

**Vector-Borne Disease Dynamics of Alabama White-tailed Deer  
(*Odocoileus virginianus*)**

by

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A thesis submitted to the Graduate Faculty of  
Auburn University  
in partial fulfillment of the  
requirements for the Degree of  
Master of Science

Auburn, Alabama  
August 4, 2018

Keywords: Disease ecology, arbovectors, ectoparasites, white-tailed deer

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## Abstract

Understanding long-term dynamics of ectoparasite populations on hosts is essential to mapping the potential transmission of disease causing agents and pathogens. Blood feeding ectoparasites such as ticks, lice and keds have a great capability to transmit pathogens throughout a wildlife system. Here, we use a semi-wild white-tailed deer (*Odocoileus virginianus*) population in an enclosed facility to better understand the role of high-density host populations with improved body conditions in facilitating parasite dynamics. As definitive hosts and breeding grounds for arthropods that may transmit blood-borne pathogens, this population may also be used as a sentinel system of pathogens in the ecosystem. This also mimics systems where populations are fragmented due to human encroachment or through specialized management techniques. We noted a significant increase in ectoparasitism by ticks ( $p=0.04$ ) over a nine-year study period where deer were collected, and ticks quantified. Beginning in 2016 we implemented a comparison of quantification methods for ectoparasites in addition to ticks and noted that white-tailed deer within the enclosure were more likely to be parasitized by the neotropical deer ked (*Lipoptena mazamae*) than any tick or louse species. Additionally, analysis of blood collected from sampled deer between 2016 and 2018 by PCR isolated four *Bartonella* spp. present within the blood of enclosed deer. Together, these works inform us about the potential dynamics of ectoparasite communities long term, and how host populations could affect ectoparasite communities, providing insight into potential disease transmission.

## Acknowledgments

First, thank you to my parents, who never seemed bothered by the ragged haired wild-creature they learned to tolerate during my childhood. For fostering a love of the outdoors, and science that I would nurture throughout my life, and for all the encouragement I needed, gentle or otherwise. Second to Dr. Summer Arrigo-Nelson and California University of Pennsylvania without whom I wouldn't have had half my opportunities or connections. Lastly to the staff and faculty of Forestry and Wildlife Sciences at Auburn University, who have aided and guided me these last two years during my graduate education, most importantly Dr. Sarah Zohdy who offered me the first place in her lab at Auburn University – and truly began my post-secondary scientific journey.

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## **Chapter 1:**

### Long-Term Dynamics of Ticks (Acari: Ixodidae) in a Semi-Wild Population of White-Tailed Deer (*Odocoileus virginianus*)

#### **Introduction**

Ectoparasites, organisms living on or attaching to the surface of an organism, have the great potential to negatively impact their hosts' health directly through infestation related costs (Norval 1990) and indirectly by facilitating blood-borne pathogen transmission as arthropod vectors (Bock et al. 2004). Ectoparasites are of importance because of their potential to vector any number of diseases. Nearly 30% of new and emerging infectious diseases are vector-borne, and potentially harbored in wildlife hosts (Jones et al. 2002). Ectoparasites such as ticks (Acari: Ixodidae) undergo multiple life-stages throughout their maturation and life cycle. Larval ticks are hatched from eggs and quest for their first host (Paskewitz 2015) and after feeding molt into nymphs. Nymphal ticks will feed and then drop from a host to molt into adults. Adult ticks feed preferentially on hosts such as white-tailed deer (*Odocoileus virginianus*) (Wilson et al. 1995). Adult ticks will then mate, and females will oviposit. Mating in ticks may occur in the environment after they have dropped from a host, but more commonly occurs on the host (Jongejan and Uilenberg 2004). Ectoparasite communities can influence host abundance and distribution in this way. Host community composition can also greatly affect ectoparasite communities (Keesing et al. 2013), by altering the availability of food sources and mating grounds necessary for ectoparasites to thrive. It has also been suggested that a negative relationship exists between parasite abundance and the diversity of hosts (Ostfeld and Keesing

2012). Weinstein et al. (2017) show a relationship between the removal of large mammals in an enclosed habitat and a drastic increase in blood-borne pathogens and ectoparasitism in rodents. Several studies performed in a Kenya long term enclosure project (KLEE) have also noted this relationship between the removal of large-bodied animals and the rise of small mammals and their associated ectoparasites and pathogens (McCauley et al. 2008; Keesing et al. 2013; Young et al. 2014, Bordes et al. 2015). In addition, living in habitats with high host densities can increase the likelihood of transmitting parasites and their associated pathogens due to the proximity of hosts and density dependent transmission (Cote and Poulin 1995).

Ectoparasites can affect many factors of a host, including influencing body condition, reproductive success, or physiology (Booth et al. 1993; Neuhaus 2003). In cases where parasites are transmitted directly, a higher density of hosts could contribute to increased infection, as the likelihood of contact and transmission increases in proportion to the density of the population within its occupied area (Wilson et al. 2002). However, ectoparasitism can also be affected by host factors like population structure (McCoy et al. 2005), age, and sex (Schalk and Forbes 1997). In many mammal species males are more heavily parasitized than females and considered accountable for more parasite and pathogen transmission within the population (Bacelar et al. 2011; Gorrell and Schulte-Hostedde 2008; Harrison et al. 2010; Moore and Wilson 2002; Perkins et al. 2003; Skørping and Jensen 2004). There are several competing hypotheses that may suggest reasoning for this imbalance toward males. Patterns of male-bias may be driven by sexual dimorphisms in species where males are a larger and more ornamental sex (Clutton-Brock et al. 1977); or by the male's ability to provide more resources to parasites, whether that be nutrients or simply space (Christe et al. 2003; Hawlena et al. 2005) or may simply be greater targets by ectoparasites because of trade-offs that occur between large

ornamentation and immune function (Hamilton and Zuk 1982; Maynard Smith 1985). It is also believed that males who must compete for mating opportunities generally lead a riskier lifestyle which presents more opportunity for the acquisition of parasites and pathogens (Moore and Wilson 2002). Risks are associated with the increased testosterone output of males and subsequent immune suppression (Salvador et al. 1996), the stress of a rutting period, and the male-male competition that occurs when searching for mating opportunities (Krause and Godin 1994). Trade-offs between male ornamentation and immune-competence are a direct relation to inflated levels of testosterone, known to suppress the immune function (Zuk and McKean 1996). This immunosuppression by testosterone could limit the ability of males to fight off parasitic infection (Folstad and Karter 1992; Roberts et al. 2007). Competition between males could also present opportunities for parasites to be exchanged through physical contact. These associations between host ecology and ectoparasitism have the potential to reveal the ecological drivers of parasite infestation and vector-borne pathogens and how host biodiversity can influence parasite ecology. To better understand the relationships between ectoparasites and hosts, experimental systems where specific host groups are included in or excluded from the environment may be used to document shifts in transmission dynamics.

Exclosure experiments in which boundaries are established to exclude certain species have been traditionally used longitudinally to better understand community ecology. Experiments have helped to better explore many aspects of community interaction, specifically the effect of species removal on other ecological aspects of a community (Bakker et al. 2016). Among notable exclosure experiments is the KLEE which began in 1995 by Young et al. and is still ongoing today. These exclosures are used to address fundamental questions in ecology relating to defense, compensation and competition of domestic livestock, mega-herbivores, other

native wildlife, parasitism, and plant and insect communities. Experiments like these enclosure treatments have provided many insights into the conservation and management of ecosystems, as the applications and understandings are broad in nature and applicable on a large scale. However, less traditionally approached are enclosure experiments. Enclosures are often utilized as a part of management or conservation situations where populations may be monitored long-term or to facilitate reintroduction or recovery (Rubio 2017; Bos 2017). Sorci et al. 1997 noted a significant positive correlation between ectoparasite load and host density per enclosed patch in the common lizard (*Lacerta vivipara*). Another study by McCoy et al. 2012 noted an increase in ectoparasites on three enclosed lizard species in the first few years following prescribed fire. There was also a slight correlation between increased host abundance within the enclosures and ectoparasitism by larvae of the genus the *Eutrombicula* (McCoy et al. 2012). Enclosure experiments have been used in a variety of other systems, however less so with large-mammals. Enclosures provide useful tools for understanding how an increase in host density could influence the ectoparasite community, which in turn could influence the health of both the host and ecosystem. Systems utilizing exclosures and enclosures have been used in a variety of ecosystems, including bluegill fish to assess the effect of predation on parasitism (Duffy 2007), white footed mice to better understand the affects of parasitism on host breeding effort (Vandergrift et al. 2008), butterflies (Lepidoptera) to look at how predation can change the population dynamics and genetic landscape of a prey species (Holmes et al. 1979), and Soay sheep to understand how parasitism may affect genetic variation within a host population (Coltman et al. 1999).

The white-tailed deer is a common and widespread species in the southeastern United States, used and maintained as a game species. White-tailed deer are a commonly ectoparasitized

and serve as important hosts for a variety of ectoparasites and pathogens (Forrester et al. 1996). Thirty-four species of ectoparasite have been recorded on white-tailed deer in North America and Canada (Kellog et al. 1971; Kennedy and Newman 1986). Due to their reliance on hosts and the feeding that is required throughout each life stage, ticks have a great capability to transmit pathogens across host groups. White-tailed deer also serve as important sentinel species as the last host that many adult ticks feed on and thusly a sink for any pathogens that tick may have encountered throughout their life cycle. Understanding parasite dynamics is more complicated than simply studying the abundance of ectoparasites within a population but has the capability to provide valuable insights into disease ecology and the health of humans and wildlife. Several studies have shown male-biased parasitism in white-tailed deer (Schulze et al. 1984; Kitron et al. 1992; Schmidtmann et al. 1998). Immunocompetence principles have shown that males with larger body mass and ornaments who can simultaneously harbor ectoparasites despite the immunocompromising effects of testosterone will be honest signals of genetic potential (Folstad and Karter 1992). However, parasitism is not distributed equally across a population. Individual variation drives differential infection based on factors even within-sex when operating under a male-bias. Body size or condition is thought to be a specific driver of parasitism, however the exact relationship between host size and parasite burden has limited and contradictory evidence. Some studies have shown a positive relationship with parasitism and body mass, where larger individuals are more likely to be parasitized and harbor more parasites (Christe et al. 2003; Kiffner et al. 2011; Durkin et al. 2015) while other shows a negative relationship, where parasites are more likely to gravitate toward smaller individuals with potentially lower immune responses (Navarro-Gonzalez et al. 2011; Milner et al. 2013; Debeffe 2014; Eads 2016) and even

still some showed confounding effects of body mass that varied depending on the time of year or other factors of host condition (Kowalski et al. 2015; Sackett 2018).

Due to conflicted evidence, associations between ectoparasitism and body mass remain relatively unclear in white-tailed deer. Additionally, these questions remain unclear when considering density of host populations with other factors that affect ectoparasitism. We seek to better understand how host-parasite community dynamics can change with increased host density, how host-parasite communities change over time in an ecosystem where large mammals are found in high densities and how factors such as body size, antler size and age affect the parasitism of an already male-biased system when the community is parasite rich. Will larger, healthier deer who may be able to support a larger number of ectoparasites and produce large antlers be more ectoparasitized as a result of immune system suppression or will smaller young males be more parasitized due to their more juvenile nature?

