2005). Approximately 600 cases of ehrlichiosis were reported to the Centers for Disease Control in 2006. Symptoms in humans include fever, rash, headache, malaise and muscle ache (Demma et al. 2005). Symptoms are unknown in birds, but the pathogen can be identified by PCR from collected blood samples and ticks. Since ticks are known carriers of this pathogen, identification of the presence or absence of *E. chaffeensis* will allow for the determination of tick parasitizing even if no ticks are discovered on the host at the time.

The transmission of diseases by ticks is achieved during feeding. Once a tick begins feeding, it injects saliva and a cement-like compound to adhere the mouthparts into the host. These secretions carry the disease causing agent. Transmission of viral or bacterial pathogens is not always certain. The chance of infection increases with the length of time the tick feds and/or with the number of feeding ticks (Bowman and Sauer 2004).

This phase of the research focused on the relationship between migratory birds and ticks and asked two related questions: (1) Do ticks infesting migratory songbirds that arrive on the northern coast of the Gulf of Mexico following a trans-gulf flight serve as vectors for disease causing agents to humans and animals, and (2) do intercontinental songbird migrants carry typically tick-borne pathogens from non-breeding areas in the Caribbean Basin, Central, and South America to North America? The answer to both
questions represents an important step in understanding the role of migratory birds in the spread infectious diseases.

Methods

Bird, Tick, and Blood Collection

Migratory birds were visually and systematically inspected for the presence of ticks. This inspection was accomplished by blowing apart the feathers of the head, neck, breast, and lower ventral surfaces as well as the nape, bill, eyes, wings and cloaca as ticks prefer these hard to preen areas (Gregoire et al. 2002). The feathers were also pushed apart using forceps and parted with a fine tooth comb when warranted (Kinsey et al. 2000). When a tick was discovered, it was carefully removed using fine-tipped forceps. Ticks were placed in a glass vial containing a 70% ethanol solution. The vial was labeled with a number corresponding to the bird’s band number. The researcher noted the time, date, species, presence/absence of ticks, where the tick(s) were located on the body, whether blood was taken, which vials were used to store the specimens, the bird’s band number, and any additional comments concerning the birds, ticks, or bleeding process.

The researcher surveyed as many species of migratory birds as possible in order to obtain the best understanding of tick distribution and pathogen prevalence. Birds with ticks had blood collected for analysis. A sterile 26 or 27 gauge Precision Glide Needle was used to puncture the left alar vein. A heparanized micro-hematocrit capillary tube was used to collect the blood. Once the tube was filled, the blood was blown into a storage vial containing 100 µL of a lysis buffer. These vials were stored in a cool, dry container and later in a freezer until they could be taken to the lab for processing. The bird’s wound was then covered with cotton until bleeding stopped. The researcher
observed each bird briefly to ensure the collection had no ill effect and then released the bird.

Detection of Tick-Borne Pathogens by PCR

Genomic DNA was extracted using a Promega DNA extraction kit. Samples were assayed for *E. chaffeensis* infection using nested PCR with primers specific for the VLPT gene. DNA was extracted from the blood samples in the laboratory of Dr. Shahid Karim (USM) and amplified using Polymerase Chain Reaction (PCR). Gel electrophoresis was then performed in order to compare the samples to established pathogens.

The reaction mixture for the nested PCR contained 12.5 µl of PCR Mastermix (Promega), 1.0 µl of each primer (forward and reverse), 2.5 µl of DNA and 8.0 µl of nuclease free water. The following PCR thermocycler protocol was used: three cycles of 94° for 1 minute, 52° for 2 minutes, 70° for 1 minute 30 seconds, followed by 37 cycles of 88° for 1 minute, 52° for 2 minutes, 70° for 90 seconds, followed by 32 cycles of 88°C for 1 minute, 55°C for 2 minutes, 70°C for 70 seconds, an extension of 72°C for 7 minutes and a 4°C hold indefinitely. After PCR amplification, all samples were separated by electrophoresis through a 2% agarose gel with 4 µl of ethidium bromide and visualized using UV transillumination. Negative controls were 2.5 μL of nuclease free water. Genomic DNA was extracted from *A. americanum* ticks known to be infected with *E. chaffeensis*, and this DNA was used as a positive control.

