EPIZOOTIOLOGY OF CRANIAL ABSCESS DISEASE

IN WHITE-TAILED DEER

by

BRADLEY STEPHEN COHEN

(Under the Direction of Karl V. Miller)

ABSTRACT

Cranial abscess disease is a cause of morbidity and mortality, particularly for mature, male white-tailed deer (*Odocoileus virginianus*). Most cases of cranial abscesses are associated with infection by *Trueperella* (*Arcanobacterium*) *pyogenes* but little is known about the epidemiology of this disease. I isolated *T. pyogenes* 46 of 65 (71%) active cranial abscess infections. All of these isolates had at least six of eight virulence genes which further implicates this bacterium as an important etiological agent. I also examined 7,545 white-tailed deer from 60 sites across Georgia for signs of cranial abscess disease to determine the distribution and risk factors for the disease. None of the 2,562 female deer examined had the disease, whereas 91 of the 4,983 (1.8%) male white-tailed deer had abscesses. A generalized linear mixed model examining the risk of a male having cranial abscess disease and treating property as a random effect suggested that age was the most important risk factor. Habitat variables (e.g., evergreen forest cover, agriculture, etc.) and soil features were not strongly associated with increasing risk. Because the model suggested that a large amount of variance occurred at the property level, I examined variation in the occurrence virulence determinants in the genome of *T. pyogenes*

between sites affected and unaffected by the disease. Using polymerase Chain Reaction (PCR), six of seven virulence determinants, all of which code for proteins promoting bacterial adhesion to epithelium, were more common on properties where abscesses were found ($p<0.05$). Thus the patchy distribution of cranial abscess disease across Georgia is likely caused by differences in the genetics of the causative agent, *T. pyogenes*. White-tailed deer managers must recognize the potential to transport pathogenic bacteria and disease when relocating white-tailed deer.

INDEX WORDS: brain abscess, cranial abscess, disease, intracranial abscessation, *Odocoileus virginianus*, *Trueperella pyogenes*, white-tailed deer

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by

Bradley Stephen Cohen

B.A., State University of New York College at Geneseo, 2009

M.S., University of Georgia, 2011

A Dissertation Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment of the Requirements for the Degree

DOCTOR OF PHILOSOPHY

ATHENS, GEORGIA

2014

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by

BRADLEY STEPHEN COHEN

Major Professor: Karl V. Miller

Committee: John R. Fischer

Robert J. Warren

Michael J. Yabsley

Electronic Version Approved:

Maureen Grasso
Dean of the Graduate School
The University of Georgia
May 2014

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

INTRODUCTION

The wide variety of bacterial pathogens that infect human and animal hosts, despite the broad capabilities of the immune system, shows the eloquent nature of bacteria to avoid, block, defeat, and overcome immune defenses (Hodgins and Shewen 2010). While the progression of our understanding of pathogenic bacteria and their interactions with their host is providing novel perspectives to pathogens at an almost overwhelming rate, bacterial infections seem to be increasing and changing (Gyles and Prescott 2010). The infection process is dynamic, and the fight against it requires vigilance, increasing knowledge, and the efficient application of that knowledge (Gyles and Prescott 2010).

The virulence of some bacteria and the difference between pathogenic and nonpathogenic bacteria is somewhat controversial (Boerlin 2010). Virulence is defined as "the relative capacity of a microbe to cause damage in a host" (Casadevall and Pirofski 1999). Thus, the capacity of a bacterium to cause damage is not only a function of the pathogen, but of the environment in which the bacterium reside, and the interactions between a complexity of other micro and macro circumstances (Casadevall and Pirofski 2003). Delineating what is a pathogenic bacterium becomes more difficult because of the increasing importance of opportunistic infections in

animals and humans, which blur the distinction between pathogenic and commensal bacterium (Casadevall and Pirofski 2000).

As infection is becoming recognized as a more dynamic process, concepts such as "One Health", a theory that underscores the intricacies and complexity of interactions between the environment, humans, and domestic and wild animals in pathogenesis have become the focus of much recent research (Kahn et al. 2007, Kaplan et al. 2009, Zinsstag et al. 2011). The majority of emerging infectious diseases in humans are zoonotic (Taylor et al. 2001), and thus originate or are being augmented through wild animal hosts (Jones et al. 2008, Rhyan and Spraker 2010). Therefore, understanding the pathogenesis of a bacterium, which are the basic steps in which infections are established, is critical in fighting against and successful prevention of bacterial infection.

The basic steps in pathogenesis are: 1) attachment or other means of entry into the body; 2) evasion of normal host defenses against infection; 3) multiplication to significant numbers at the site of infection and/or spread to other sites; 4) damage to the host, either directly or through immune host responses to the bacterium; and lastly 5) transmission of the bacterium from the infected animal to other susceptible animals, thus continuing the infection cycle. While each step provides the conceptual skeleton of infection and allows for easier understanding, the infection process is a continuum that lacks a clear series of steps (Gyles and Prescott 2010). Therefore, regardless of the steps, the outcome of infection is dependent on a complex process involving the pathogen, host, environment and their interactions (Gyles and Prescott 2010).

Recent studies in white-tailed deer (*Odocoileus virginianus*) have illustrated the uncertainty between pathogenic and nonpathogenic bacteria. They also underscore the importance of opportunistic infections as a health threat and call attention to the need to

understand the pathogenesis of bacteria. The intracranial abscessation-suppurative meningitis disease complex is generally considered a minor cause of population-wide mortality among white-tailed deer (Davidson et al. 1990, Baumann et al. 2001), but a recent study indicated that it can account for nearly 35% of annual mortality of mature bucks in some regions (Karns et al. 2009). Interestingly, *Trueperella pyogenes* (formerly *Arcanobacterium pyogenes*, *Actinomyces pyogenes* and *Corynebacterium pyogenes*, Yassin et al. 2011), a resident bacterium found among the microbiota on mucosal membranes and skin of healthy animals (Moore et al. 2010), has been isolated from 65 of 100 reported cases of intracranial abscesses in white-tailed deer for which culture was attempted (Davidson et al. 1990, Baumann et al. 2001, Nettles et al. 2002). Human-deer and livestock-deer interactions have increased as the interface between these species increased (Zhang et al. 2008, Baker 2010). Thus, expanding and urbanizing deer populations have implications beyond resource stewardship, including the potential for increased deer-human interactions (Messmer 2009) and for deer populations to serve as reservoirs for infectious diseases (Rhyan and Spraker 2010).

However, little is known about the epidemiology of intracranial and cranial abscessation in white-tailed deer, with only 4 studies published to date on the subject (Davidson et al. 1990, Baumann et al. 2001, Karns et al. 2009, Turner et al. 2013), and little attention has been given to the pathogenesis of *T. pyogenes* in this disease. As bacterial infections seem to be increasing and changing, disciplined research of the associated pathogens is required to aid in prevention and treatment if need arises (Gyles and Prescott 2010). Most aspects of pathogenesis of infection caused by *T. pyogenes* remain poorly characterized and understood (Jost and Billington 2005), and this seems to be especially the case in regards to *T. pyogenes*-associated cranial abscessation

in white-tailed deer. Therefore, my research examined the pathogenesis of cranial abscesses in white-tailed deer, particularly *T. pyogenes*-associated cranial abscessation.

LITERATURE REVIEW

Pathogenesis of T. pyogenes and cranial abscesses

Entry into the body - Successful colonization of the skin or a mucosal surface of the host is the first step in the infection process. Clearly, the bacterium must be present, either in the environment or on an infected animal for initial contact to occur. Successful adhesion to, and invasion of the host cell is usually mediated by fimbrial and nonfimbrial adhesions along the bacterial surface (Kline et al. 2009). These adhesions can be host and organ specific, and thus differences in infection rates may be determined by the differences in the host animal receptors. Bacterial pathogens that are associated with wound infections, such as *T. pyogenes,* may bind to other extracellular matrix models such as collagen and fibronectin, often facilitating entry by rearranging the host cell's cytoskeleton (Galán and Cossart 2005). Also, many host receptors are developmentally regulated, meaning age can also determine whether a pathogen successfully binds to the host cells.

T. pyogenes is thought to be the most widely distributed and most common opportunistic pathogen of mucosal surfaces in domestic animals (Jost and Billington 2005, Moore et al. 2010). It is commonly found among the normal microflora of susceptible species such as cattle (*Bos taurus*; Narayanan et al. 1998, Madsen et al. 1992), swine (*Sus scrofa domesticus*; Jost et al. 2002) and even the bearded dragon (*Pogona vitticeps*; Ülbegi-Mohyla et al. 2010). It is commonly isolated from the digestive tract, udders, urogenital region, and upper respiratory tracts of healthy animals (Natterman and Horsch 1977, Timoney et al. 1988, Carter and Chengappa 1991, Queen et al. 1994, Narayanan et al. 1998, Jost et al. 2002). *T. pyogenes* has

several virulence factors such as collagen-binding protein (cbpA), fibrinogen-binding protein, fibronectin-binding protein, neuraminidases (or sialidases) and fimbriae which are involved in adherence to host cells once it has come in contact with the host (Jost and Billington 2005). Important unknowns include how susceptible animals come into contact with *T. pyogene*s, its environmental prevalence, and whether contact with conspecifics is a mechanism for increased transmission and infection risk.