Here, we utilize a system where semi-wild white-tailed deer are enclosed in a fenced in facility in high densities. Other animals and ectoparasites can move freely in and outside of the fencing, but a maintained deer population allows us the capability to understand how a community of large bodied hosts in high densities can affect the ectoparasite community. This study system allows us to monitor, long-term, the dynamics of this enclosed high-density deer population and better understand the dynamics of its ectoparasite community. We focus on white-tailed deer and their associated ecosystem as important hosts for several ectoparasites that act as vectors of disease causing pathogens (Wilson et al. 1985, 1988). White-tailed deer serve as important breeding grounds for several tick species and are hosts to other ectoparasites such as lice, and hippoboscids flies also known as deer keds (Wedincamp and Durden 2016). Additionally, ectoparasites may utilize other hosts like small mammals that act as amplifying

hosts for pathogens such as *Borrelia burgdorferi*, causative agent of Lyme disease (Levine et al. 1985). In habitats where the distribution of potential hosts is altered, whether through exclusion or selective inclusion, ectoparasite communities may be affected by these changes. In situations where habitats are fragmented, and white-tailed deer populations are present in high densities – there is an increase in the utilization of small mammals by ectoparasites – and a subsequent increase in the transmission of arthropod vectored pathogens throughout the system (Rand et al. 2004). Biodiversity has also been linked to differential pathogen transmission (Schmidt and Ostfeld 2001). These conceptual models are called the ‘dilution effect’ first proposed in 2001 by K. Schmidt and R. Ostfeld. Dilution effect refers to the idea that increased diversity of potential hosts can limit the transmission of pathogens through a population. This population presents a low diversity ecosystem dominated by late-stage hosts of ectoparasites and should reflect a potential increase in pathogen transmission.

## **Methods**

This study was performed at Auburn University’s Deer Research Facility through Piedmont Substation. The facility was constructed in 2007 and consists of approximately 174 hectares. Surrounded by 2.6m fencing, it maintains a semi-wild population of between 100-120 deer. The population consists of individuals captured at construction of the fencing and their descendants. This population is regulated through natural and capture-related mortality. Ten to fifteen young deer per year are captured and released outside the facility at 6 months of age to control density and maintain the desired number of individuals. Deer are fed *ad libitum* a 16-18% extruded protein feed (Record Rack, Nutrena Feeds, Minneapolis, MN). During the capture period of September-February deer were also fed from four timed feeders that provided approximately 2kg of cracked corn per day. Two 0.8-ha fences plots were planted annually in

various warm and cool season forages as part of other ongoing research projects. Deer could rotationally graze at regular intervals throughout the year as prescribed by management projects.

Deer were chemically immobilized and darted after 6 months of age. Capture began in 2007 and continued from September to February each year, and the research is still ongoing as of 2018. Deer were darted once per season at elevated stands, with sex, age, and activity patterns deciding what sex or size were captured. Two hundred and fifteen male deer were included within this study from those sampled 2007-2018. All methods were approved by the Auburn University Institutional Animal Care and Use Committee (2008-1417, 2008-1421, 2010-1785, 2011-1971, and 2013-2372) and followed the American Society of Mammologists' guidelines (Sikes & Gannon 2011). An anesthetization solution was prepared daily during the darting season for between 2-4 volunteers. This solution consisted of a 4ml combination of Telazol (Fort Dodge Animal Health, Fort Dodge, IA; 100 mg/ml given at a rate of 4.5 mg/kg) and xylazine (Lloyd Laboratories, Shenandoah, IA; 100 mg/ml given at a rate of 2.2 mg/kg). The solution was loaded into an impact release dart mounted with a radio telemetry transmitter (Pneu-Dart, Williamsport, PA). Sampled deer ranged between 1.5 and 10.5 years of age, with preference given to males who have not previously been darted to gain genetic material.

Once immobilized and recovered, metrics of body condition and health were measured. Measurements included; skull length, total body length (from nose to tail base), tail length, neck girth (at halfway), chest girth (posterior of the shoulder), and right hind foot length (with hoof pointed, from the tip to the posterior end of the tuber calcis). Antler measurements were also taken for sampled males, using a Boone and Crockett scoring system which measures: inside spread, main beam length and circumference, and the length and circumference of any tines longer than 1 inch (Nesbitt, 1981). Abnormal points and antler malformation were also recorded.

At initial capture, sex and tooth wear and replacement were recorded which was then used to estimate age. Tissue samples were collected from an ear notch to record genetic information and determine parentage. Additionally, a measure of ectoparasitism was calculated by completing point counts of ticks present on specific areas of the body. Ticks were counted on the eyes and ears, sternum and anus (Figure 1).

Data were analyzed using Program R (version 3.0.3, [www.r-project.org](http://www.r-project.org), accessed 01 Jan 2018) to conduct a principle component analysis (PCA) of body measurements to determine a standardized score as a measure of Body Size and Condition, as completed by Newbolt et al. 2017. We anticipated that measures would be highly correlated. Using chest girth, hind foot length, and body length to generate a single term that represented annual body size. Gross Boone and Crockett antler scores (Nesbitt 1981) to represent an individuals' annual antler size, and the estimated age during the corresponding year to represent age. Variance Inflation Factors (VIFs) and were calculated for age, annual antler size and annual body size to evaluate collinearity. We used generalized mixed-effect regression in Program R (package glmmTMB) to analyze models. We tested for over dispersion in the dataset and selected the family of error distribution (i.e. Poisson, binomial, or negative binomial) that best suited the data. We evaluated out data for zero inflation and selected model specifications based on these. All models used included a random term to control for the observed changes in population demographics and the repeated sampling of males as well as the time and year of sampling. T-tests were completed to compare the number of ticks quantified on animals throughout the sampling years.

## **Results**

Five-hundred and ninety deer were captured between December 2007 and February 2018. Of these, only males were included in this analysis, which was then limited to 215 individuals that had complete body measurements, antler measurements and an estimation of ectoparasite

abundance on that individual (Table 1). The PCA analysis of 3 measured body measures; chest girth, body length and hind foot length, indicated that while they were highly correlated with one another, component 1 explained 84% of variation within our dataset, and was thus used as a score for annual 'body size'. Estimates of collinearity among the three variables used in the generalized model (annual body size, gross Boone and Crockett antler score, and age) were moderate to high (VIF: body size: 3.56, antler score: 5.02 and age: 2.65). Because ectoparasite data consists of discrete counts, a Poisson distribution was used in statistical analysis. (Package: glmmTMB family: Poisson).

Models indicated that there is a significant effect of, annual body size, gross Boone and Crockett antler score, and deer age on the abundance of ticks' present. We observed that for each increase in annual body size (larger chest girth, longer hind foot length and longer body length), an individual was 0.062 times less likely to have ticks present on the body ( $p=4.47e-07$ ). We also noted that for each increase in the gross antler score assigned to an animal, and thusly the larger antlers an individual was 0.037 times more likely to have ticks present on the body ( $p=2.39e-08$ ). Younger deer were also more likely to be burdened by ticks than older deer, with each 1 year increase male deer were 0.311 times less likely to be parasitized ( $p=0.0056$ ). While these relationships are significant, the effect of these measures of health are small and unlikely to show clear trends when viewed in the environment. These findings do suggest a trend toward healthier, larger males having less ticks, or a smaller likelihood of discovering those ticks they are preliminary in nature and limited by the method of ectoparasite quantification.

Significantly more ticks were found on animals in 2017 than the first sampling in 2007 (Figure 2). Mean tick quantification for all collected animals in 2007 was 0.0 ticks per male deer (SE 0.8) compared to 42 per male deer in 2017 (SE 2.75). We observed that for each passing

year there were 2.23 more ticks quantified from captured deer ( $p=0.0413$ ). The total number of ticks collected has increased steadily throughout the sampling period – exponentially in 2016 with the implementation of new quantification methods. Throughout the course of ectoparasite sampling, we collected a variety of tick species (Figure 3) Species collected were: *Ixodes scapularis* (blacklegged tick), *Amblyomma americanum* (lone star tick), *Amblyomma maculatum* (gulf coast tick), *Rhipicephalus sanguineus*, (brown dog tick), and *Dermacenter variabilis* (American dog tick).

## **Discussion**

Increased host density has significantly changed the abundance of ectoparasitizing ticks within this system. Increased deer density over time has provided greater mating opportunities and resource for ticks. However, these increased tick numbers may simply reflect the inflated number of mating sites and opportunities. Because there are high density deer, upon which ticks can mate – we could see an inflated number of tick offspring within this enclosure simply because of increased deer density. Where there is an increased density of large bodied animals we would expect to see an increase in the ectoparasitism of these animals. Where Keesing et al. 2013 and Young et al. 2014 note a distinct increase in the ectoparasitism of small-mammals in the absence of large bodied mammals, we would expect the opposite in our community because of the higher than typical deer density. We would expect to see increased ectoparasitism of late-stage hosts such as white-tailed deer and an abundance of ticks in the environment. Small mammals and other animals outside of the enclosure would also be expected to host fewer ectoparasites – as a majority would be sustained by the enclosed population. Logical next steps would be sampling ectoparasites within the enclosure not on a host and sampling ectoparasites on hosts other than our large-bodied deer, such as small mammals or meso-mammals.

Additionally, sampling outside the enclosure would also provide a point of comparison for lower deer density populations and how ectoparasite communities can be changed based on this composition. Broadly, our understanding of host dynamics is currently limited. With the increased fragmentation of wildlife habitats, and subsequent higher density of potential hosts within that fragmented habitat, we could be moving toward situations where ectoparasite numbers will skyrocket in response to increased host density – furthering the transmission of disease causing vector-borne pathogens.

While the variables included relating to host health and heterogeneity were highly collinear for males within this study population the analysis performed did provide insights into greater understanding of ectoparasitism within this system. Multicollinearity in this case did not appear to affect the validity of the results. These results demonstrated that both annual body size and increasing age were negatively related to the number of ticks found on the body, but that the gross antler score assigned to an individual was positively related to ticks found. This suggests that antler size could be used as a predictor of ectoparasitism. However, the relationship between antler size and body size complicates these results. Larger antler size is typically indicative of a male in greater condition, and as a result that male presents with a larger body size than his competitors might. However, we note that larger body size is negatively related with the number of ticks according to our best fit model. This means then, that deer with smaller body sizes, but larger antlers are most likely to be parasitized - males of median age, who have experienced several years of antler growth, but have not yet reached peak size and weight were most heavily ectoparasitized.