Tick Identification

The collected ticks were sent to Lorenza Beati Ziegler at Georgia Southern University in 2009 and to Rich Robin at the Smithsonian Institute in 2010 for expert
Identification. Identification was accomplished by PCR amplification of DNA from collected ticks compared to DNA samples drawn from known species of tick.

Results

From 2009 - 2010, 2064 birds were surveyed out of the total 6039 that were banded (34%) and 252 (12%) blood samples were taken. The researcher observed 293 ticks attached to 49 (2%) surveyed birds, though only 112 of the ticks were collected for further analysis. Observed ticks were either larval (76%) or nymphal life history stages (24%) and almost all belonged to the genus *Amblyomma*. One *Ixodes* genus nymph, one *Haemaphysalis leporispalustris* (Packard) larvae and one *Haemaphysalis leporispalustris* (Packard) nymph were also found (Appendix B). Almost all of the 112 ticks (97%) were determined to be tropical in origin and non-native (i.e., not indigenous to North America) by experts at the Smithsonian Institute and Georgia Southern University.

Six *Amblyomma* genus nymphs and one *A. longirostre* nymph tested positive for *E. chaffeensis* (26% of the total number of nymphal ticks sampled) while all 27 *Amblyomma* genus larvae (30% of all collected ticks) tested positive for *E. chaffeensis* (Fig. 9). Twenty-seven (11%) blood samples taken from migratory birds tested positive for *E. chaffeensis* (Fig. 10). Species infected included COYE, GRCA, HOWA, INBU, NOWA, OVEN, REVI, SCTA, SUTA, SWTH, WEVI, WEWA and WOTH. Only one bird, a Wood Thrush, was found to be infected with *E. chaffeensis* while carrying infected, feeding ticks (three larvae and one nymph). The same bird also was infested with seven additional feeding ticks (six larvae and one nymph) that were not infected (Appendix B).
Figure 9. Gel electrophoresis illumination showing positive and negative samples from ticks for *Ehrlichia chafeensis* identification collected at Johnson Bayou, LA. Positive samples are illuminated.

Figure 10. Gel electrophoresis illumination showing positive and negative blood samples for *Ehrlichia chaffeensis* identification from migratory birds collected at Johnson Bayou, LA from 2009-2010. Positive samples are illuminated.
Discussion

Results reported here support my hypothesis that migrating birds are capable of transporting a tick-borne pathogen, *Ehrlichia chaffeensis*, the causative agent of human monocytic ehrlichiosis and ticks species not native to North America, not to mention harboring the same disease causing organism. All birds sampled in this study were intercontinental migratory songbirds that spend the temperate winter in the Caribbean Basin, Mexico, and/or Central and South America and had within hours of sampling engaged in a non-stop flight of 18–24 hours across the Gulf of Mexico. The migratory birds infected with the tick-borne pathogen represented 13 phylogenetically diverse passerine species characterized by a wide range of migratory routes and varied breeding and wintering geographic distributions. Although the biogeography of parasites, especially microparasites, such as viruses, bacteria, and protozoa, is poorly understood, the application of PCR and DNA sequencing will help to characterize parasite lineages and describe their geographical distribution and host affiliation (see Ricklefs et al. 2005). Using these techniques, we hope to determine where these birds spent the temperate winter and where they may have stopped during migration in order to further our knowledge of emerging disease origination and movement.