Little research has focused on the spread of *T. pyogenes* among individuals, so neither the route of bacterial transmission nor the mechanism of penetration is fully understood (Jost and Billington 2005). This reflects the assumption that *T. pyogenes* is spread through contact with the environment and with other animals (Jost and Billington 2005). Because *T. pyogenes* is a common bacterium in the environment, transmission could occur among males when they rub their foreheads and antlers against trees as a socio-sexual scent communication mechanism (Kile and Marchinton 1977). However, *T. pyogenes* was not cultured from trees in a study area where 82% of 22 male deer harbored *T. pyogenes* on their mucosal membranes and forehead (Karns et al. 2009, Turner et al. 2013). In addition, *T. pyogenes* was not cultured from 11 neonate fawns sampled from the same property despite 6-month-old fawns being colonized by the bacterium (Turner et al. 2013). Therefore, the mechanism of *T. pyogenes* colonization of white-tailed deer is unknown. With the indications that *T. pyogenes*-associated intracranial abscesses can account for significant natural mortality among mature male deer (Karns et al. 2009), and because colonization is the first step of pathogenesis, an examination of the roles that the environment or cohort-specific contact play in the initial colonization of *T. pyogenes* to white-tailed deer, specifically during their first six months of life is prudent.

Multiplication, evasion and damage to the host - After the initial association with the host, bacterial pathogens must evade the host's defenses and multiply sufficiently for a self-sustaining infection. Disease is only one of the possible outcomes of bacteria-host interactions, and a number of factors such as the bacterial environment and flora, the host physiology, and the host immune system play a role in the infection process (Gyles et al. 2010). To avoid being destroyed by the host immune response, "defensins" are used to defend against various host immune mechanisms. Bacteria also produce a variety of toxins that promote apoptosis of host cells, coined "offensins". As a commensal organism, *T. pyogenes* is usually found along the outer mucosal membrane of host species, and only when *T. pyogenes* disseminates beyond the outer membrane does it begin to multiply rapidly and illicit a host immune response (Jost and Billington 2005). Thus, diseases caused by *T. pyogenes* are believed to be often autogenous and opportunistic, usually preceded by direct physical trauma, immunological action, or infection by other microorganisms that allow *T. pyogenes* to penetrate past the outer mucosal membranes (Jost and Billington 2005, Moore et al. 2010).

When *T. pyogenes* disseminates into host tissue, innate immune defenses of the host such as natural killer cells, neutrophils, and macrophages (collectively known as phagocytes), rapidly respond (Hodgins and Shewen 2010). Pyolysin (plo) is an offensin secreted by all strains of *T. pyogenes*, and is a cholesterol-dependent cytolysin which plays an important role in disease formation (Jost et al. 1999, Jost and Billington 2005, Gyles et al. 2010). Plo binds to cholesterol-containing rafts in the host cell membrane, forming pores and resulting in apoptosis of the host cell (Giddings et al. 2004, Rosado et al. 2008). Plo also promotes cytolysis of immune cells in the host body (Houldsworth et al. 1994, Nishibori et al. 1996, Ruiz et al. 1998). Thus, plo damages host cell membranes and aids in the manifestation of the disease. By utilizing defensins

and offensins, *T. pyogenes* can evade the host's immune system and rapidly multiply within a heavily encapsulated abscess that contains phagocytes, natural killer cells, and dead and living bacteria (Moore et al. 2010). Thus, *T. pyogenes* induces suppurative infections, in the form of abscesses, empyemas, and pyogranulomas which can result in abortion, arthritis, endocarditis, mastitis, pneumonia, infertility, osteomyelitis, and vesiculitis (Moore et al. 2010). In white-tailed deer, *T. pyogenes* has been reported to cause pneumonia, mastitis, sepsis and most commonly, cranial abscesses (Davidson et al. 1990, Turnquist and Fales 1998, Palmer and Whipple 1999, Baumann et al. 2001, Nettles et al. 2002, Dyer et al. 2004, Karns et al. 2009).

Tissue damage and impairment of host function is often due to the inflammatory response by the host in response to the pathogen (Gyles and Prescott 2010). Cell-membrane-degrading toxins released by neutrophils combined with offensins released by *T. pyogenes* causes necrosis of encapsulated and surrounding tissue. Abscesses along the cranial region cause erosion and pitting of the cranium and necrosis of the tissue connecting the cranial bones in white-tailed deer (Davidson et al. 1990). As the infection persists, and the abscess enlarges, the connecting tissue of the cranial bones is eventually eroded, allowing the abscess to enter the cranial chamber. Once the abscess becomes intracranial, the pressure on the brain from the abscess coupled with the necrosis of the brain tissue usually results in death. The intracranial abscessation-suppurative meningitis disease complex disproportionately affects adult ($\geq$ 3.5 yrs old) male white-tailed deer, accounting for approximately 9% of natural mortality compared to <1% of natural mortality in juvenile or female deer (Baumann et al. 2001). However, in some areas, intracranial abscesses can account for up to 35% of annual mortality of adult bucks (Karns et al. 2009). An unknown percentage of individuals with these purulent infections may recover, albeit with varying degrees of necrosis and erosion of the frontal bones and antler pedicel (Karns and

Ditchkoff 2013). Little is understood about this disease complex in white-tailed deer and research should focus on environmental and cohort-specific factors that may increase the susceptibility of deer to form cranial abscesses, what role these risk-factors have in the prevalence of the disease and how to predict areas that could experience high prevalence. Also, research should examine how *T. pyogenes* disseminates into the cranial area to cause infection, and what role these risk factors have in propagating these abscesses to become intracranial abscesses.

Clinical Infections - *T. pyogenes* can cause disease in a variety of animal species, including blackbuck antelope (*Antilope cervicarpa*), European bison (*Bison bonasus*), camels (*Camelus dromedarius*), chicken (*Gallas gallas domesticus*), white-tailed deer, elephants (*Elephas maximus*), gazelles (*Gazella dorcas*), horses (*Equus ferus caballus*), reindeer (*Rangifer tarandus*), turkeys (*Meleagris gallopavo*), and wildebeest (*Connochaetes taurinus albojubatus*), and also in companion animals such as dogs and cats (Jost and Billington 2005, Moore et al. 2010). It has also been a reported pathogen in an extensive range of human disease conditions including abdominal abscessation, otitis media, cystitis, mastoiditis, septicemia, sigmoiditis, appendicitis, cholecystitis, peritonitis, endocarditis, meningitis, arthritis, empyema, pneumonia and sepsis (Ballard et al. 1947, Chlosta et al. 1970, Vega and Gavan 1970, Jootar et al. 1978, Norenberg et al. 1978, Lipton and Isalska 1983, Gahrn-Hansen and Frederiksen 1992, Levy et al. 2009). *T. pyogenes* is an under-recognized and frequently misdiagnosed human pathogen, and although it is commonly isolated as part of a mixed infection in humans, it has the potential to serve as a primary pathogen (Gahrn-Hansen and Frederiksen 1992, Kavitha et al. 2010). *T. pyogenes* could also be under reported because *T. pyogenes* has a similar biochemical profile to *Arcanobacterium hemolyticus* (Vega and Gava 1970, Gahrn-Hansen and Frederiksen 1992).

T. pyogenes infections in dairy and beef cattle are extremely costly, with *T. pyogenes* being associated with 27-69% of bovine uterine infections (Lewis 1997), leading to poor reproductive performance, and also causing mastitis in dairy cows, resulting in lower milk production (Gröhn et al. 2004). Among the diseases caused by *T. pyogenes*, the mostly costly is liver abscessation in beef cattle, which results in less efficient weight gain in cattle (Lechtenberg et al. 1988, Brink et al. 1990). Thus, *T. pyogenes* is an important pathogenic bacterium in many economically important animals.

Concepts such as "One Health" stress the interactions among humans, domestic and wild animals, and the environment in producing infection and disease (Kahn et al. 2007, Kaplan et al. 2009, Zinsstag et al. 2011). Wildlife species such as white-tailed deer can play important roles in disease interactions, especially as the interface between humans, domestic animals and deer increase with the coinciding expansion of deer populations and the suburban-wilderness edge (Baker 2010). Most emerging infectious diseases are zoonotic in nature (Taylor et al. 2001) and understanding the spatiotemporal distribution of pathogens and disease, as well as the virulence of these pathogens is critical in the fight against disease (Cutler et al. 2010, Gyles and Prescott 2010). Therefore, virulence potential of *T. pyogenes* found in cranial abscesses must be examined to better identify *T. pyogenes* as the causative agent of cranial abscess disease.