It should be noted that body size and mass varied greatly for male deer throughout the season, and our estimates of ectoparasite burden are valuable only for the period directly before

and during the beginning of the breeding season within the state of Alabama. We provide evidence that suggests the most reproductively successful males may not be those who are most parasitized, and instead younger males experiencing their first substantial antler growths (and subsequent testosterone spikes) are more likely to be parasitized. In that way, our study population seems less likely to follow the tenants of the immunocompetence handicap hypothesis. However, we also cannot support the ‘well-fed’ hypothesis as proposed by Christie et al.2003. These trends however, may vary at points throughout the year – and a longer term, in depth analysis of condition trends for males over the course of a yearly cycle and throughout their lives would be greatly beneficial to better understanding this preliminary evidence. It is also possible that within this study system, a relative lack of natural variation may skew results. All deer within the facility are supplemented with feed in addition to natural dietary intake. All deer have *ad libitum* access to extruded protein feed, in addition to timed feeders that hold cracked corn from September to February. Thusly, deer are generally in greater condition than wild, free-living individuals may otherwise be. Along with this, we may see an inflation in measures of condition and antler growth that do not necessarily reflect trends we would see outside of this quasi-experimental setting. Ticks were not sampled within the environment or anywhere other than on host deer, next steps would be to sample ticks within the environment and enclosure not necessarily on white-tailed deer, and sampling ticks outside of the fenced area. Accurate quantification of ectoparasites not only on the deer themselves, but also within and outside the facility is an important next step. With such an increase in tick numbers within the facility due to the high density of hosts and breeding sites, ectoparasite communities outside of the fence are likely to be altered. We provide ample evidence however, to note that tick density has increased significantly over the nine sampling years both on all deer and per individual.

These results themselves are limited by the methods of ectoparasite detection. Detection of ectoparasite infestation was limited to only ticks in several small areas, and very likely left out a large portion of the ectoparasites present on any individual. There was also an overabundance of zero counts within the dataset, where ticks were not quantified in the areas collected and so the animal is listed as ‘not ectoparasitized’ which is very unlikely in practice. Moving forward, we propose a more complete method of ectoparasite quantification in addition to the analysis of these measures of host health. One that includes ectoparasites in addition to ticks, as these are not the only ectoparasites capable of presented disease risk and implications for wildlife hosts, but also a quantification method that increases the chances of obtaining an accurate understanding of that individuals’ parasite burden. Through the adjustment of these quantification methods, we may be better able to understand the factors of male hosts effecting ectoparasitism within this study system. Additionally, species was not recorded for any ticks counted on individuals, and counts were skewed toward animals with large, engorged ticks easily noticed within the fur of the animals.

This study system does provide insight into the management implications of ecosystems with high-densities of hosts important to ectoparasites. Not necessarily limited to white-tailed deer, but of note for deer because they have been historically managed in high-fence facilities maintained for hunting. Bosler et al. (1983) studied a similar system where deer on long-island NY were kept in high density. On this island, they noted an increased tick present and subsequently an abundance of Lyme disease in the form of *B. burgdorferi* spirochetes. While we did not focus on the presence of Lyme within this system, it is of notable importance because of its threat to human health. Hosts in enclosed systems such as this could serve as reservoirs of Lyme disease for humans and wildlife in the nearby vicinity.

## **Chapter 2:**

### Comparison of Ectoparasite Quantification Methods on White-Tailed Deer

*(Odocoileus virginianus)*

#### **Introduction**

Ectoparasites, such as ticks (Acari: Ixodidae) and lice (Insecta: Phthiraptera), are parasitic arthropods found in great numbers across a large variety of animal taxa (Marshall 1981). These ectoparasites are dependent on animal hosts for all or part of their life cycle and can be blood feeders. Ectoparasites can also transmit a wide-variety of disease causing agents, and hosts with greater numbers of ectoparasites may harbor greater infection of vector-borne illness (Tian 1998, Zhang et al. 2002, Wang et al. 2007). Understanding the composition and abundance of ectoparasite communities on hosts can provide valuable insight into ecological roles they play in relation to those hosts (Krasnov et al. 2004). Host-parasite relationships are particularly important because of their dependency on one another. Host population composition, age, sex, or other factors could affect the demographics and dynamics of an ectoparasite community. On the other hand, ectoparasites can affect the dynamics of a host population through the potential transmission of the parasites themselves, associated pathogens, or fitness consequences. These ectoparasite species may also be of risk to humans. Understanding which ectoparasites are present on hosts is the first step in understanding disease transmission and community dynamics (Bloemer 1988). This understanding comes through estimations of abundance or physical quantification of any present ectoparasites.

Ectoparasite quantification is traditionally a challenging process due to the small size and coloration of arthropod ectoparasites but is necessary for understanding ectoparasite abundance

on the host. There are a wide variety of techniques that use different applications, products, and methods. Broadly, they can be classified into two large groups: dead or live quantification. Dead quantification refers to quantification or collection of ectoparasites from the pelt or hide of a recently deceased animal. Here, efforts do not have to be taken to minimize stress or desecration of the materials. Time limits exist only in that ectoparasites may migrate away from a deceased animal. Live quantification requires the capture and handling of an individual and has greater time constraints. The most common method is to comb through the fur or feathers of the animal (Waterson 1912, 1913), which is tedious and generally erroneous if not completed carefully. Brushing is often also used as an alternative to a basic search and has been amended to increase efficiency for decreased contamination (Hopkins 1949) and on live animals (Dunn 1932). Fumigates and acaricides are commonly used (Hopkins 1949, Cook 1954, Kiffner et al. 2010) with smaller bodied mammals and birds, while washing is often used for removed pelts despite its destructive nature (Gering and Thomas 1953, McIvor 1987). Dust ruffling and feather combing are also common methods for quantification in birds (Walther & Clayton, 1997). Method comparisons have been previously completed for bird species (Clayton & Drown 2001, Clayton & Walther 1997), but less so for large bodied mammals. A comparison of quantification is difficult due to the large body size and methods of capture. Quantification of ectoparasites is more accurate when using a pelt or hide of a deceased animal, but methods cannot be considered foolproof due to detection probability and the varied abundance of ectoparasites.

Here, we seek to validate the accuracy of methods introduced for other large bodied mammals without desecration of the pelt and hide and introduce methods that increase efficiency and decrease total handling time. The half body estimation method, first introduced by Keiser (1987), was used to quantify the ectoparasite burden on livestock cattle. These methods, while

more time consuming than the use of acaricides or other parasitic spray-and-sheet methods, are not harmful to both the animal and the environment in which they are used. Combing methods preserve the hide and pelt, as well as the respiratory health of animal and scientist. This half body burden estimation technique has been used often with cattle or other large mammals (Fourie 1995, L'Hostis 1994, Mathee 1997), but not with white-tailed deer or in direct comparison with a full-body live count method. Field-based techniques that are both accurate and efficient are highly valuable. The introduction of methods that both limit capture stress and total handling time, but also serve as an accurate method for quantifying ectoparasites present on an animal, would be of great help to studies that examine ectoparasite abundance. In addition, we seek to better understand the composition of ectoparasite communities through a more thorough approach to quantification.

This study was performed in a semi-wild population of white-tailed deer enclosed in 2.6m fencing that hosts approximately 100-120 individuals. There is an inflated density of white-tailed deer within this fenced enclosure, which serve as important hosts for a variety of ectoparasite species and thusly potential vectors of disease transmitted by ectoparasites (Forrester et al. 1996). It has been proven that high biodiversity dilutes disease transmission (Schmidt and Ostfeld 2001) and that a lack of white-tailed deer within an ecosystem leads to decreased abundance of ectoparasites (Stafford et al. 2003). Given the unnaturally high deer density and lack of biodiversity, we would expect to see increased ectoparasitism of these hosts and subsequently increased transmission of vector-borne disease. Preliminary quantification of tick species on these animals has been performed from 2007, when the enclosed facility was constructed, to present (2018). These counts were basic in nature – encompassing four small regions of the body and searching only for notable ticks, most commonly of the family Ixodidae.

However, it was noted over the course of this nine-year quantification that ectoparasitism by ticks had significantly increased within the facility, especially on male white-tailed deer. We seek to build upon these previously compiled data to gain a more complete understanding of the ectoparasite community, and how increased host density could affect not only the aggregation of ectoparasites but also the potential transmission of relevant pathogens. In doing so, we would also like to analyze the validity of commonly used ectoparasite quantification techniques for large-bodied mammals and introduce methods that increase efficiency and decrease capture stress for these animals. Obtaining accurate quantification of ectoparasite communities on these deer could help clarify the community dynamics and ecology that occur in habitats of high host density and low biodiversity.

## **Methods**

Deer sampling and ectoparasite collection was completed at Piedmont Substation in collaboration with Auburn University Deer Lab, and Dr. Stephen Ditchkoff. The substation is approximately 570 hectares of Auburn University owned and operated land that hosts a variety of agricultural experiments and livestock, as well as a captive deer facility of just over 12 hectares. The field lab was constructed in 2007 and maintains this population of captive deer year-round, containing approximately 100-120 individuals within 2.6 m fencing. Population numbers are maintained within the facility through natural mortality, removal of 10-15, 6-month old fawns per year, and myopathies'. Sex ratios are approximately 1:1. 90% of contained deer are marked with two cattle ear tags and an electronic identification tag. We chose this facility because of the high-density of deer within. White-tailed deer serve as both important breeding hosts for ectoparasites but also excellent sentinel species for vector-borne illness. Thusly, within the facility we would expect a larger ectoparasite population, which provides the ideal

environment for comparison of quantification methods. Deer are darted from September to February of each year, with population demographics determining the sex breakdown of animals sampled. Deer were darted once per season at elevated stands located near feeders, with sex, age, and activity patterns deciding what sex or size were captured. From September to January, primarily bucks were darted, with doe darting occurring usually between February and March. While data from the facility is available from 2007 and ticks have been historically quantified on these animals – we sought to obtain as accurate a quantification of ectoparasites as possible through a comparison of quantification methods. As such this study contains only deer sampled from September to February of 2016–2017 and September to January of 2017-2018. This dataset encompasses 63 male white-tailed deer between the ages of 1.5 years to 10.5 years.

A tranquilizing solution was prepared with a 4mL combination of Telazol (Fort Dodge Animal Health, Fort Dodge, IA; 100 mg/ml given at a rate of 4.5 mg/kg) and xylazine (Lloyd Laboratories, Shenandoah, IA; 100 mg/ml given at a rate of 2.2 mg/kg). This protocol was approved through Auburn University’s Institutional Animal Care and Use Committee (2008-1417, 2008-1421, 2010-1785, 2011-1971, and 2013-2372) and followed the American Society of Mammologists’ guidelines (Sikes & Gannon 2011). Tranquilizing solution was then loaded into a dart with a telemetry transmitter mounted in the opposite end (Pneu-Dart, Williamsport, PA) which releases a drug dosage on impact. Immobilized deer were recovered using radio telemetry. Once recovered metrics of body condition and health were measured. Measurements included; skull length, total body length (from nose to tail base), tail length, neck girth (at halfway), chest girth (posterior of the shoulder), and right hind foot length (with hoof pointed, from the tip to the posterior end of the tuber calcis). Antler measurements were taken for captured males, using a

Boone and Crockett scoring system which measures: inside spread, main beam length and circumference, and the length and circumference of any tines longer than 1inch (Nesbitt, 1981).