It might be argued that the instances of pathogen transmission was lower than expected in light of the fact that migratory birds may be immunocompromised in relation to the energetic demands of migration (Owen 2004, Williams 2008). That aside, the lack of infection in some birds that were infested with infected ticks may indicate that an insufficient period of time has elapsed for transmission of the pathogen to the host, which usually requires minimum of 12–24 hours (Jongejan and Uilenberg 2004, Bowman and
Sauer 2004). Conversely, some ticks tested negative for the pathogen while feeding on an infected host, which may again be a function of elapsed feeding time. It is also possible that a bird acquired the pathogen from a tick that had since engorged and detached prior to arrival along the northern gulf coast. Moreover, there did not seem to be a relationship between nymph burden and infection prevalence (see James et al. 2011). Only one heavily burdened WOTH was positive for *E. chaffeensis* in this study. Other individuals regardless of species that were positive for *E. chaffeensis* either did not have a tick when sampled or had more tick(s) that were not positive for the pathogen.

My results may indicate that migrants do not become ill from infection by *E. chaffeensis* but may be competent reservoirs for it. Additionally, if *E. chaffeensis* does cause illness, those individuals who were impacted by the symptoms may not have made it across the Gulf of Mexico. This possibility may indicate that migrants who did cross and who were infected were able to overcome any negative impacts or had not had time to be impacted.

Regardless of the potential of migratory birds to carry disease organisms between geographic regions, the spread of an emerging disease by migration depends on several factors that were not addressed in this study:

1. Number of individuals with high parasite burdens (Anderson and May 1991), and high burdens should be more likely when the stress of migration has suppressed immune function (see Owen and Moore 2008) such as movement across the Gulf of Mexico (Owen 2004).
2. Suitable vectors must be present when migratory birds arrive in North America to transmit the disease and maintain the infection in the population (Shroyer 1986, Mitchell 1991).

3. Resident species must be susceptible to infection, which depends on the complexity of the parasite life cycle, specialization of vectors, and the pathogen itself. Ultimately, the barriers, or lack thereof, to emergent disease are not completely understood as these requirements are not fully understood in terms of the ecology of host-parasite relationships.

Influxes of non-native ticks and pathogens could have a detrimental effect on local wildlife, domestic animals, and even humans as the transmission of tick-borne diseases becomes a better understood epidemiological phenomenon (Reed et al. 2002). These findings have implications both from an ecological and an epidemiological viewpoint. *Ehrlichia chaffeensis* is known to vector by way of tick bites. As non-native ticks are being introduced by migratory birds, the chances of introducing ehrlichiosis to areas where the pathogen is not native are increasing (Bisgard 2009). A novel pathogen from an unexpected vector could be difficult to identify in wildlife and humans, and tick-borne pathogens can take several days to manifest and identify. In that time, individuals can begin to develop symptoms and suffer consequences (Chapman et al. 2006) while the pathogen continues unchecked.
APPENDIX A