Application for Deer Management

Quality Deer Management (QDM), a deer management strategy to promote more natural population demographics as well as deer densities suitable for the available habitat, has increased in popularity over the past two decades (Miller and Marchinton 1995; Adams and Ross 2013). QDM emphasizes restraint in harvesting fawns and immature bucks (≤2.5 yrs old) to allow those males to reach maturity, resulting in a more balanced sex ratio in the deer population. However,

increased intrasexual competition among males for mates results in disproportionately higher rates of natural mortality than females. Mature adult males (≥3.5 yrs old) also have higher rates of natural mortality than younger males (Ditchkoff et al. 2001).

Intracranial abscesses have been reported as a cause of natural mortality among male white-tailed deer across portions of the United States and Canada. Davidson et al. (1990) examined the necropsy records of white-tailed deer mortalities submitted to the Southeastern Cooperative Wildlife Disease Study, of which, 24 of 683 (4%) deer had intracranial abscesses. Most (88%) cases occurred in males, with highest prevalence in older males. Intracranial abscesses accounted for 20% of the diagnoses among 56 bucks ≥3 years old. Subsequently, Baumann et al. (2001) reported that 9% of 119 male skulls from deer found dead in the southeastern US had lesions characteristic of intracranial abscesses, whereas none of the 299 males examined from Texas had characteristic lesions. Other reports of the prevalence of intracranial abscesses include 35% of known-fate mature male deer necropsied from Maryland (Karns et al. 2009). In a sample of 170 Key deer (*O. v. clavium*) of mixed sex and ages submitted for necropsy, Nettles et al. (2002) reported neurologic disease caused by intracranial abscesses in 17 adult males. Importantly, not all abscesses result in mortality because the abscess may fail to penetrate into the cranial cavity and male deer may have severe antler malformations for the rest of their lives (Karns and Ditchkoff 2013).

Because of the high variability in prevalence rates reported, as well as the purported impacts of climatic (Baumann et al. 2001, Karns et al. 2009) and demographic (Karns et al. 2009) conditions on the prevalence of this disease, the impacts of cranial abscesses on white-tailed population demographics across Georgia is speculative. Anecdotal observations suggest that prevalence may vary across physiographic regions, and may likewise be greater in herds

managed to increase the occurrence of mature males in the population. If intracranial abscesses are additive to other natural and harvest mortality, it could be a significant impediment to the success of management efforts, especially those practicing QDM. If strategies such as QDM contribute to cranial abscessation, management decisions may also have broader impacts on deer population health than previously recognized. It is the host-pathogen-environment triad that determines the outcome of infection, and all of these must be examined in the context of cranial abscesses in white-tailed deer so managers can make informed and strategic management decisions. A well-designed series of experiments must be done to examine the epizootiology of cranial abscesses, so this disease can be better understood and high risk areas can be identified, allowing adaptive management in these situations.

OBJECTIVES

Based on the literature review, my objectives are as follows:

1. Determine the virulence potential of *T. pyogenes* isolated from active cranial abscess infections and suggest its ability to be the causative agent of cranial abscess disease.
2. Assess the prevalence and influential factors of cranial abscess disease across Georgia.
3. Determine the prevalence of known and putative genes associated with increased virulence from *T. pyogenes* in white-tailed deer at sites affected and unaffected by cranial abscess disease.

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CHAPTER 2

GENOTYPIC VIRULENCE CHARACTERIZATION OF *TRUEPERELLA* (*ARCANOBACTERIUM*) *PYOGENES* ISOLATES RECOVERED FROM CRANIAL ABSCESSES OF WHITE-TAILED DEER[1]

[1]Cohen, B.S., E.H. Belser, S.P. Keeler, M.J. Yabsley, K.V. Miller. 2014. To be submitted to *Veterinary Microbiology* .

ABSTRACT: *Trueperella* (*Arcanobacterium*) *pyogenes* is a causative agent of numerous suppurative infections in domestic and wild animals. In white-tailed deer (*Odocoileus virginianus*), cranial abscess disease is an important source of mortality of males in some populations. Although the pathogenesis of this disease is poorly understood, *T. pyogenes* has been isolated from active infections alongside other opportunistic bacteria. In this study we sought to identify bacteria associated with cranial abscess infections, determine the prevalence of *T. pyogenes* in these cultures, and report the presence of virulence genes in *T. pyogenes* isolates to suggest if *T. pyogenes* could be the causative agent of this disease. Identification of bacterial species was determined by typical biochemical techniques. The presence of known and putative virulence genes *plo, nanH, nanP, cbpA, fimA, fimC, fimE, and fimG* was examined by conventional PCR. *T. pyogenes* was recovered from 71% (n = 46) of samples, and was often the only bacterium species detected. *Staphylococcus aureus* was detected in 40% (n = 26) of samples. All *T. pyogenes* isolates were positive for the pyolysin gene *plo*, *nanP* and *fimA*. Furthermore, *nanH*, *fimA*, *fimC*, and *fimE* were found in over 70% of isolates. Of the isolates tested, 48% had genotypes containing all virulence genes except *cbpA*. The suggestive virulence potential of all isolates, coupled with the large number of pure cultures obtained, implies *T. pyogenes* is a causative agent of cranial abscess disease.

KEY WORDS: cranial abscess, disease, intracranial abscessation, *Odocoileus virginianus*, white-tailed deer

INTRODUCTION

Trueperella pyogenes (Yassin et al. 2011), formerly *Arcanobacterium pyogenes*, is a Gram positive, irregular, non-motile, non-spore forming bacterium. It is believed to be the most widely distributed and common opportunistic pathogen of mucosal surfaces in domestic animals (Jost and Billington 2005, Moore et al. 2010). *T. pyogenes* induces suppurative infections, in the form of abscesses, empyemas, and pyogranulomas (Moore et al. 2010). These infections can result in abortion, arthritis, endocarditis, mastitis, pneumonia, infertility, osteomyelitis, and vesiculitis (Moore et al. 2010) and may result in serious economic losses in livestock operations. *T. pyogenes* is also widespread in wild animals, as it has been associated with disease in numerous bovids and cervids (e.g., blackbuck antelope (*Antilope cervicarpa*), bison (*Bison bonasus*), camels (*Camelus dromedarius*), and white-tailed deer (*Odocoileus virginianus*)) (Davidson et al. 1990, Jost and Billington 2005).

In white-tailed deer, *T. pyogenes* is most commonly associated with abscesses along the cranium, sometimes penetrating intracranially and accounting for mortality rates as high as 35% in free-ranging adult males (Karns et al. 2009). In captive white-tailed deer, these *T. pyogenes*-associated cranial and intracranial abscesses are also a reported source of morbidity and mortality (Hattel et al. 2004, Brooks 2010). These abscesses can also result in permanent antler malformations (Karns et al. 2013), resulting in economic losses for deer farm operations. Only recently has this disease been recognized as a possible health problem in wild herds (Baumann et al. 2001).

Several studies have indicated that *T. pyogenes* is a significant cause of intracranial abscesses. This bacterium was isolated from 65 of 100 reported cases of intracranial abscesses in white-tailed deer for which bacterial culture was attempted (Davidson et al. 1990, Baumann et al.

2001, Nettles et al. 2002) but other bacteria, such as *Staphylococcus* spp., *Streptococcus* spp. and other opportunistic pathogens were often found in mixed infections with *T. pyogenes* (Davidson et al. 1990, Baumann et al. 2001, Nettles et al. 2002). Although *T. pyogenes* can act as a primary pathogen, infection usually follows physical or microbial trauma to the mucosal membrane (Jost and Billington 2005), thus, the pathogenesis of cranial abscessation and the role of *T. pyogenes* as the etiological agent are poorly understood. If *T. pyogenes* is a primary etiological agent in this disease, isolates from infections would be expected to contain genes encoding virulence factors that aid in its ability to adhere and colonize host tissue (Jost and Billington 2005). Herein, we report the presence of virulence factor genes in *T. pyogenes* isolates from active cranial abscess infections in male white-tailed deer.

MATERIALS AND METHODS

Origin of bacterial isolates and growth conditions

A total of 65 samples was obtained from clinical specimens of cranial abscesses from hunter-harvested male white-tailed deer. Samples were collected in 2012-2013 from 14 locations across Georgia, USA. Samples of active suppurative infections along the cranium were obtained with a sterile rayon-tipped cotton swab (Puritan Medical Products, ME, USA), placed in a 2.5 mL Cryosaver vial (Cyosaver, Hardy Diagnostics, CA, USA), and frozen at -20°C until they were processed. After thawing, each sample was streaked onto Columbia Agar supplemented with 5% defibrinated sheep blood (bioMérieux, France) for 48 h at 37°C. Colonies of T. pyogenes were isolated based on morphology and post-incubation hemolysis and identification was confirmed by biologic tests. To ensure a representative sample, three colonies of *T. pyogenes* per sample were randomly selected for analysis, when available. After

identification, colonies were mixed in a glycerol-brucellaw solution and stored at -20°C until further analysis.

DNA isolation

DNA was extracted from 40µl of the isolate solution using the DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA, USA), per manufacturer's instructions. Extracted DNA was stored at -20°C until PCR testing.