Ectoparasite quantification occurred in three separate stages. First, photographs were taken of the anus, anogenital region, scrotum, ventrum, ear, neck and face which are cataloged as preferred index sites for ectoparasites (Watson and Anderson, 1975, 1976) and are areas of high ectoparasite concentration, due to hair length, access to skin, and bloodmeal. Photographs were taken at 12-14 inches from each focal area. Photographs were later analyzed to obtain a count of all visible ectoparasites. Images were analyzed using imaging processes software in RAW formatting to preserve high quality and magnification ability. A grid overlay was used, and images magnified to 200% (Figure 4). Ectoparasites were counted per grid cell and summed per photograph. The number of ectoparasites was recorded per photograph.

Using techniques described by Keiser (1987), an expedited estimation of ectoparasites was collected from each captured animal. Specific regions of each captured animal were combed through with a single flea comb and fine-toothed steel forceps. These areas were contained on  $\frac{1}{2}$  of the body surface, with the animal laying on its side. Quantification numbers were then multiplied by 2 to obtain an estimate of burden across the entire body. Areas of focus for  $\frac{1}{2}$  estimation were; the face and neck (to the shoulder), ears (inside and outside), along the spine (2-4 inches in width on one side) and the anogenital region (the anus to base of tail, surrounding scrotum and to the femoral region).

A full body count was then conducted using the same comb and forceps method as a standard number for ectoparasite burden. A count was completed on one side of the body, excluding areas previously counted during a  $\frac{1}{2}$  body estimation. This included: from the shoulder to the rear flank, and from the sternum to the femoral region. A count was then completed for the

entirety of the side that had not been included in the ½ body estimation. All visible ectoparasites were sorted into respective categories (lice, ticks, keds, fleas, other, etc.)

Following the completion of quantification methods, representative samples were collected from each captured animal to identify ectoparasites at the species level when possible. Seven species were collected from deer; including tick species described in the previous chapter with the additional keds and louse, *Lipoptena mazamae* (Neotropical deer ked) and *Tricholipeurus lipeuroides* (chewing lice) (Figure 5). All ectoparasite quantities were collected into a combined worksheet that compared the three methods to one another directly. It should be noted that ectoparasites were not distinguished to species during quantification, and that representatives were taken based on identification.

Using program R (version 3.0.3, [www.project-r.org](http://www.project-r.org), accessed Oct 2017) two-sampled t-tests were completed to compare the ectoparasite counts obtained through each method. A Welch's two sampled t-test was used to compare the values collected for individuals. Tests were run comparing each method; full body to half body, full body to photograph and half body to photograph individually for each ectoparasite category. Additional analysis was performed relating to the heterogeneity of ectoparasite abundance in order to look at how ectoparasite aggregation using generalized mixed-effect regression packages (glmmTMB) in program R. Using a principle component analysis (PCA) to create a body score from three measured metrics (body length, chest girth, hind foot length) each deer was assigned a 'body size' score correlating to these three collinear variables. In addition to the generated body size score, annual antler size, determined from a Gross Boone and Crocket score (Nesbitt 1981), and estimated age at capture were used in the models. These models were run separately for each quantified ectoparasite group (ticks, lice and keds) After testing for over-dispersion in the dataset, we selected an

appropriate family measurement for the statistical argument (Poisson) and evaluated for zero-inflation within the dataset. All models included a random term to control for the repeating sample of male deer over the two study years and to account for variation that occurs in ectoparasitism over the seasonal period.

## **Results**

Sixty-three male deer were sampled throughout the course of September 2016 – February 2017 and September 2017 – December 2017. Comparisons were made between methods to discern if differences in the quantified number were statistically significant. A significant difference would denote that methods being compared did not yield similar ectoparasite counts. There was no significant difference between the full body and half body estimation techniques (Table 2). However, there was a significant difference in each ectoparasite group between the full body count and the number obtained from photographs. A direct comparison of quantifications between the full body and half body method showed that these counts mirror each other closely, again showing that these methods are directly comparable (Figure 6). The ½ body estimation technique, based on these results, is a valid method of more efficient ectoparasite quantification for this population of white-tailed deer.

Preliminary analysis of ectoparasite aggregation shows several emerging trends throughout the deer sampled (Figure 7). In all 63 deer over the course of two study seasons deer keds were found on the animal, and in 100% of sampling were found along the spine and anogenital region of the animal, less often (50%) on the head and neck. Keds were most often concentrated in the anogenital region but also found throughout the body. Seventy-five percent of quantifications found ticks on the neck and ears of the animal, often on the backside of the ear, around the eyes, at the base of the skull and near the sternum. Twenty-five percent of

quantifications found ticks on the spine, though generally closer to the animals' head, and 25% of the time ticks were found in the ano-genital region, located on the bare skin surrounding the surrounding the anus. Lice were the least prevalent target species throughout the course of the study, with a much lower quantification rate than the other two ectoparasite targets, likely due to their smaller size. Fifty percent of quantifications found lice on the anogenital region, in the course fur of the tail or surrounding the anus. Lice were found in < 10% of quantifications along the spine, but in 25% of quantification counts on the face and neck, often within or surrounding the ear.

The principle component analysis used to generate a 'Body Size' metric explained 80% of the variation within the sampled population, and the variables were highly collinear as expected for metrics of health. Measures of morphometrics were variable, but on average increased until sexual maturity and males reached prime breeding success (Table 3).

Analysis of host factors that could be affecting ectoparasitism was completed using glmmTMB models in program R. Because ectoparasite data consists of quantification counts, a Poisson distribution was used for all included models. Models indicated that there was a significant effect of 'body size' and annual antler score on the number of ticks present on an individual. We observed that for each increase in body size (increased chest girth and body length) an individual was 0.314 times more likely to have ticks present ( $p=0.0496$ ). The effect of 'body size' on tick abundance is significant, but with weak effect. Models indicated that there was a significant negative effect of annual antler size on tick quantification. We observed that for each increase in annual antler score (cm) an individual was 0.336 times less likely to have ticks present ( $p=3.91e^{-08}$ ). These results suggest that tick abundance is affected by both 'body size' and annual antler size, but that annual antler size has a much more significant effect. Ticks were found in fewer

numbers on deer with larger antlers when compared to their body size. There was not a significant effect of age on the abundance of ticks found on sampled males. Models used for the analysis of deer keds (*I. mazamae*) indicated that there was a significant effect of antler size on the number of keds present on an individual. We observed that for each increase in annual antler score (cm) an individual was .005 times more likely to harbor keds ( $p=0.0331$ ). We did not however, note a significant effect of ‘body size’ or age on the abundance of deer keds. Models also indicated that there was a significant effect of annual antler size on lice abundance. We observed that for each increase in annual antler size an individual was 0.059 times less likely to harbor lice ( $p=1.7e^{-05}$ ). There was no significant difference noted between years for ticks or keds quantified between sampling years, but significantly less lice ( $p=0.001$ ) were found on hosts in 2017 than during the 2016 sampling (Figure 8).

## **Discussion**

Full body counts, when compared to the experimental standard method for quantification on large bodied animals were similar. The half body estimation method of measuring specific regions of an animal and doubling those numbers proves accurate in comparison to full body searches. There was variation present between individuals, where the half body estimation would under or overestimate the presence of ectoparasites, but there was no significant difference between the quantification of ectoparasites using either method.

The photography method presented did not prove to be an accurate method of quantification for ticks, lice or keds species. While largely insignificant for ticks and lice, the p-value for deer keds was much closer to significant. Photographs were most useful in areas where contrast allowed ectoparasites to be more visible through the fur – as the photography method lacked the more precise brushing that the full and half body estimation methods used. This

means that ectoparasites close to the skin, or deep within the fur of the animal were often overlooked or not visible in photographs. However, ectoparasites on the skin surface or visible against the lighter fur on the ventrum and inner ear of the animal were very easy to quantify using this methodology. *L. mazamae* were concentrated on the ventrum and anogenital region of the sampled animals, meaning a large portion of these ectoparasites were recorded using the photography method. These results can be considered of value for the population studied, and while no statistically significant difference was detected between methods, a separate researcher performing these methodologies may obtain different results. Interobserver repeatability would have increased the reproducibility of a study of this nature, but due to limited time and lack of volunteers was not possible. A more comprehensive comparison could be completed with multiple researchers that compares similar methods.

Collection of representative samples was done based on sight, with individual ectoparasites chosen from the body. In this way, our representative samples may be biased toward individuals that were larger or easier to see against the landscape of the deer. Chewing lice and sucking lice were the most difficult to collect and differentiate on the body, due to their small size and close-skin contact.

Over the course of this study, however, we were able to catalog the patterns of infection and prevalence on white-tailed deer. We noted an increase in ectoparasite infestation by ticks and keds as male deer moved into the period of rut and during mating. Deer within the capture facility mate from late November to January, with a peak in early January. We note that male necks begin to swell in early November, likely due to an influx of testosterone as they prepare for mating competition. Further study of hormone patterns in captive deer would further elucidate this hormone cycle. Infestation numbers of ectoparasites peak in mid-November, and

we also began to note ectoparasites mating on hosts. Keds found along the spine and ano-genital region were often seen clumped in groups of mating individuals, and mating ticks were found on the neck and sternum. It is possible that these mating ectoparasites are utilizing increasing levels of testosterone in male animals, and the increased contact with potential hosts that occurs during mating and sexual competition.

We also note that keds are the only group where annual antler size has a significant positive effect. For both ticks and lice, as annual antler size increased these ectoparasites were less likely to be quantified. This could be attributed to community dynamics of ectoparasites. As keds were more likely to be located on males with larger antlers – ticks and lice may be utilizing other hosts to minimize resource competition for blood meal or mating opportunities.

This population lends itself to controlled ectoparasite study due to its contained nature and long-term dataset, as well as the understanding of parentage and genetic makeup. Within this high-density population, we note an increased density of ectoparasites. However, the demographics of this population and results obtained are limited by the nature of the contained facility. Animals within are fed in addition to natural intake, and most males are of increased health and age than what may be found in natural populations. Deer within the facility face no hunting pressure, and often die of old-age and more rarely, capture myopathy. These deer are likely in better condition than their same-age wild counterparts would be, as a result can host an increased number of ectoparasites. Factors affecting heterogenous infection throughout the population may not be as pronounced as they would be in a wild population, as the deer are in exceptional shape per age class.