<table>
<thead>
<tr>
<th>Bird Species</th>
<th>Foraging Guild</th>
<th>Number of ticks/bird</th>
<th>Attachment site</th>
<th>Tick life stage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acadian Flycatcher <em>(Empidonax virescens)</em></td>
<td>Canopy</td>
<td>4</td>
<td>Eyes</td>
<td>Nymph</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>Chin/bill</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>Eyes</td>
<td></td>
</tr>
<tr>
<td>Baltimore Oriole <em>(Icterus galbula)</em></td>
<td>Canopy</td>
<td>1</td>
<td>Throat</td>
<td>Nymph</td>
</tr>
<tr>
<td>Black-and-white Warbler <em>(Mniotilta varia)</em></td>
<td>Canopy</td>
<td>1</td>
<td>Chin/bill</td>
<td></td>
</tr>
<tr>
<td>Eastern Kingbird <em>(Tyrannus tyrannus)</em></td>
<td>Canopy</td>
<td>8</td>
<td>Chin/bill</td>
<td></td>
</tr>
<tr>
<td>Great Crested Flycatcher <em>(Myiarchus crinitus)</em></td>
<td>Canopy</td>
<td>2</td>
<td>Eyes</td>
<td>Larvae</td>
</tr>
<tr>
<td>Orchard oriole <em>(Icterus spurius)</em></td>
<td>Canopy</td>
<td>1</td>
<td>crown</td>
<td></td>
</tr>
<tr>
<td>Painted Bunting <em>(Passerina ciris)</em></td>
<td>Canopy</td>
<td>2</td>
<td>Throat</td>
<td>Nymph</td>
</tr>
<tr>
<td>Rose-breasted Grosbeak <em>(Pheucticus ludovicianus)</em></td>
<td>Canopy</td>
<td>1</td>
<td>Breast</td>
<td>Nymph</td>
</tr>
<tr>
<td>Red-eyed Vireo <em>(Vireo olivaceous)</em></td>
<td>Canopy</td>
<td>1</td>
<td>Eyes</td>
<td>Larvae</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>Chin/bill</td>
<td>Nymph</td>
</tr>
<tr>
<td>Species</td>
<td>Habitat</td>
<td>Area</td>
<td>Location</td>
<td>Diet</td>
</tr>
<tr>
<td>------------------------------------------</td>
<td>----------</td>
<td>------</td>
<td>----------</td>
<td>--------</td>
</tr>
<tr>
<td>Scarlet tanager (Piranga olivacea)</td>
<td>Canopy</td>
<td>1</td>
<td>Nape</td>
<td>Nymph</td>
</tr>
<tr>
<td>Summer Tanager (Piranga rubra)</td>
<td>Canopy</td>
<td>2</td>
<td>Throat</td>
<td>Nymph</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>Neck</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>Crown</td>
<td></td>
</tr>
<tr>
<td>Tennessee Warbler (Oreothlypis peregrina)</td>
<td>Canopy</td>
<td>1</td>
<td>Eyes</td>
<td>Larvae</td>
</tr>
<tr>
<td>Grey-cheeked/Bicknell’s thrush (Catharus minimus/C. bicknelli)</td>
<td>Ground</td>
<td>2</td>
<td>Eyes</td>
<td>Nymph</td>
</tr>
<tr>
<td>Indigo Bunting (Passerina cyanea)</td>
<td>Ground</td>
<td>1</td>
<td>Eyes</td>
<td>Nymph</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>Crown</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>Neck</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>Nape</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>Nape</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>Chin/bill</td>
<td></td>
</tr>
<tr>
<td>Kentucky Warbler (Oporornis formosus)</td>
<td>Ground</td>
<td>1</td>
<td>Shoulders</td>
<td>Nymph</td>
</tr>
<tr>
<td>Ovenbird (Seiurus aurocapilla)</td>
<td>Ground</td>
<td>1</td>
<td>Chin/bill</td>
<td>Nymph</td>
</tr>
<tr>
<td>Swamp Sparrow (Melospiza georgiana)</td>
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<td>1</td>
<td>Crown</td>
<td>Nymph</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>Chin/bill</td>
<td></td>
</tr>
<tr>
<td>Swainson’s Thrush (Catharus ustulatus)</td>
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<td>Eyes</td>
<td>Nymph</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>Larvae</td>
<td></td>
</tr>
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<td></td>
<td>1</td>
<td>Larvae</td>
<td></td>
</tr>
<tr>
<td>Species</td>
<td>Habitat</td>
<td>Adult</td>
<td>Location</td>
<td>Juvenile</td>
</tr>
<tr>
<td>-------------------------------</td>
<td>----------</td>
<td>-------</td>
<td>-----------------------------------------------</td>
<td>----------</td>
</tr>
<tr>
<td>Wood Thrush (Hylocichla mustelina)</td>
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<td>1</td>
<td>Cloaca</td>
<td>Larvae</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>Chin/bill</td>
<td>Larvae</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>Cloaca</td>
<td>Larvae</td>
</tr>
<tr>
<td></td>
<td></td>
<td>~100, 19 retrieved</td>
<td>Flank (cloaca, wings, shoulders, neck)</td>
<td>Larvae</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>Wing</td>
<td>Nymph</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>Chin/bill</td>
<td>Larvae</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>Cloaca</td>
<td>Larvae</td>
</tr>
<tr>
<td></td>
<td></td>
<td>60, 11 retrieved</td>
<td>Cloaca</td>
<td>2 Nymphs, 9 Larvae</td>
</tr>
<tr>
<td>Common Yellowthroat (Geothlypis trichas)</td>
<td>Understory</td>
<td>1/1</td>
<td>crown</td>
<td>Larvae</td>
</tr>
<tr>
<td>Hooded Warbler (Wilsonia citrina)</td>
<td>Understory</td>
<td>3</td>
<td>Nape</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>Nape</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>Nape</td>
<td>Nymph</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>Shoulders</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>Crown</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>Nape</td>
<td>Nymph</td>
</tr>
<tr>
<td>White-eyed Vireo (Vireo griseus)</td>
<td>Understory</td>
<td>1</td>
<td>eyes</td>
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<tr>
<td>Worm-eating Warbler (Helmitheros vermivorum)</td>
<td>Understory</td>
<td>39</td>
<td>Eyes</td>
<td>Larvae</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>Ear</td>
<td>Nymph</td>
</tr>
</tbody>
</table>
APPENDIX B