Screening of virulence factor genes

T. pyogenes isolates were evaluated for the presence of known and putative virulence factor genes (*plo, nanH, nanP, cbpA, fimA, fimC, fimE, fimG*) by standard PCRs. The reaction mixtures, in final volumes of 25µl, contained 11 µl of DNA-free PCR water (MO BIO Laboratories, Inc., Carlsbad, CA, USA), 0.25 µl of dNTP, 7.75 µl of GoTaq Flexi DNA Polymerase (Promega Corporation, Madison, WI, USA), 0.5µl of each appropriate primer for each gene target, and 5 µl of extracted DNA. The PCR conditions, sequences of PCR primers for each gene target and expected amplicon size are shown in Table 2.1. Amplifications were performed in a thermal cycler (BioRad DNA Engine, Hercules, CA, USA). The PCR products were electrophoresed in ethidium bromide stained 1.5 (w/v) agarose gels. Precautions were taken to avoid and detect contamination, including separation of the areas for DNA extraction and PCR preparation, regular changing of gloves, and use of DNA-away (Molecular BioProducts, Inc., San Diego, CA, USA) on equipment. In addition, all PCR batches included negative controls of DNA-free PCR water (MO BIO Laboratories, Inc.) without DNA template to ensure there was no contamination in the reaction mixture. *T. pyogenes* reference strains JGS 189 and JGS 230 were used as positive controls.

Statistical analysis

We conducted chi square tests to analyze differences in occurrence of virulence genes between *T. pyogenes* isolates found in pure culture and *T. pyogenes* isolates found in mixed cultures with other species of bacteria. Statistical significance was considered when $P < 0.05$ was observed. Analyses were done in program R version 2.11.1 (R Development Core Team 2010).

RESULTS

Bacterial isolates

T. pyogenes was the predominant bacterium isolated from our clinical samples and was detected in pure culture from 32 samples, mixed culture with *S. aereus* from 12 samples, and mixed culture with *Enterococcus* sp(p). from two samples (Table 2.2). Other bacteria isolated included 24 *Staphylococcus aureus*, seven *Enterococcus* sp(p)., one *Corynebacterium* sp., two *Serratia protemaculans*, one *Ralstonia picketti*, and one *Enterobacter* sp.

Detection of virulence factor genes

We isolated 113 colonies of *T. pyogenes* from the 46 cultures in which *T. pyogenes* was detected. Results of PCR testing for the eight virulence-determinant genes showed that 26 samples had only one detectable genotype while 20 samples had two different genotypes of *T. pyogenes* within the same culture. Thus, we confirmed 66 different isolates of *T. pyogenes* from our clinical samples. Of these, 52 originated from pure cultures whereas 14 originated from mixed cultures.

Of the 66 unique isolates, all possessed the *plo*, *nanP*, and *fimA* genes (Table 2.3). Fifty-two (79%) were positive for *nanH*, 53 (80%) were positive for *fimC*, 65 (98%) were positive for *fimE*, and 46 (70%) were positive for *fimG*. The *cbpA* gene was only detected in one isolate

(2%). The reference strains tested (*T. pyogenes* JGS 189 and *T. pyogenes* JGS 237) carried all genes. Among the seven genotypes detected, the most frequent was *plo nanH nanP fimA fimC fimE fimG* which was found in 48% of isolates (Table 2.3). No statistical differences in the occurrence of virulence determinant genes between isolates detected in pure vs. mixed cultures were noted (Table 2.4).

DISCUSSION

T. pyogenes was the most common bacteria species isolated from active cranial abscess infections of white-tailed deer and was commonly found in pure culture. All isolates exhibited β-hemolysis on blood agar and as expected tested positive for the pyolysin gene (*plo*). Unlike previous studies which suggested virulence potential may not related to diseases such as metritis in cows (Silva et al. 2008), isolates from our study commonly tested positive for all virulence determinant genes except *cbpA*.

In addition to *plo*, both *nanH* and *fimA* are dominating genes for neuraminidase and fimbriae expression (Zhao et al. 2013a). These genes are heavily upregulated at the beginning of infection, and seem critical for promoting initial adhesion to the epithelial linings (Zhao et al. 2013a). *FimA* has been detected in 100% of samples from bovine mastitis (Zastempowska and Lassa 2012) and infections in European bison (*Bison bonasus*; Rzewuska et al. 2012). Meanwhile, nanP seems important for inducing latent infections, and enhancing epithelial attachment and subsequent colonization (Zhao et al. 2013a). Thus, *nanP* and *fimA* may be important in the pathogenesis of cranial abscess disease in white-tailed deer as we detected both in all isolates. Additionally, we detected *nanH* in 80% of isolates.

FimC, *fimE*, and *fimG* are reportedly important for recognition of host specific tissue (Jost et al. 2002, Zhao et al. 2013a). Of these 3, *fimE* occurred most frequently (98%). Although

it seems all fimbriae subunits C, E, and G could account for some differences in site variability, the higher occurrence of *fimE* suggests it may play a more significant role in adhesion to the epithelial linings of white-tailed deer and is important for the ability of *T. pyogenes* to cause cranial abscesses.

CbpA was only detected in one isolate. CbpA is expressed at the surface of *T. pyogenes* and is believed to promote adherence to collagen-rich tissue. However, the importance of this virulence factor seems speculative. For example, a *cbpA* knockout strain lost up to 57% of its adherence ability to human fibroblasts but most studies have found it unrelated to pathogenicity of *T. pyogenes* in wild animals such as bison and forest musk deer (*Moschus berezovskii*; Esmay et al. 2003, Rzewuska et al. 2012, Zhao et al. 2013b). Our results suggest *cbpA* is not important for *T. pyogenes*-associated cranial abscess disease in white-tailed deer.

The presence of virulence genes did not differ between isolates from pure and mixed culture; occurrence of these genes was high for seven of the eight genes evaluated. Because the prevalence of virulence determinant genes of *T. pyogenes* did not differ whether found alone or with other bacteria in these infections, and these isolates have virulence potential, it seems *T. pyogenes* is the putative causative agent in cranial abscess disease. While our evidence suggests multiple genotypes of *T. pyogenes* may occur even within the same infection, all genotypes appeared to have high virulence potential.

T. pyogenes was detected in 71% of samples, similar in incidence to the summation of three previously published reports in which *T. pyogenes* was cultured from 65 of 100 clinical cases of intracranial abscesses (Davidson et al. 1990, Baumann et al. 2001, Nettles et al. 2002). *S. aureus* was the only other bacterium isolated in pure culture, and was also found in mixed cultures with *T. pyogenes*. Given it was detected in pure culture in 17% of samples, and is a

resident along the mucosal linings of white-tailed deer (Turner et al. 2013), *S. aureus* may be an important opportunistic pathogen in this disease.

T. pyogenes infections in dairy and beef cattle can be costly, with *T. pyogenes* being associated with 27-69% of bovine uterine infections (Lewis 1997), leading to poor reproductive performance, and mastitis in dairy cows, resulting in reduced milk production (Gröhn et al. 2004). Additive to these diseases is liver abscessation in beef cattle, which hinders efficient weight gain (Lechtenberg et al. 1988, Brink et al. 1990). White-tailed deer may serve as potential reservoirs of pathogenic *T. pyogenes* for livestock.

Our study indicates cranial abscess infections in white-tailed deer are typically associated with *T. pyogenes*. The strains of *T. pyogenes* isolated from infections have high virulence potential and are likely an important etiological agent in this disease. Mortality as a result of cranial abscess disease seems to vary across North America, with reported prevalence ranging from 0% in Texas (Baumann et al. 2001) to as high as 35% in mature male deer in Maryland (Karns et al. 2009). Future studies should identify whether the differences in reported prevalence may be related to the prevalence of *T. pyogenes*, differing virulence in *T. pyogenes* among white-tailed deer populations, or potential environmental factors.

ACKNOWLEDGEMENTS

Funding for this project was provided by the Georgia Wildlife Resources Division through the Wildlife Restoration Program, which derives monies through an excise tax on sporting arms and ammunition paid by hunters and recreational shooters. We thank all the regional wildlife biologists as well as the technicians who assisted in data collection. We thank all of the private landowners for allowing us access to their properties and for their help with

data collection. We thank J. Fischer, the Southeastern Cooperative Wildlife Diseases Study and the Veterinary Diagnostics Laboratory at the University of Georgia for their equipment and assistance. We thank M. MacKenzie for her assistance in the lab.

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Table 2.1. Sequence of PCR primers and cycling conditions used to amplify virulence determinants of *T. pyogenes*.