### **Chapter 3:**

*Bartonella* Spp. In Alabama White-Tailed Deer (*Odocoileus virginianus*)

## **Introduction**

Bartonellosis is a vector-borne disease caused by the gram-negative bacteria of the genus *Bartonella*. These bacteria infect erythrocytes, endothelial cells and macrophages, which can lead to persistent blood-borne infections. Bacteria of the genus *Bartonella* are variable and there is the significant opportunity for the pathogenic bacteria to be ingested by arthropod vectors that feed on humans and wildlife (Billiter et al.2008). *Bartonella* has been confirmed to be transmitted through lice, fleas or sandflies, with significant evidence that also suggests it may be transmitted through tick species. Several species of *Bartonella* have been identified as zoonotic agents, including *Bartonella henselae*, *Bartonella quintana*, and *Bartonella bacilliformis* the causative agents for Cat-scratch fever, Trench fever and Carrion's disease, respectively (Boulouis et al.2005; Chomel et al.2006; Raoult et al.2006). Historically little was known about members of the genus *Bartonella*, but more recently it has become of increasing importance due to its emerging status as a zoonotic disease the potential of transmission by arthropod vectors. *Bartonella* is of notable importance because of the nature of infection by bacteria of this genus. *B. henselae* manifests as a chronic infection associated with swollen lymph nodes, reoccurring fever, fatigue and headaches, memory loss, disorientation and immune suppression (Bass et al.1997). While immunocompetent individuals typically clear infection without treatment, *B. henselae* can be a danger to the young or sick. The disease can also disseminate into body organs (Carithers 1985). The causative agent of Carrion's disease, *B. bacilliformis*, however persists as a

sudden and life-threatening fever and decreased red-blood cell circulation. It is considered the deadliest species of *Bartonella* to date (Huarcaya et al.2004). *Bartonella quintana* is also an infection capable of relatively benign bacteremia or severe illness and fever. It is seen more often in chronic cases of reoccurring fever (Brouqui et al.2006). These species of *Bartonella* have been found historically in wildlife, and several others including *B. schoenbuchensis* and *B. bovis* are also thought to cause dermatitis in humans. Because of the zoonotic potential of *Bartonella* species, infected wildlife could pose a significant risk to humans through hunting and butchering or the antagonistic contact that often occurs with urbanized wildlife. Traditionally, it was thought that only *B. quintana* (trench fever) and *B. bacilliformis* (Carrion's disease) were disease causing in humans but since the 1900's six species of *Bartonella* have been associated with clinical manifestation of symptoms (Jacomio et al.2002). *Bartonella* has currently been described in 20 species (Dehio and Sander 1999).

*Bartonella* transmission has been confirmed in only four species *Lutzomyia verrucarum* (sandfly) (Battistini 1929, 1931), *Pediculus humanus humanus* (body louse) (Swift 1920), *Ctenocephalides felis* (cat flea) (Chomel et al.1996), and *Ctenophthalmus nobilis nobilis* (rodent flea) (Parola et al.2003). However, *Bartonella* has many suspected vectors, including several dog and rodent fleas, members of the genus *Lipoptena*, biting flies, and ticks (Ishida et al.2001; Parola et al.2003; Kim et al.2005; Dehio et al.2004; Halos et al.2004; Chung et al.2004). The competency of these vectors where *Bartonella* species have been isolated still requires considerable research. Halos et al.2004 found that 94% of collected keds and other *Hippoboscidae* harbored *Bartonella* DNA. Additionally, Dehio et al.2004 were able to isolate *B. schoenbuchensis* in the midgut of *Lipoptena* keds and speculated that *B. schoenbuchensis* could be the cause of deer ked dermatitis in humans. Vertical transmission of *B. schoenbuchensis* has

been noted by *Lipoptena* keds, further suggesting that they are also a competent vector of *Bartonella* (Bruin et al.2015). Ticks have also been of increasing focus in relation to members of the *Bartonella* genus. Relatively few studies have been completed that isolate *Bartonella* DNA in tick species, mainly through PCR reaction. *Ixodes scapularis* (blacklegged tick) and *Dermacenter variabilis* (dog tick), two of four ticks commonly found in the Southeastern United States, are among several tick species where *Bartonella* spp. have been identified.

Here we seek to better understand the role of *Bartonella* in wildlife, explore how this *Bartonella* spp. may be transmitted through wildlife, and what isolation of *Bartonella* DNA in collected samples could mean for the local disease ecology. We use a novel study system of semi-wild white-tailed deer to explore the prevalence of blood-borne pathogens in Alabama. These semi-wild deer are enclosed completely by 2.6m fencing and present a significant area for ectoparasitism by ticks, keds, fleas, lice and other obligate bloodfeeding ectoparasites. Within this high-fence facility deer are present in much higher density than would otherwise be found in the wild and provide a unique opportunity to study the dynamics of this system. Ectoparasites have been noted to show preference for animals that are in greater nutritional condition (Christe et al.2003). White-tailed deer are also known host species for a wide range of suspected vector species for *Bartonella* bacteria, including lice, biting flies, *Lipoptena* keds, and *Ixodes* ticks (Wedincamp and Durden 2015). We are interested in investigating what species of *Bartonella* are harbored in white-tailed deer that may be fed on by ectoparasites capable of traversing outside of their captive facility. Specifically, we wanted to know how infected animals were parasitized, and if they were more fed on by a group of potential vector species than another. We use white-tailed deer as sentinel species to identify what species of *Bartonella* are present in

central Alabama, but also to help elucidate potential or suspected vectors that may serve as the main arbovectors of *Bartonella* species.

## **Methods**

Deer sampling and ectoparasite collection was completed at Piedmont Substation in collaboration with Auburn University Deer Lab. The substation is approximately 570 hectares of Auburn University owned and operated land that hosts a variety of agricultural experiments and livestock, as well as a captive deer facility of just over 174 hectares. The field lab was constructed in 2007 and maintains this population of captive deer year-round, containing approximately 100-120 individuals within 2.6 m fencing. The captive population consists of wild animals captured during the construction of the fencing and their descendants. Bordering the facility are kept domestic livestock, unmanaged forest, and rural neighborhoods (Figure 9). Deer are free fed 16-18% extruded protein pellets (Record Rack, Nutrena Feeds, Minneapolis, MN) year-round from three feeding stations, and during darting periods from September to February three timed feeders provided ~2kg of shelled corn per day. Population numbers are maintained within the facility through natural mortality, removal of 10-15, 6-month old fawns per year, and myopathies'. Sex ratios are approximately 1:1. Breeding occurs from mid-December to mid-February, with a peak in fawn conception at approximately January 18. Deer sampled for *Bartonella* were darted between September of 2016 – January of 2018 – in two respective trapping seasons. All sampled deer were male, and 63 deer were included in this study. Ages of collected males ranged from 1.5 – 10.5 years.

An anesthetization serum was prepared each day during the darting season. The solution consisted of a 4ml combination of xylazine (Lloyd Laboratories, Shenandoah, IA; 100mg/ml given at a rate of 2.2 mg/kg) and Telazol (Fort Dodge Animal Health, Fort Dodge, IA; 100 mg/ml given at a rate of 4.5 mg/kg). This 4ml was loaded into an impact release dart with a

mounted telemetry transmitter (Pneu-Dart, Williamsport PA). Each night, 2-4 darters were deployed to elevated stands with modified rifles ranged at approximately 20m. Deer were darted once per season at elevated stands located near feeders, with sex, age, and activity patterns deciding what sex or size were captured. From September to January, primarily bucks were darted, with doe darting occurring usually between February and March. Once immobilized and recovered, metrics of body condition and health were measured. Measurements included; skull length, total body length (from nose to tail base), tail length, neck girth (at halfway), chest girth (posterior of the shoulder), and right hind foot length (with hoof pointed, from the tip to the posterior end of the tuber calcis). Antler measurements were also taken for captured males, using a Boone and Crockett scoring system which measures: inside spread, main beam length and circumference, and the length and circumference of any tines longer than 1inch (Nesbitt, 1981). At initial capture, sex and tooth wear and replacement were recorded. Animals were given a unique 3-digit identifier, that was displayed on ear tags and freeze branded on the front shoulder and hindquarter. Tissue samples were collected from an ear notch to record genetic information and determine parentage of yearly borne fawns.

Approximately 10ml of whole blood was collected from the jugular vein of each captured animal. 5ml of this was spun down to serum and frozen for later analysis, the other half kept whole and stored in EDTA vials. Blood was then kept frozen until needed for further analysis. DNA extraction was performed on 100 $\mu$ l of whole blood, using a Qiagen DNAeasy Blood and Tissue Extraction Kit (Qiagen # 69506, Georgia, USA) which is designed for rapid and total purification of present DNA and optimizes for PCR identification. To do this, 100 $\mu$ l of blood was mixed with 20 $\mu$ l of Proteinase K and 100 $\mu$ l of Phosphate Buffered Saline Solution. This solution was vortexed to mix, and then 200 $\mu$ l of Buffer AL (kit provided) added. Samples were

then incubated at 56°C and 300rpm for 10-15 minutes. Following this, 200µl of 100% Ethanol was added and vortexed again to ensure thorough mixing. Samples were then transferred to DNAeasy mini spin columns and collected tubes (2ml). All samples were centrifuged and 8000rpm for 1 minute, and then the flow through into the collection tube was discarded. This washing occurred twice more, were 500µl of pre-prepared buffers (AW1 and AW2, respectively) were used and centrifuged at 8,000rpm for 1 minute and then at 13,300rpm for 3 minutes during the second washing. Each time the flow through and collection tube were discarded and replaced. Samples were then washed with a final 200µl of AE buffer and incubated at room temperature for 1 minutes before being centrifuged a final time at 8000rpm for 1 minute. This eluted the extracted DNA. We completed this final washing step an additional two times with molecular water in place of AE buffer to increase DNA yield and create duplicates of samples.

The resulting samples were then analyzed through traditional PCR using a Qiagen HotStar Taq Plus Master Mix Kit (Qiagen # 203645, Georgia, USA) where DNA extract samples were combined with pre-prepared Master Mix and RNA free water, as well as primers created through NCBI to broad genus level specification of the focus pathogens. Primers were created based on 16S RNA genes and made to detect as many species as possible (Table 4). Gel electrophoresis was performed on a 2% gel and run at a consistent 100v for 35-40 minutes before being imaged using Azure Blue Software. Gel electrophoresis was performed to screen for positives, and then following further amplification and verification of those positive samples was completed through Sanger Sequencing by Eurofins.

Quantification of ectoparasites present on animals was performed as previous chapter of this project. During this, ectoparasites were counted on the animal in three ways to compare the validity of quantification methods. First was a photography method, then a ½ body estimation

technique based on work began by Keiser (1987) and finally a full body quantification. We focused on three blood-feeding vector species for a variety of wildlife and human pathogens, ticks, lice and keds. From each deer, representative samples were collected and preserved in 90% ethanol for later study. Individuals were speciated and sexed when possible.

Using program R (version 3.0.3, <http://www.rproject.com>, accessed January 2018) we analyzed the number of ectoparasites (ticks, lice and keds) present on an animal to determine if ectoparasite burden differed between infected and non-infected individuals.

## Results

From white-tailed deer we collected a variety of ectoparasites, some of which are considered suspected vectors of the genus *Bartonella*; *Ixodes scapularis* (blacklegged tick), *Amblyomma americanum* (lone star tick), *Amblyomma maculatum* (gulf coast tick), *Rhipicephalus sanguineus* (brown dog tick), and *Dermacenter variabilis* (American dog tick), *Lipoptena mazamae* (neotropical deer ked), and *Tricholipeurus lipeuroides* (chewing lice). Because of the correlation of *Bartonella* infected with deer keds, *L. mazamae* we have included photographs of representative specimens and mating keds on male deer (Figure 10).