BIRD SPECIES AND IDENTIFICATION OF TICKS REMOVED AT JOHNSON BAYOU, LA FROM 2009-2010.

*INDICATES INDIVIDUAL WAS POSITIVE FOR _EHRLICHIA CHAFFEENSIS_.

<table>
<thead>
<tr>
<th>Bird species</th>
<th>Ticks removed from bird</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACFL</td>
<td>Amblyomma nymph</td>
</tr>
<tr>
<td>BAOR</td>
<td>Amblyomma longirostre (Koch) nymph</td>
</tr>
<tr>
<td>COYE</td>
<td>Haemaphysalis leporispalustris (Packard) larva</td>
</tr>
<tr>
<td>COYE*</td>
<td>N/A</td>
</tr>
<tr>
<td>GCBT</td>
<td>Amblyomma nymph*</td>
</tr>
<tr>
<td>GCFL</td>
<td>2 Amblyomma larva</td>
</tr>
<tr>
<td>GRCA*</td>
<td>N/A</td>
</tr>
<tr>
<td>GRCA*</td>
<td>N/A</td>
</tr>
<tr>
<td>HOWA*</td>
<td>N/A</td>
</tr>
<tr>
<td>HOWA*</td>
<td>N/A</td>
</tr>
<tr>
<td>HOWA</td>
<td>Amblyomma nymph*</td>
</tr>
<tr>
<td></td>
<td>2 Amblyomma nymph</td>
</tr>
<tr>
<td>HOWA*</td>
<td>N/A</td>
</tr>
<tr>
<td>HOWA*</td>
<td>N/A</td>
</tr>
<tr>
<td>INBU</td>
<td>Amblyomma nymph</td>
</tr>
<tr>
<td>INBU</td>
<td>Amblyomma nymph</td>
</tr>
<tr>
<td>INBU</td>
<td>Amblyomma maculatum (Koch) nymph</td>
</tr>
<tr>
<td>KEWA</td>
<td>2 Amblyomma nymph</td>
</tr>
<tr>
<td>NOWA*</td>
<td>N/A</td>
</tr>
<tr>
<td>OVEN*</td>
<td>Amblyomma nymph*</td>
</tr>
<tr>
<td>PABU</td>
<td>2 Amblyomma nymph*</td>
</tr>
<tr>
<td>RBGR</td>
<td>Amblyomma nymph</td>
</tr>
<tr>
<td>REVI</td>
<td>Amblyomma larva</td>
</tr>
<tr>
<td>REVI</td>
<td>Amblyomma longirostre (Koch) nymph*</td>
</tr>
<tr>
<td>REVI*</td>
<td>N/A</td>
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<td>SCTA*</td>
<td>N/A</td>
</tr>
<tr>
<td>SCTA*</td>
<td>N/A</td>
</tr>
<tr>
<td>SCTA</td>
<td>Amblyomma larva</td>
</tr>
<tr>
<td>SCTA*</td>
<td>Amblyomma nymph</td>
</tr>
<tr>
<td>SUTA</td>
<td>Amblyomma longirostre (Koch) nymph</td>
</tr>
<tr>
<td>SUTA*</td>
<td>N/A</td>
</tr>
<tr>
<td>SUTA*</td>
<td>N/A</td>
</tr>
<tr>
<td>Location</td>
<td>Species</td>
</tr>
<tr>
<td>----------</td>
<td>---------</td>
</tr>
<tr>
<td>SUTA*</td>
<td>N/A</td>
</tr>
<tr>
<td>SWSP*</td>
<td><em>Amblyomma</em> nymph</td>
</tr>
<tr>
<td>SWSP</td>
<td><em>Haemaphysalis leporispalustris</em> (Packard) nymph</td>
</tr>
<tr>
<td>SWTH</td>
<td><em>Amblyomma</em> larva</td>
</tr>
<tr>
<td>SWTH</td>
<td><em>Amblyomma</em> larva</td>
</tr>
<tr>
<td>SWTH</td>
<td><em>Amblyomma</em> nymph</td>
</tr>
<tr>
<td>SWTH*</td>
<td>N/A</td>
</tr>
<tr>
<td>SWTH</td>
<td><em>Amblyomma</em> larva</td>