Target gene	Virulence factor	Oligonucleotide sequence (5' to 3')	Amplicon size (bp)	Reference
plo	Pyolysin	Fw: GGCCCGAATGTCACCGC	270[a]	Jost et al. (2002)
		Rv: AACTCCGCCTCTAGCGC		
nanH	Neuraminidase H	Fw: CGCTAGTGCTGTAGCGTTGTTAAGT	781[b]	Silva et al. (2008)
		Rv: CCGAGGAGTTTTGACTGACTTTGT		
nanP	Neuraminidase P	Fw: TTGAGCGTACGCAGCTCTTC	150[b]	Silva et al. (2008)
		Rv: CCACGAAATCGGCCTTATTG		
cbpA	Collagen-binding protein	Fw: GCAGGGTTGGTGAAAGAGTTTACT	124[b]	Silva et al. (2008)
		Rv: GCTTGATATAACCTTCAGAATTTGCA		
fimA	Subunit of type A fimbriae	Fw: CACTACGCTCACCATTCACAAG	605[c]	Silva et al. (2008)
		Rv: GCTGTAATCCGCTTTGTCTGTG		
fimC	Subunit of type C fimbriae	Fw: TGTCGAAGGTGACGTTCTTCG	843[b]	Silva et al. (2008)
		Rv: CAAGGTCACCGAGACTGCTGG		
fimE	Subunit of type E fimbriae	Fw: GCCCAGGACCGAGAGCGAGGGC	775[d]	Silva et al. (2008)
		Rv: GCCTTCACAAATAACAGCAACC		
fimG	Subunit of type G fimbriae	Fw: ACGCTTCAGAAGGTCACCAGG	929[c]	Silva et al. (2008)
		Rv: ATCTTGATCTGCCCCCATGCG		

[a] Cycling conditions: 35 cycles of 94°C (60 s), 55°C (60 s), 72°C (60 s); final extension 72° C (300 s)

[b] Cycling conditions: 94°C (180 s), 35 cycles of 94°C (60 s), 60°C (60 s), 72°C (60 s); final extension 72° C (420 s)

[c] Cycling conditions: 94°C (180 s), 35 cycles of 94°C (60 s), 57°C (60 s), 72°C (60 s); final extension 72° C (420 s)

[d] Cycling conditions: 94°C (180 s), 35 cycles of 94°C (60 s), 55°C (60 s), 72°C (60 s); final extension 72° C (420 s)

Table 2.2. The species and number of identified bacterial isolates from 65 active cranial abscess infections in male white-tailed deer. Bacteria were identified using classic biologic tests and were found in samples containing either pure cultures or mixed cultures with one other predominant bacteria. Samples were taken with a sterile-rayon tipped swab from diseased deer during 2012-2013 from 14 locations across Georgia, USA.

Bacterial species	Isolates	
	Number of samples in which bacteria was detected (%)	Number of pure cultures (%)
Gram positive:		
Trueperella pyogenes	46 (71)	32 (49)
Staphylococcus aureus	26 (40)	11 (17)
Enterococcus sp(p).	7 (11)	0 (0)
Corynebacterium sp.	1 (2)	0 (0)
Gram negative:		
Serratia proteamaculans	2 (3)	0 (0)
Ralstonia picketii	1 (2)	0 (0)
Enterobacter sp.	1 (2)	0 (0)

Table 2.3. Genotypic virulence of 66 *T. pyogenes* isolates from the cranial abscesses of male white-tailed deer. All 46 samples in which *T. pyogenes* was identified had up to three colonies subsampled and genotypic virulence for each isolate was examined. 26 samples had only one detectable genotype. In 20 samples two different genotypes of *T. pyogenes* occurred within the same culture. Thus, 66 different isolates of *T. pyogenes* from our clinical samples were confirmed.

Genotype	Isolates	
	Number	%
plo nanH nanP cbpA fimA fimC fimE fimG	1	1
plo nanH nanP fimA fimC fimE fimG	32	48
plo nanH nanP fimA fimC fimE	7	11
plo nanH nanP fimA fimE fimG	6	9
plo nanH nanP fimA fimE	5	8
plo nanP fimA fimC fimE fimG	7	11
plo nanP cbpA fimA fimC fimE	7	11

Table 2.4. Presence of known putative virulence genes in *T. pyogenes* isolated from active cranial abscess infections of male white-tailed deer. The 66 genotypic isolates were confirmed from 45 samples in which *T. pyogenes* was present. Of these isolates, 52 originated from samples containing a pure culture of *T. pyogenes*, whereas 14 originated from samples containing mixed cultures of *T. pyogenes* and other bacterial species. When appropriate, a chi-square test was used to determine if differences in occurrence for virulence gene occurred between isolates from pure cultures and mixed cultures (*df* = 1 for each test).

Virulence factor	Number of isolates (% of isolates)		X^2	*P-value*
	From pure culture (n=52)	From mixed culture (n=14)		
plo	52 (100)	14 (100)	–	–
nanH	41 (79)	11 (79)	0.00	1.00
nanP	52 (100)	14 (100)	–	–
cbpA	0 (0)	1 (7)	0.44	0.50
fimA	52 (100)	14 (100)	–	–
fimC	41 (79)	12 (86)	0.04	0.85
fimE	51 (98)	14 (100)	0.00	1.00
fimG	33 (63)	13 (93)	3.23	0.07

CHAPTER 3

EPIZOOTIOLOGY OF CRANIAL ABSCESS DISEASE IN WHITE-TAILED DEER OF GEORGIA[2]

[2]Cohen, B.S., E.H. Belser, C.H. Killmaster, B.J. Irwin, M.J. Yabsley, K.V. Miller. 2014. To be submitted to *Journal of Wildlife Diseases*.

ABSTRACT: Intracranial abscess disease is a reported cause of natural mortality for mature, male white-tailed deer (*Odocoileus virginianus*). Most cases of abscesses are associated with infection by *Trueperella* (*Arcanobacterium*) *pyogenes* but little else is known about the disease. We examined 7,545 white-tailed deer from 60 sites throughout Georgia (USA) for signs of cranial abscesses, the predecessor of intracranial abscesses, to model risk factors that may contribute to incidence of the disease. Factors examined included environmental factors (soil and forest type), demographic factors (deer density and male to female ratio), and individual host factors (deer gender and age). We found no cases of cranial abscesses in the 2,562 female deer examined but they were found in 91 of the 4,983 (1.8%) male deer examined. A generalized linear mixed model treating site as a random effect indicated age was the most important risk factor for male deer. Adult sex ratio also at the site was also important. Land cover and soil types were not strongly associated with risk of the disease. However, the model indicated a large amount of variance occurred at the site level, suggesting the occurrence of other unknown, region-specific risk factor(s) may exist. Our results suggest the high incidence of cranial abscesses in some populations could interfere with deer management strategies attempting to increase demographic representation of older males in the population. Future studies should investigate additional variables that might explain site-to-site differences, such as the prevalence of the presumed causative agent, *Trueperella pyogenes*.

KEYWORDS: cranial abscess, disease, intracranial abscessation, *Odocoileus virginianus*, white-tailed deer

INTRODUCTION

Understanding risk factors that contribute to the incidence of disease in wildlife populations can be an important determinant in molding management efforts. In addition, discerning risk factors can help identify at-risk segments of the population. Thus, understanding landscape and population level processes impacting disease incidence is critical for sound wildlife management.

Intracranial abscesses are a reported cause of mortality among white-tailed deer (*Odocoileus virginianus*) across portions of the United States and Canada (Baumann et al. 2001). Beginning as a cranial abscess, these suppurative infections along the cranial region cause erosion and pitting of the cranium and necrosis of the tissue connecting the cranial bones (Davidson et al. 1990). An unknown percentage of individuals with these purulent infections may recover, albeit with varying degrees of necrosis and erosion of the frontal bones and antler pedicel. However, persistent infection results in erosion of the connecting tissue of the cranial bones allowing the abscess to enter the cranial chamber. Pressure on the brain from these intracranial abscess coupled with necrosis of brain tissue is fatal. Intracranial abscesses disproportionately affect adult (≥ 3.5 yrs old) male white-tailed deer, accounting for approximately 9% of natural mortality compared to <1% of natural mortality in juvenile or female deer (Davidson et al. 1990, Baumann et al. 2001). However, some geographically limited studies have reported up to 35% of natural mortality in adult males (Karns et al. 2009).

Trueperella (*Arcanobacterium*) *pyogenes* (Yassin et al. 2011), a resident bacterium found on mucosal membranes and skin of healthy animals (Moore et al. 2010), has been isolated from 65 of the 100 published cases of intracranial abscesses from white-tailed deer in which culture was attempted (Davidson et al. 1990, Baumann et al. 2001, Nettles et al. 2002). *T. pyogenes* may

be the most widely distributed and most common opportunistic pathogen of mucosal surfaces in domestic animals (Jost and Billington 2005, Moore et al. 2010), and is commonly found along the digestive tract, udders, urogenital region, and upper respiratory tracts of healthy animals (Natterman and Horsch 1977, Queen et al. 1994, Narayanan et al. 1998, Jost et al. 2002). Neither the route of *T. pyogenes* transmission nor the infective mechanism is fully understood although it is assumed the bacterium are spread environmentally and through contact with other animals (Jost and Billington 2005). Karns et al. (2009) examined if *T. pyogenes* could be transmitted among deer through scent-marking trees during the breeding season; however, they were unable to culture *T. pyogenes* from marked trees in an area where 18 of 22 male deer harbored *T. pyogenes* on the forehead region (Karns et al. 2009, Turner et al. 2013). Although no studies have examined landscape-related features in relation to *T. pyogenes* transmission or disease incidence in wildlife, research in cattle has suggested populations of *T. pyogenes* and similar suppurative opportunistic pathogens are influenced by bedding soil-type (Zdanowicz et al. 2004, Ericsson Unnerstad et al. 2009). Thus, edaphic factors may influence the occurrence of the bacteria, as well as the incidence of cranial abscesses in white-tailed deer.