Ten of the 63 samples tested positive for *Bartonella* spp (15.8%). It was hypothesized that the most common *Bartonella* infection would be that of *Bartonella schoenbuchensis* as it had previously been isolated in other ruminants such as roe deer (Dehio et al.2001). However, we noted a wide variety of species isolates. Most prevalent was *Bartonella melophagi* (6.3%) with four positives, generalized *Bartonella* spp. (4.7%) with three positives, *Bartonella bovis* (3.2%) with two positives, and finally *Bartonella schoenbuchensis* (1.5%) with one positive (Table 5). Positive *Bartonella* samples had a best match to four species of the *Bartonella* genus (Table 5); between 98-100% query coverage; 95.9-99.8% identity; (Accession number pending, Submission: 2099212). It should be noted that the highest sequence match of samples positive

for *B. melophagi* is identified as *Wolbachia melophagi*, which has since been determined to be more closely related to the *Bartonella* genus (Dulmer et al. 2001; Lo et al. 2007). Of those infected, 3 were symptomatic with dermatitis of the skin (4.76%). We noted a positive trend of infection in relation to infestation by neotropical deer keds, which may play a role in the transmission of *Bartonella* through this system, however all deer infected with *Bartonella* were also infested by ticks (Table 6). There were no deer with *Bartonella* infection that tested positive both sampling years, of the deer that were collected multiple times.

Rankins et al. (in prep) had previously tested a subset of this population for *Anaplasma* spp. and found a high occurrence of the pathogen within those deer. Of the ten deer that tested positive for *Bartonella*, four of these also tested positive for *Anaplasma plays*. *Bartonella* species found in deer coinfecting with *Anaplasma* were: one *B. bovis*, one *B. schoenbuchensis*, and two generalized *Bartonella* spp. These deer showed coinfection with *Bartonella* and *Anaplasma* within the same time-period, potentially vectored by the same ectoparasites.

Sampled deer had on average 27.87 ticks (SE 3.05), 9.65 lice (SE 1.38) and 92.82 keds (SE 6.455). Deer infected with *Bartonella* had an average of 8.5 (SE 2.75) lice which was lower than 9.9 (SE 1.56) on non-infected individuals but not statistically significant. Deer infected with *Bartonella* had significantly more ticks and deer keds than non-infected individuals. Infected individuals had an average of 43.9 (SE 9.73) ticks as compared to 24.5 (SE 2.91) in non-infected individuals ( $p=0.015$ ). Deer infected with *Bartonella* also had an average of 119.6 (SE 14.23) keds per individuals as compared to 87.3 (SE 4.34) keds per individual found on those that were not infected ( $p=0.006$ ) (Figure 11).

## **Discussion**

Here, we document the infection of white-tailed deer with various *Bartonella* spp. Infection by *Bartonella* has been documented in a wide variety of taxa and has been known to cause disease

in both humans and wildlife. *Bartonella* has also been associated with transmission by a wide variety of arthropod vectors, including lice, fleas, ticks, and keds. *Bartonella schoenbuchensis* has been found in high prevalence in other ruminants, in both Canada and Germany, though has been lesser studied in the United States.

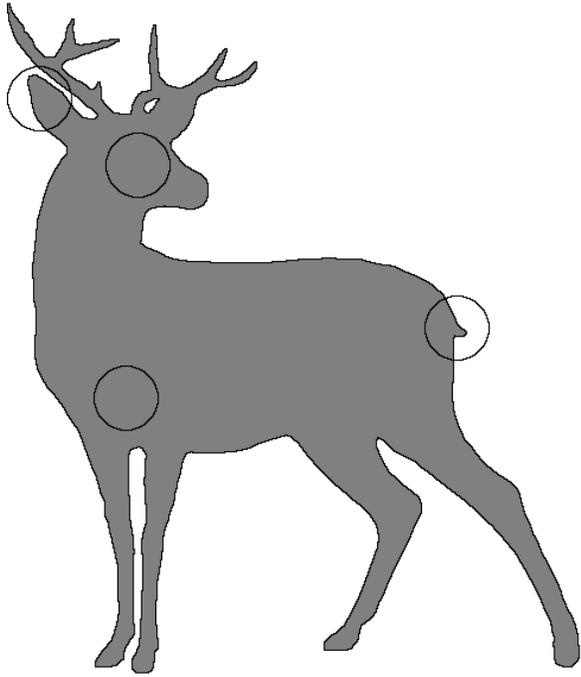
It was expected that we would find *B. schoenbuchensis* in our deer population due to its nature as a ruminant disease and that it has been suspected to be transmitted by a wide variety of vectors, including ticks and deer keds. However, we noted a wide variety of *Bartonella* species found within our deer, least of which was the expected *B. schoenbuchensis*. The identity matches of sequences were very high, however the nature of closely related *Bartonella* genus is that differences in product length from PCR reactions are very small. qPCR techniques with specific probing for *B. schoenbuchensis* could be the next step to confirming or refuting this wide variety of isolates from our deer population. Additionally, it is hypothesized here that white-tailed deer within this population could be served as reservoir hosts for non-typical species of *Bartonella* to cervids because of the transient ectoparasite population. We also note the occurrence of coinfection with *Anaplasma platys* and *Bartonella* species within this population. *Anaplasma* is most commonly vectored by *R. sanguineus*, from which *B. henselae* has been previously isolated in California (Wikswow et al. 2007). Coinfection has been previously identified in white-tailed deer population in British Columbia by both *Anaplasma* and *Bartonella* species (Lobanov et al. 2012).

Ectoparasites and wildlife smaller than the enclosed deer, including coyotes, feral cats, skunks, raccoons, opossums, squirrels, bobcats, and foxes have been noted on camera traps within the facility but also have the capability to move through the fencing. Livestock cattle are kept on-facility just outside the fencing and could contribute to the presence of *B. bovis* within

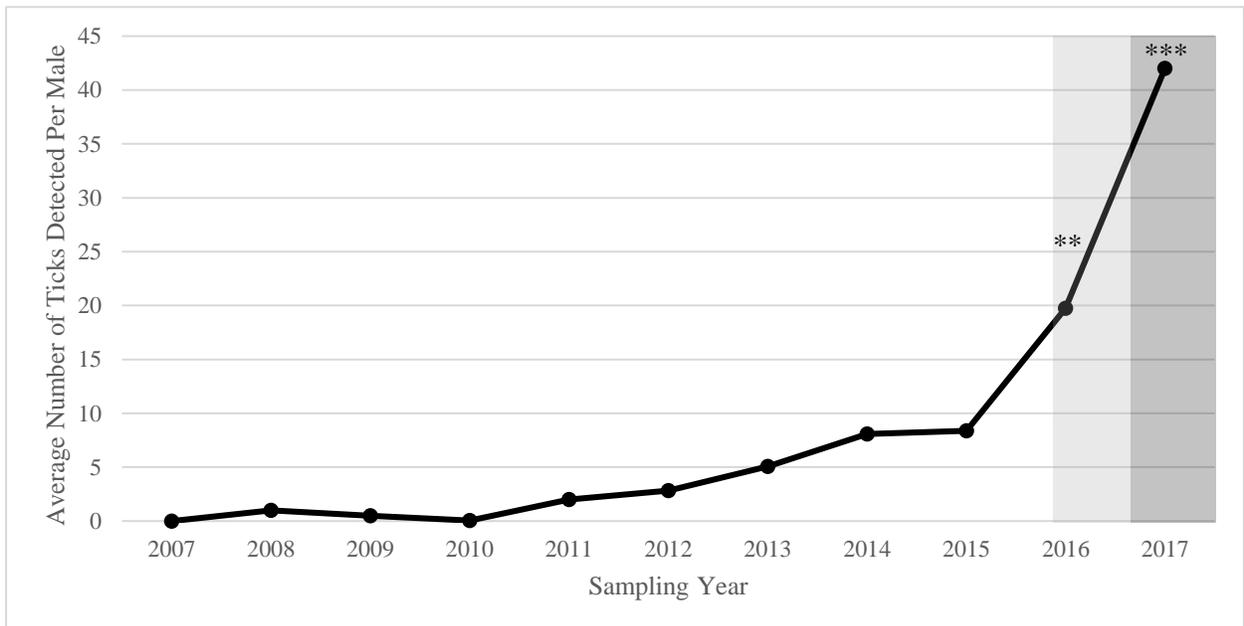
the fence. Additionally, the research facility is surrounded by urban neighborhoods, where ectoparasites could encounter domestic pets and humans. Understanding the movement of ectoparasites within this population, and sampling species just outside and within the facility vicinity could help to better elucidate the nature of the situation. Are livestock cattle infected with *B. bovis*? Do domestic pets and wildlife near the capture facility carry other members of the *Bartonella* genus into the fence? Do we note the parasitism of *Lipoptena mazamae* and Ixodid ticks on these cattle, wildlife and domestic pets? Because keds were found on every individual captured in a relatively high number, we believed that *Bartonella* has great capability to move through this system if transmitted by keds. However, isolation of *Bartonella* spp. in *L. mazamae* cannot confirm vector competency in this case, only that keds had ingested infected blood from host animals.

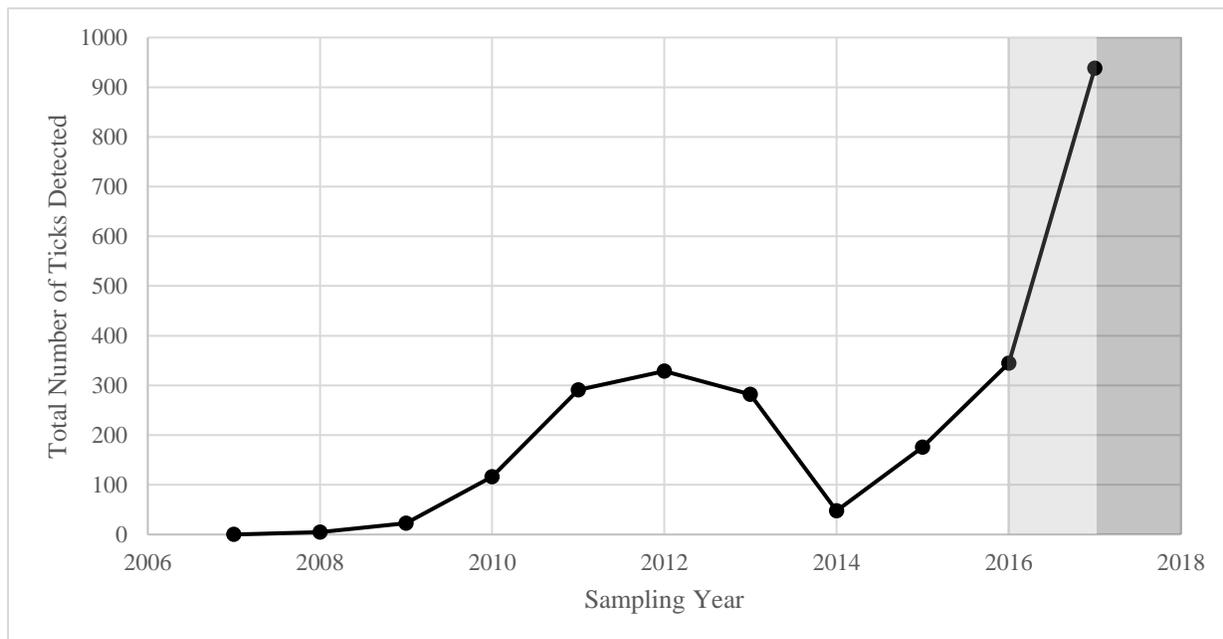
## Figures and Tables

**Figure 1:** Representation of tick detection on darted white-tailed deer. Ticks were counted on the sternum in a 5in x 5in area, on the bare skin and short fur surrounding the anus, on the anterior and posterior surface of both ears, and surrounding both eyes.