</tr>
<tr>
<td>TEWA</td>
<td><em>Amblyomma</em> larva</td>
</tr>
<tr>
<td>WEVI*</td>
<td>N/A</td>
</tr>
<tr>
<td>WEVI*</td>
<td>N/A</td>
</tr>
<tr>
<td>WEVI*</td>
<td>N/A</td>
</tr>
<tr>
<td>WEWA</td>
<td>24 <em>Amblyomma</em> larva</td>
</tr>
<tr>
<td>WEWA*</td>
<td><em>Amblyomma maculatum</em> (Koch) nymph</td>
</tr>
<tr>
<td>WOTH</td>
<td><em>Amblyomma</em> larva</td>
</tr>
<tr>
<td>WOTH</td>
<td>14 <em>Amblyomma</em> larva</td>
</tr>
<tr>
<td>WOTH</td>
<td><em>Amblyomma</em> nymph</td>
</tr>
<tr>
<td>WOTH</td>
<td><em>Amblyomma</em> larva*</td>
</tr>
<tr>
<td>WOTH</td>
<td><em>Amblyomma</em> nymph</td>
</tr>
<tr>
<td>WOTH</td>
<td>2 <em>Amblyomma</em> larva</td>
</tr>
<tr>
<td>WOTH*</td>
<td><em>Amblyomma</em> larva</td>
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<tr>
<td>WOTH*</td>
<td>N/A</td>
</tr>
<tr>
<td>WOTH*</td>
<td>N/A</td>
</tr>
<tr>
<td>WOTH*</td>
<td>N/A</td>
</tr>
<tr>
<td>WOTH*</td>
<td>6 <em>Amblyomma</em> larva</td>
</tr>
</tbody>
</table>
APPENDIX C

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE APPROVAL FORM

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE
NOTICE OF COMMITTEE ACTION

The proposal noted below was reviewed and approved by The University of Southern Mississippi Institutional Animal Care and Use Committee (IACUC) in accordance with regulations by the United States Department of Agriculture and the Public Health Service Office of Laboratory Animal Welfare. The project expiration date is noted below. If for some reason the project is not completed by the end of the three year approval period, your protocol must be reactivated (a new protocol must be submitted and approved) before further work involving the use of animals can be done.

Any significant changes (see attached) should be brought to the attention of the committee at the earliest possible time. If you should have any questions, please contact me.

PROTOCOL NUMBER: 09012801
PROJECT TITLE: Migratory Connectivity and the En Route Migration Strategies of Migratory Birds
PROPOSED PROJECT DATES: 03/15/09 to 03/14/11
PROJECT TYPE: Renewal of a Previously Approved Project
PRINCIPAL INVESTIGATOR(S): Frank R. Moore, Ph.D.
COLLEGE/DIVISION: College of Science & Technology
DEPARTMENT: Biological Sciences
FUNDING AGENCY/SPONSOR: National Science Foundation
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Date 2-2-09
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