Evidence suggests the population-level influences of cranial abscess may vary across physiographic regions, and may be greater in herds managed to increase the occurrence of mature males in the population (Davidson et al. 1990). If intracranial abscesses are additive to other mortality factors, then it could affect the success of some deer management efforts and may have broader impacts on deer population health than previously recognized. Therefore, we evaluated environmental and demographic factors that may influence the prevalence of cranial abscesses across the varied physiographic regions of Georgia. We characterized disease prevalence among populations and then used information criteria modeling to evaluate

landscape, demographic and individual factors that may contribute to the occurrence of cranial abscesses in male white-tailed deer.

MATERIALS AND METHODS

During 2011 and 2012, we examined hunter-harvested deer from 60 sites across all physiographic provinces in Georgia (Blue Ridge, Ridge and Valley, Piedmont, Upper Coastal Plain, and Lower Coastal Plain) for signs of cranial abscesses. We chose sites to ensure a thorough spatial distribution across the state (Figure 3.1) and to represent a range of deer herd demographics.

For each deer, we recorded the sex, location, and estimated age (Severinghaus 1949). Because cranial abscesses are the predecessor of intracranial abscesses and are grossly observable, we used cranial abscesses as a surrogate measure of the potential occurrence of intracranial abscesses. We thoroughly inspected the cranium of each deer, with specific focus on the pedicles on males and the area overlaying the frontal bones on females. We visually examined each pedicle, looking for signs of infection such as necrosis of the skin, scabbing, or pus as described in Davidson et al. (1990). In addition, a finger or flat item was used to push at the skin around the base of the pedicle, checking for release of purulent discharge. Any grossly visible signs of an infection on the forehead were classified as a cranial abscess. Thus, the presence or absence of an abscess was recorded for each individual deer examined.

We obtained land cover data from the 2006 National Land Cover Database (NLCD) at a scale of 30 m and adopted their land-cover classification scale (deciduous forest, evergreen forest, and agriculture). For each of the 60 sites, we assessed land cover by calculating the percentage of each NLCD category within the property borders of each site. Similarly, we obtained soil type data from the 2012 Soil Survey Geographic Database (SSURGO) and

combined all SSURGO-classified, sand- and clay-predominated soils into ‘sand’ or ‘clay’ associations based on their ability to hold moisture which may influence occurrence of environmental bacteria. SSURGO data were unavailable at three sites; however, to ensure these sites were not excluded from analysis, we averaged sand and clay values from all other sites and placed these as "dummy values." This procedure allowed these sites to be included in our analysis and not affect analyses regarding soil type.

On private lands (n = 6), we used infrared-triggered camera surveys (Jacobson et al. 1997) to estimate deer density and adult sex ratio. On public lands (n = 54), we obtained deer density from Georgia Department of Natural Resources’ estimates based on population reconstruction (Downing 1980) which uses harvest-by-age data and backward addition of cohorts to estimate population size over time. Based on recommendations from Davis et al. (2007), we averaged five years (2008-2012) of population data to obtain an estimated deer density on these sites. Additional estimates of deer density were provided by Georgia Department of Natural Resources regional biologists. The Downing population estimates and biologist’s estimates did not concur at three sites but we utilized the biologist's estimate because reconstruction can produce variable results in areas where populations are changing (Davis et al. 2007). Because of the coarseness of these measurements, we grouped population density on all sites into three categories: low (10-30 deer/km^2), medium (30-60 deer/km^2), and high (>60 deer/km).

To estimate the adult sex ratio on public land we averaged the number of 3.5 year-old males and females harvested on each site for the previous five years (2008-2012) because this was the most stable age group harvested across sites. We then categorized adult sex ratios into three categories: low (<0.75 males per female), medium (0.75-1.50 males per female), and high

(>1.50 males per female). Our categorization of demographic variables allowed comparisons of their relative importance as risk factors for cranial abscesses across our study areas.

Generalized linear mixed model

All analyses for this study were conducted using program R version 2.11.1 (R Development Core Team 2010). We conducted generalized linear mixed modeling (GLMM) analyses using the lme4 (Bates et al. 2011) and LMER Convenience Functions (Tremblay 2011) to investigate what variables increased the risk of cranial abscesses in male deer. GLMMs allow incorporation of a flexible covariance structure into the modeling framework, resulting in better estimates of variability than standard generalized linear models (Clayton and Kaldor 1987, Breslow and Clayton 1993). Analyses used a logit link with binomial errors and models were fitted using Laplace approximation. The dependent variable was a binary measure, with cranial abscess presence or absence on the individual deer classified as 1 or 0, respectively. Fixed response variables were the deer's age, and the landscape and demographic variables. To examine for potential multicollinearity, Pearson correlations (r) were performed on all pairs of predictor variables. No variables that were significantly correlated (cutoff of $r = \pm 0.60$) were included in the same model. To account for the unknown variation of each site, we treated site as a random effect with a mean of zero and an estimated variance.

While some debate remains about the appropriateness of information criteria approaches with GLMMs (Vaida and Blanchard 2005), we conducted an exploratory analysis using Akaike's Information Criteria (AIC; Akaike 1973) with small sample adjustment (AICc) (Hurvich and Tsai 1989) to compare four models could be the most appropriate for our data set (Table 3.1). Our four models were the aforementioned model which was considered the global model, a Landscape model consisting of percent land cover and soil types as fixed effects, a Herd

Demographic model consisting solely of deer density and buck-to-doe ratio as fixed effects, and an Age model consisting of age of the deer as the fixed effect. We treated site as a random effect in all of our models. The model with the smallest AICc value was considered the most plausible model. Akaike weights (w_i) were calculated to determine the relative importance of the models, with the best approximating possessing the highest w_i value.

RESULTS

We examined 7,545 deer for signs of cranial abscessation on 60 sites during the two-year study period. Of the 4,983 male deer examined, 91 showed visible signs of cranial abscesses (Table 3.2). We found no evidence of cranial abscesses in any of the 2,562 female deer examined. Therefore, hereafter all analyses refer only to male deer. Apparent prevalence averaged 1.8% but ranged from 0.0% (44 sites) up to 33.3% (1 site). However, this apparent prevalence number is skewed for some sites due to the selective harvest of only mature bucks.

Only percent evergreen and percent sand were used as fixed effects in our global GLMM because other landscape variables were removed due to multicollinearity. Results of our global GLMM suggested forest and soil type had little effect on cranial abscess incidence, with parameter estimates of 0.06 and -0.02 respectively (Table 3.3). This was supported by our model rank exercise in which Landscape was the worst performing model (Table 3.4). Furthermore, this model had very low w_i values at <0.01.

Herd density and adult sex ratio were positively related to the incidence of cranial abscess disease (parameter estimates of 0.40 and 1.48 respectively; Table 3.3). However, our GLMM suggests herd density did not significantly contribute to incidence. There was no support for the Demographic model in our modeling exercise, further contrasting the apparent lack of contribution of herd density with the modest contributions of buck-to-doe ratio (Table 3.4).

Evidence from both our global GLMM and model ranking suggested age was the largest risk factor in our analysis. The incidence of infection increases with age, with a parameter estimate of 0.24 (Table 3.3). In addition, our Age model was ranked as the strongest model in our ranking analysis and accounted for 70% of the model sets w_i (Table 3.4)

There was a distinct spatial pattern where deer were found with cranial abscesses, clustered in the central and south-western parts of Georgia (Figure 3.1). No deer from sites in the Ridge and Valley, Blue Ridge, and Lower-Coastal Plain physiographic regions were found to have cranial abscesses. Site random effects were large in our global GLMM and had a variance estimate of 6.28. An examination of the random effects associated with each site shows a distinct partitioning amongst each other, with parameter estimates as low as -1.64 and as high as 4.91 (Figure 3.2). Thus, our model suggests very high variability between two groupings of sites, one clustered with negative variance estimates, and the other clustered with positive variance estimates, with moderate variability amongst sites within the group.

DISCUSSION

Despite previous reports of intracranial abscesses in female white-tailed deer, we did not detect any cases of cranial abscesses in the females we examined, supporting previous assertions that there is a strong sex bias to cranial abscessation (Davidson et al. 1990, Baumann et al. 2001). Furthermore, deer density does not seem to influence the incidence of cranial abscess disease whereas increasing buck-to-doe ratios seem to increase its occurrence. Coupled with our results suggesting age was the most important factor in increasing a male's chance of having a cranial abscess, the sex-skewed bias of this disease is likely behaviorally related. Antagonistic behaviors likely results in more abrasions and cuts along the cranial epidermis providing a

gateway for pathogens such as *T. pyogenes*. Older males tend to engage in these behaviors more, and also have higher natural mortality rates (Ditchkoff et al. 2001).