**Figure 2:** Average number of ticks detected per male deer, and total number of ticks detected from the beginning of sampling (2007) to the current year (2017). A new quantification method was implemented in September of 2016 – as denoted by the shading.

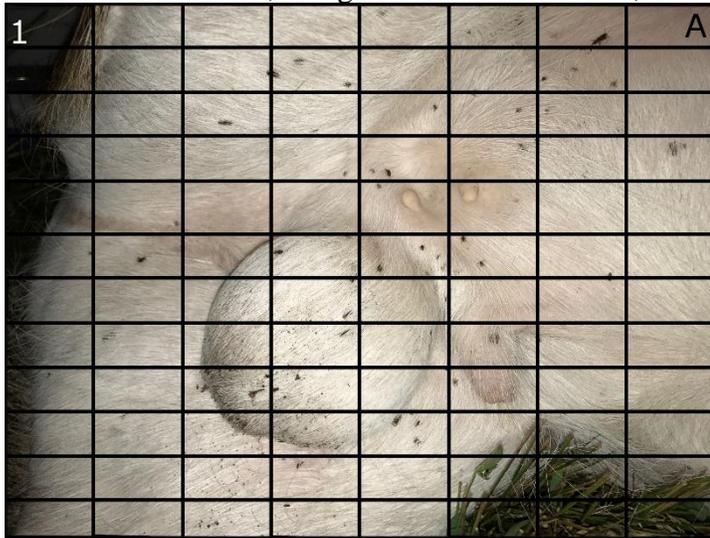




**Figure 3:** Ticks species identified at Auburn University’s Deer Lab facility. A. *Ixodes scapularis*, (male) B. *Amblyomma americanum*, (female) C. *Amblyomma maculatum*, (male) D. *Rhipicephalus sanguineus*, (female) and E. *Dermacenter variabilis* (female). Ectoparasites were viewed at 20 times magnification under a dissecting microscope.



**Figure 4:** Grid overlay of one photograph of a male-white tailed deer. Photographs are viewed at 200% magnification and ectoparasites counted per grid-cell and summed per photograph. Each individual has photographs taken of the; anogenital region, along the spine and one side, the inside and outside of the ear, along the neck and sternum, and the face/eyes.

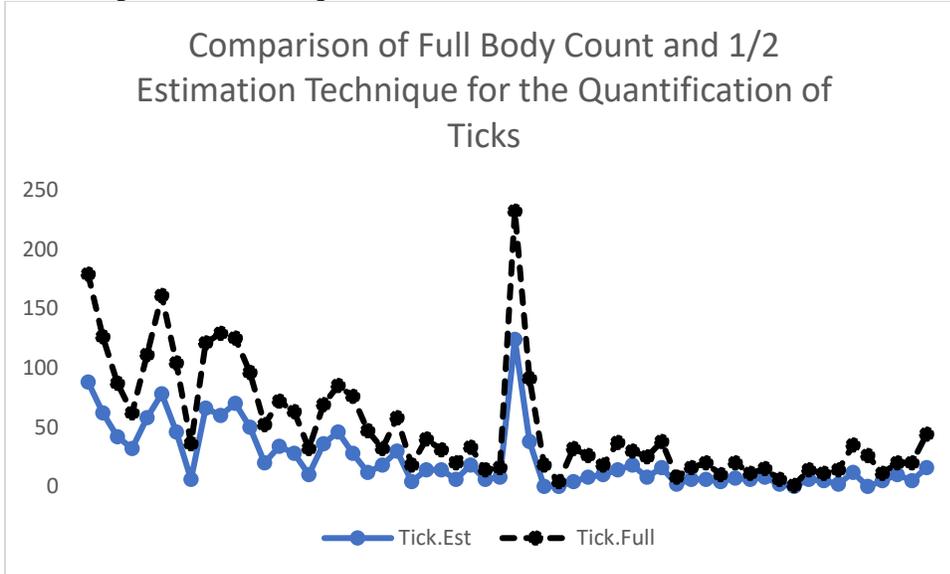


**Figure 5:** Photographs of representative ectoparasite specimens collected from white-tailed deer. A. *Ixodes scapularis*, (male) B. *Amblyomma americanum*, (female) C. *Amblyomma maculatum*, (male) D. *Rhipicephalus sanguineus*, (female) E. *Dermacenter variabilis*, (female) F. *Lipoptena mazamae*, and G. *Solenopotes binipilosus*. Ectoparasites were viewed at 20 times magnification under a dissecting microscope.

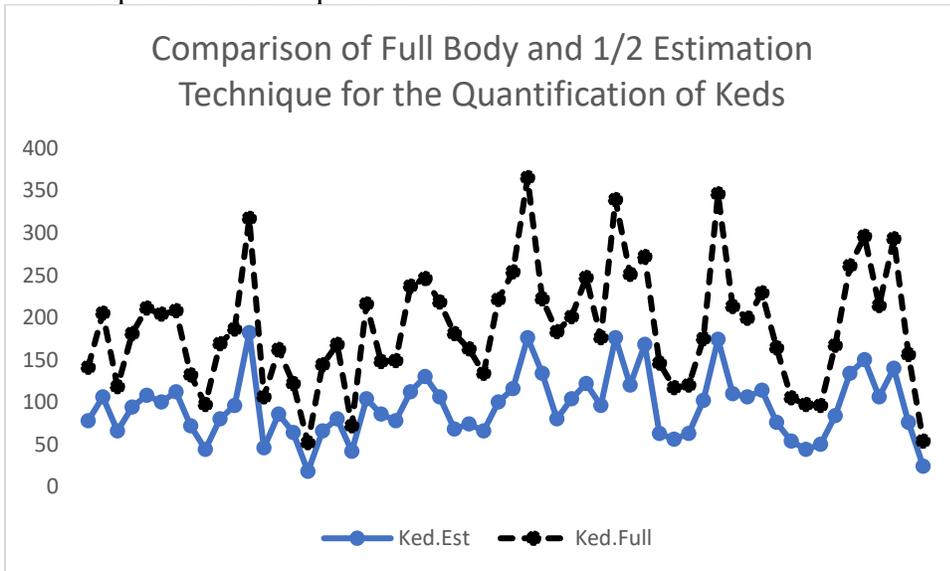


**Figure 6:** Comparison of 1/2 body estimation technique and full body quantification of ectoparasites on 63 sampled male white-tailed deer. Comparisons are broken down by ectoparasite group.

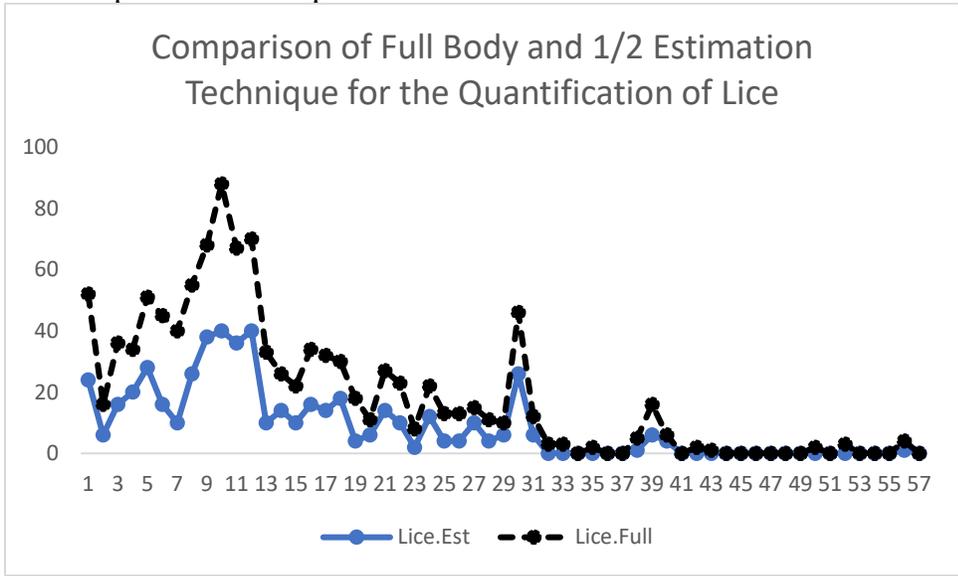
**6a:** Comparison of tick quantification.



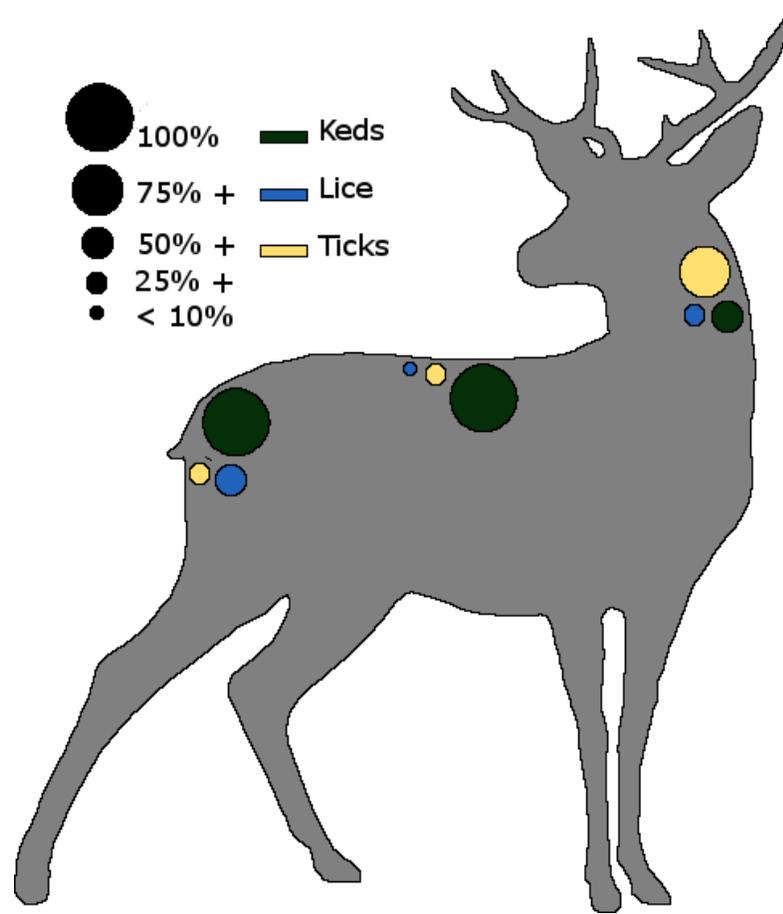
**6b:** Comparison of ked quantification.