We observed a low prevalence rate (1.8%) suggesting this is an infrequent disease across large regions. This strengthens previous assertions that this disease is not a significant mortality factor for white-tailed deer populations on a large regional basis, at least in Georgia (Davidson et al. 1990). However, all positive cases were recorded from male deer in the midwestern and southwestern regions of Georgia, in the Piedmont and Upper Coastal Plain physiographic regions. This clustering suggests this disease could be important on local scales. For example, of males harvested from sites where at least one cranial abscess was found, apparent prevalence was 5.7%.

Our results indicated the environmental factors we measured were not associated with the observed distribution or prevalence of cranial abscesses. However, apparent prevalence was highly variable among sites, ranging from 0.0% to 33.3%. The GLMM analysis subsequently revealed two groupings of sites where we did not observe deer with a cranial abscess, and sites where multiple cases were found. There was moderate variability within these two groups attributed to the range in apparent prevalence for positive sites and the assumption of a random effect distribution with a mean of zero. The lack of variability in the random effect estimate of unaffected sites suggests a similar single variable these sites may share or lack. Therefore, a site-specific factor that we did not measure likely accounts for the presence or absence of this disease across a region. Although the prevalence of *T. pyogenes* may vary across sites (Turner et al. 2013), this bacterium is a common member of the microflora on the mucosal membranes of livestock and other ruminants (Jost and Billington 2005). Future studies should investigate the

commonality of *T. pyogenes* as a resident on the mucosal linings of white-tailed deer, as well as its pathogenic potential if present.

ACKNOWLEDGEMENTS

Funding for this project was provided by the Georgia Wildlife Resources Division through the Wildlife Restoration Program, which derives monies through an excise tax on sporting arms and ammunition paid by hunters and recreational shooters. We thank all the regional wildlife biologists as well as the technicians who assisted in data collection. We thank all of the private landowners for allowing us access to their properties and for their help with data collection.

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Table 3.1. Mixed models used to evaluate relative importance of age, landscape features, and herd demographics as they relate to a male deer testing positive for cranial abscess disease.

No.	Model name	Hypothesis	Models
1	Global Model	All main effects at deer level will change response parameter	y = AGE + BD$^{\alpha}$ + EVERGREEN + SAND + Rj^{β}
2	Landscape	Response is dependent on predominant soil and forest type	y = EVERGREEN + AGRICULTURE + SAND + *Rj*
3	Herd Demographic	Response is dependent on the density and buck-to-doe ratio of the herd	y = DENSITY + BD + *Rj*
4	Age	Response is dependent solely on the age of the buck	y = AGE + *Rj*

$^{\alpha}$BD = buck-to-doe ratio

$^{\beta}$*Rj* = random effect of site $Rj \sim N(0, \sigma^2_R)$

Table 3.2. Number of hunter-harvested male white-tailed deer examined for the presence of cranial abscess disease, and the apparent prevalence of cranial abscesses at each location sampled.

Location (by county)	*n*	# positive	Location (by county)	*n*	# positive	Location (by county)	*n*	# positive
Baker 1	55	0	Harris 1	20	4	Paulding	135	0
Baker 2	73	7	Harris 2	57	0	Polk	3	0
Barrow	7	0	Houston	139	5	Putnam 1	80	6
Bartow 1	47	0	Jasper	81	4	Putnam 2	544	33
Bartow 2	15	0	Jeff Davis	27	0	Rabun	20	0
Burke 1	102	0	Laurens 1	63	0	Screven	268	0
Burke 2	87	0	Laurens 2	68	0	Talbot	83	1
Chatham	144	0	Long 1	24	0	Telfair	29	0
Coweta	21	1	Long 2	56	0	Thomas	19	0
Dawson	110	0	Lowndes	14	0	Towns	17	0
DeKalb	33	0	Lumpkin	28	0	Twiggs	209	10
Dooly	21	0	McIntosh	166	0	Union	55	0
Dougherty	75	2	Meriwether 1	16	2	Walker	288	0
Emanuel	39	0	Meriwether 2	37	0	Ware	68	0
Fannin 1	111	0	Meriwether 3	12	4	Wayne 1	97	0
Fannin 2	58	0	Monroe 1	73	3	Wayne 2	14	0
Floyd	330	0	Monroe 2	15	1	White	10	0
Glynn 1	6	0	Morgan	94	2	Whitfield	102	0
Glynn 2	115	0	Murray 1	57	0	Wilkes	36	0
Habersham	235	0	Murray 2	125	0	Worth	50	6

	Sites	*n*	# positive
Total:	60	4983	91

Table 3.3. Parameter estimates (logit scale) for the global model of the risk of an individual white-tailed deer testing positive for cranial abscess disease across 60 sites in Georgia, USA. Standard errors (SE), z values, and probabilities that a coefficient differs from 0 are also presented.

Covariate	Estimate	Coefficient (SE)	z value	Pr(>|z|)
Intercept	-12.08	3.21	-3.76	<0.01
Age	0.24	0.08	2.98	<0.01
Density	0.40	0.72	0.56	0.58
Buck:Doe Ratio	1.48	0.88	1.69	0.09
Evergreen	0.06	0.04	1.45	0.15
Sand	-0.02	0.02	-0.92	0.36
Site	6.28[α]	N/A	N/A	N/A

[α]Site was considered a random effect in the model. Thus, it is a variance estimate.

Table 3.4. Akaike information criteria with small sample bias adjustment (AICc); number of parameters (K), ΔAICc, Akaike weights (*w*) for candidate models (*i*) relating deer, land cover types, soil types, herd demographics and the deer's age to the incidence of cranial abscesses in male white-tailed deer. The age model is the best model as it has the lowest AICc score.

Candidate Model	Model No.	K	AICc	ΔAICc	w_i	% w_i
Age	4	3	762.49	0.00	0.70	100.00
Global	1	7	748.18	1.69	0.30	23.00
Demographics	3	4	772.81	10.32	0.00	0.00
Landscape	2	7	773.31	10.82	0.00	0.00