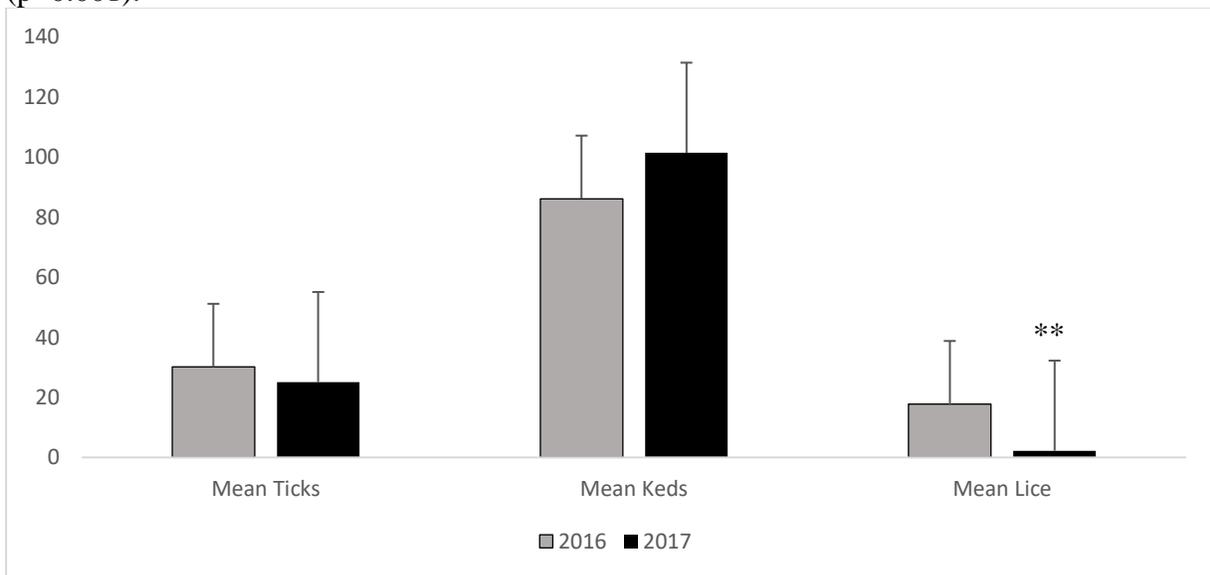
6c: Comparison of lice quantification.



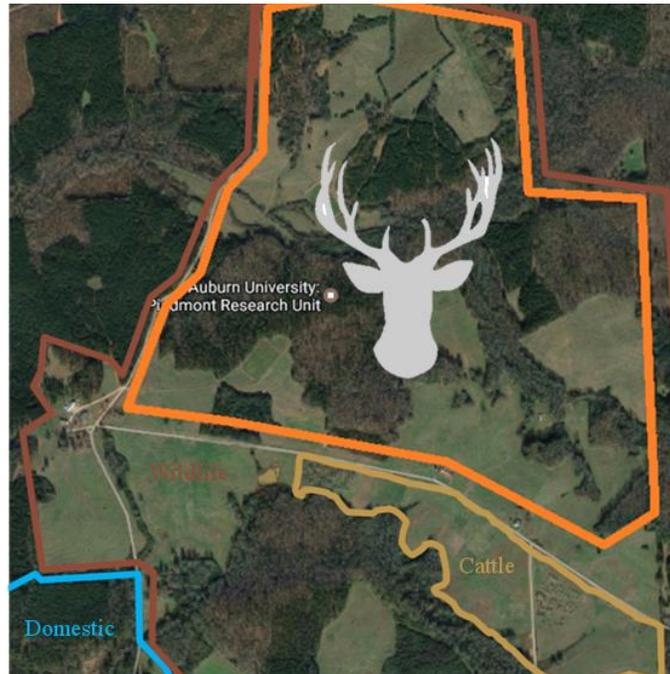
**Figure 7:** Representation of ectoparasite quantification on male white-tailed deer. Circle size correlates to the percentage of time ectoparasite groups were found at specific locations. Color denotes ectoparasite group.



**Figure 8:** ectoparasites individual. Mean quantified per individual. Significantly less lice were found on individuals in the 2017 sampling year ( $p=0.001$ ).



**Figure 9:** Satellite map that shows the boundary of the deer facility and habitat types that can be found in the vicinity.

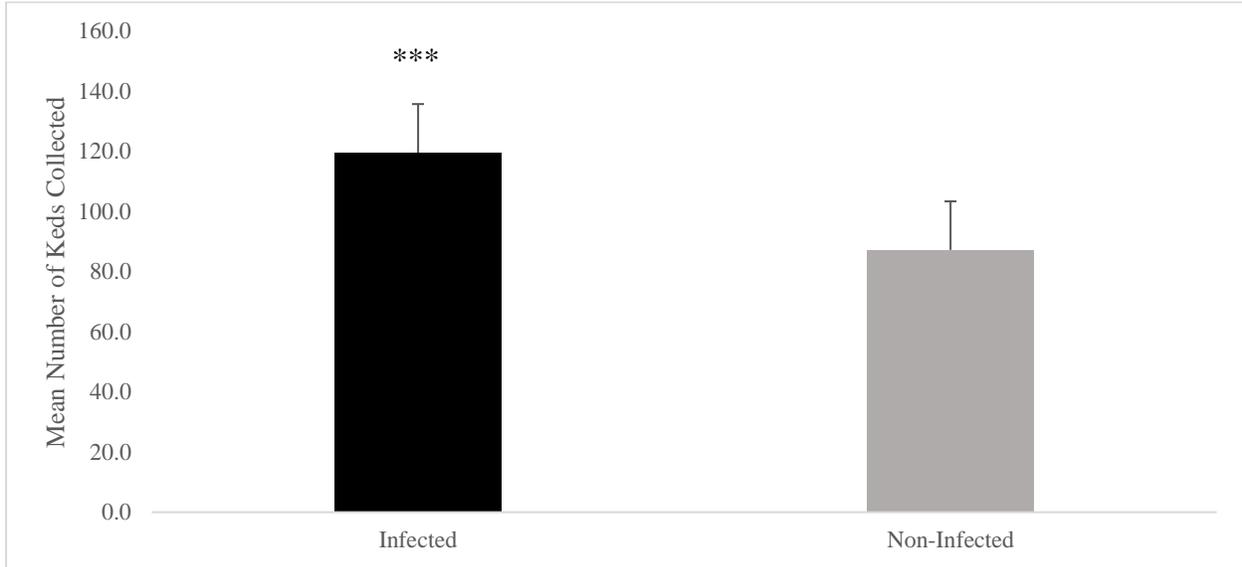


**Figure 10:** Keds species identified and quantified from semi-wild deer at Auburn University's Research Facility. A. *Lipoptena mazamae* (viewed at 20 times magnification under a dissecting scope) and B. individuals mating in groups on deer.



**Figure 11:** Comparison of neotropical deer ked (*Lipoptena mazamae*) burdens between *Bartonella* infected and non-infected white-tailed deer captured at Auburn University's deer research facility. Infected deer had significantly more keds per deer than non-infected deer

(p=0.006).



Age (year)	n	Gross Antler Score (cm)		Body Length (cm)		Hind Foot Length (cm)		Chest Girth (cm)		Mean Ticks	
		x	SE	x	SE	x	SE	x	SE	x	SE
1.5	56	29.5	1.4	127.4	1.1	42.2	0.2	78.2	0.6	7.52	1.81
2.5	27	78.7	2.7	141.1	1.1	43.6	0.3	88.3	1.3	9.22	3.33
3.5	37	98.7	2.5	148.2	1.2	43.5	0.5	93.2	0.9	7.70	2.32
4.5	28	114.8	2.6	149.2	2.3	43.8	0.3	96.5	0.8	7.21	3.08
5.5	17	127.7	2.5	153.5	1.7	44.6	0.4	98.8	1.3	3.58	1.15
6.5+	41	122.5	1.8	152.0	1.2	44.1	0.2	98.0	0.9	10.51	2.34

**Table 1:** Mean (+ SE) antler score, morphometrics, and ticks detected by age class for male white-tailed deer sampled at Auburn University's research facility 2007-2017.

Ectoparasite	Full vs. Half Estimate	Full vs. Photography
Ticks	0.2866	5.24E-12
Lice	0.5366	3.88E-08

Keds	0.803	0.00032
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**Table 2:** P-values for Welch’s two sampled t-test comparing quantification methods. Non-significant values here denote that there was no significant difference between the methods compared – and thus that method is viable. Significant values denote that there was a significant difference between the number of ectoparasites quantified by the compared methods and thus cannot be used as a viable quantification method.

		Gross Antler Score (cm)		Body Length (cm)		Hind Foot Length (cm)		Chest Girth (cm)	
Age	n	x	SE	x	SE	x	SE	x	SE
1.5	11	28.36545	3.597883	126.0727	2.220651	41.57273	0.314524	75.04545	1.424549
2.5	13	80.83182	3.526501	139.5455	2.439686	43.59091	0.499173	88.09091	1.381399
3.5	10	99.979	7.347746	144.65	2.108251	42.01	1.852353	93.05	1.571358
4.5	7	120.962	7.793745	148.2	3.776903	38.2	5.212005	99.2	1.157584
5.5	2	157.63	10.75	143	1	43.25	0.25	98.25	0.75
6.5+	20	122.5616	2.664522	150.1053	2.220182	43.75789	0.258461	96.78947	1.243883
Age	n			Mean Ticks		Mean Lice		Mean Keds	
1.5	11			x	SE	x	SE	x	SE
2.5	13			35.90909	8.068406	26.54545	3.022232	81.45455	6.127974
3.5	10			23.18182	6.325731	15.45455	2.001652	73.09091	9.299516
4.5	7			28.4	6.42426	7.9	1.523519	115.9	10.41415
5.5	2			18.6	4.284857	1	0.632456	113.6	14.29545
6.5+	20			9	3	7	3	117.5	13.5
				30.05263	6.159419	0.894737	0.30488	90.63158	8.912246

**Table 3:** Mean (+SE) antler score, morphometrics and ectoparasite quantification for male white-tailed deer by age class

Primer	Sequence
Forward	TTAGCCGTCGGGTGGTTTAC
Reverse	GCGATTCCGACTTCATGCAC

**Table 4:** Sequences for *Bartonella* primers as designed by NCBI based on 16S ribosomal RNA gene.

<i>Bartonella</i> Species	Number of positives	GenBank Matches for Submitted Sequences	Typical Host
<i>B. melophagi</i>	4	X89110	Domestic Sheep ( <i>Ovis aries</i> )
<i>B. bovis</i>	2	KM371095	Cattle (Bovidae)
<i>B. schoenbuchensis</i>	1	KJ639882	Ruminants and Cervids (Cervidae)
<i>B. spp</i>	3	JN673761	Unknown

**Table 5:** Representation of the *Bartonella* species detected by PCR from detected blood samples of male white-tailed deer.

Ectoparasite	% Individuals Infested within the Population n= 63	% Individuals Infested also Infected with <i>Bartonella</i> n= 10
Tick	100	100
Lice	68.25	60
Ked	100	100

**Table 6:** Percentage of male white-tailed deer infested with ectoparasites, and percentage of those infested with ectoparasites that were also infected with *Bartonella* spp.

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