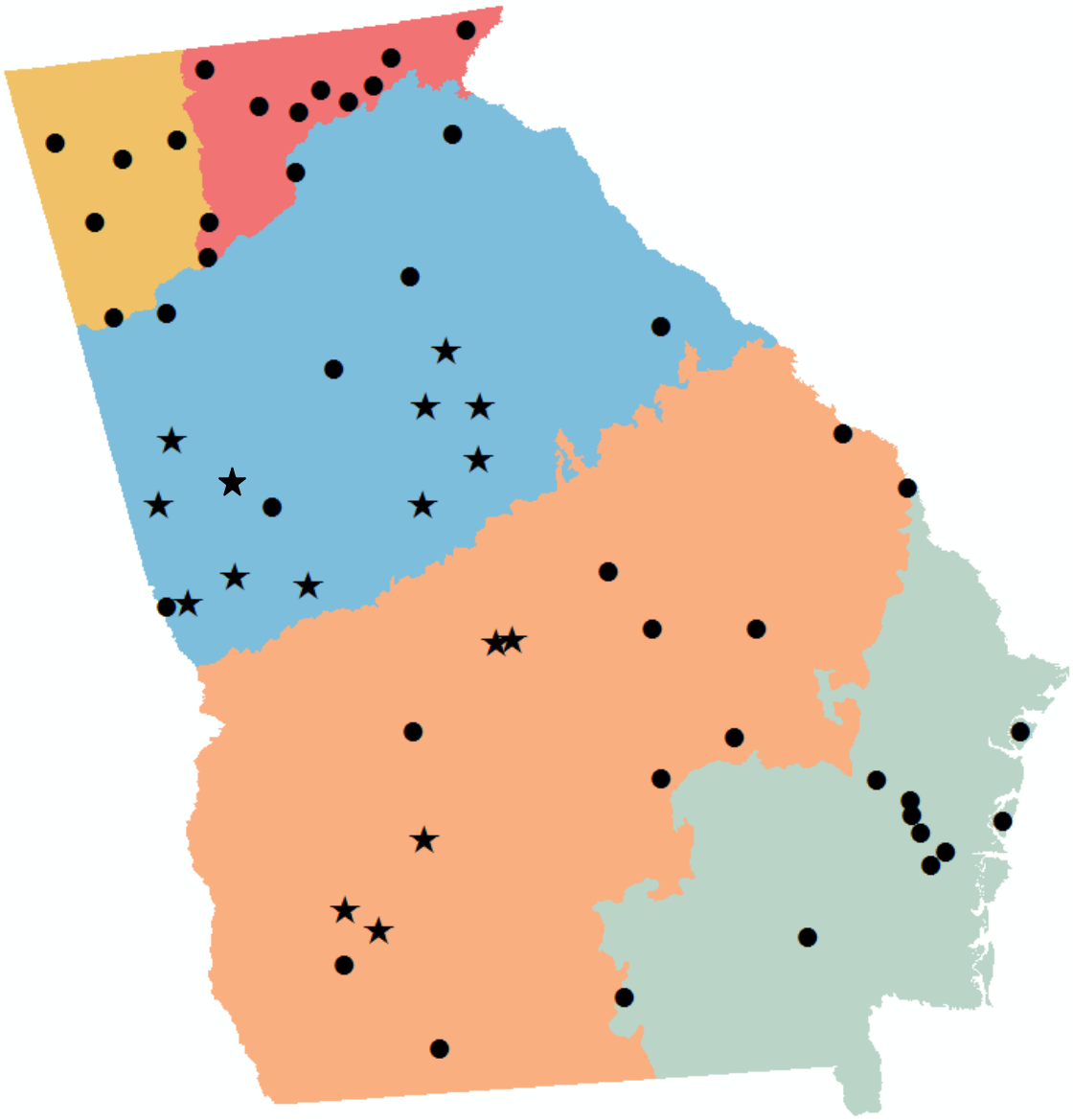

Figure 3.1. Locations where hunter-harvested deer were surveyed for incidence of cranial abscesses, 2011-2012. Stars represent sites where at least one buck tested positive for cranial abscess disease, whereas circles represent sites where no positive cases were found. The five physiographic regions of Georgia are represented by differing colors (yellow = Ridge and Valley; red = Blue Ridge; blue = Piedmont; beige = Upper Coastal Plain; grey = Lower Coastal Plain).

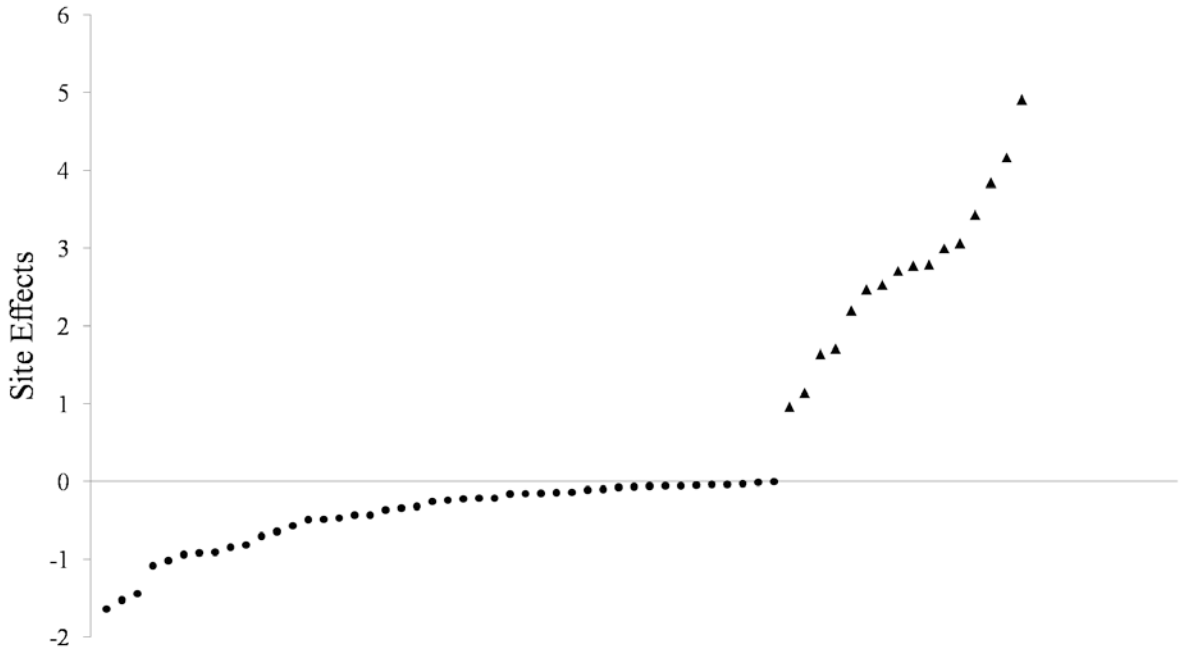

Figure 3.2. Random effect associated with each site examined. Random effects were assumed to be normally distributed with a mean of zero. Triangles represent sites where an incidence of cranial abscesses was recorded. Circles represent sites where no cranial abscesses were found.

CHAPTER 4

VIRULENCE DETERMINANTS OF *TRUEPERELLA* (*ARCANOBACTERIUM*) *PYOGENES* EXPLAINS VARIABILITY IN PRESENCE OF CRANIAL ABSCESS DISEASE IN WHITE-TAILED DEER AT SITES ACROSS GEORGIA[3]

[3]Cohen, B.S., E.H. Belser, S.P. Keeler, M.J. Yabsley, K.V. Miller. 2014. To be submitted to *Journal of Wildlife Diseases*.

ABSTRACT: *Trueperella pyogenes*, a microbial resident along the forehead of white-tailed deer, is the purported etiological agent associated with intracranial abscessation-suppurative meningitis, a cause of natural mortality in male white-tailed deer (*Odocoileus virginianus*). Incidence of this disease varies among regions even within states, perhaps due to differing prevalence or differing virulence of *T. pyogenes* residing on deer in geographically distinct population. We examined the presence of seven virulence determinants from the forehead swabs of 186 apparently health deer from Georgia, USA that tested positive for *T. pyogenes*. Six of the seven virulence determinants were detected more commonly on sites affected by cranial abscesses when compared to sites which were unaffected. In particular, *nanH* and *fimA*, genes coding proteins that promote general epithelial attachment and which are critical for initial adhesion during infection, were found approximately six and four times more frequently on affected sites. Differences in the presence of virulence determinants observed in *T. pyogenes* between affected and unaffected sites appear to explain the site-to-site variability commonly observed in this disease.

KEY WORDS: cranial abscess, disease, intracranial abscessation, *Odocoileus virginianus*, white-tailed deer

INTRODUCTION

The intracranial abscessation-suppurative meningitis disease complex is generally considered a minor cause of mortality in white-tailed deer (*Odocoileus virginianus*) populations (Davidson et al. 1990, Baumann et al. 2001), although it some regions it can account for nearly 35% of annual mortality of mature bucks (Karns et al. 2009). Due to the high variability in prevalence rates reported among deer populations (Baumann et al. 2001, Cohen 2014), as well as the purported impacts of climactic (Baumann et al. 2001, Karns et al. 2009) and demographic (Karns et al. 2009, Cohen 2014) conditions on the prevalence of this disease, the risk factors associated with this disease are speculative.

Trueperella pyogenes (formerly *Arcanobacterium pyogenes*, Yassin et al. 2011), a bacterium commonly resident on mucosal membranes and skin of healthy livestock and wild animals such as white-tailed deer (Turner et al. 2013, Moore et al. 2010), has been isolated from 65 of all 100 published cases of intracranial abscesses in white-tailed deer in which culture was attempted (Davidson et al. 1990, Baumann et al. 2001, Nettles et al. 2002). To successfully invade hosts, *T. pyogenes* relies on virulence factors such as collagen-binding protein (cbpA), neuraminidases (nanH and nanP), and fimbriae (fimA, fimC, fimE, fimG) to adhere to host cells (Jost and Billington 2005).

The prevalence of cranial abscesses (the predecessor of intracranial abscesses) may be related to the prevalence of *T. pyogenes* on male white-tailed deer among (Belser 2013, Turner et al. 2013). Furthermore, *T. pyogenes* cultured from active cranial abscess infections typically test positive for genes encoding several virulence factors (Cohen 2014). Increased genotypic virulence should allow for enhanced epithelial attachment and host avoidance, resulting in increased presence of the disease. Thus, differences in occurrence of virulence determinants in *T.*

pyogenes among sites may explain the variable spatial distribution of cranial abscesses. In an attempt to explain the site-to-site variation in cranial abscess disease observed in previous studies, we used polymerase chain reaction (PCR) to screen for the presence of seven virulence determinants of *T. pyogenes* from the forehead of white-tailed deer harvested at sites both affected and unaffected by cranial abscess disease.

MATERIAL AND METHODS

During Fall 2011 and Fall 2012, we examined all hunter-harvested deer from 29 sites selected to represent a distribution across all physiographic provinces in Georgia (Blue Ridge, Ridge and Valley, Piedmont, Upper Coastal Plain, and Lower Coastal Plain) for signs of cranial abscesses. The sex and location were recorded for each deer sampled.

Because cranial abscesses are the predecessor of intracranial abscesses and are grossly diagnosable without dissecting the skull, we used cranial abscesses as a surrogate measure of intracranial abscesses, which are difficult to inspect on a large scale. We performed a thorough examination of each deer's cranium. Each pedicle was visually examined by looking for signs of scabbing and suppurative infection along the cranium as described in Davidson et al. (1990). Any grossly visible signs of an infection on the forehead were diagnosed as a cranial abscess. Because of the relative rarity of this disease across the general deer population, sites in which cranial abscesses occurred at a prevalence of at least 1% among bucks within the two-year time frame of this project were considered to be affected sites, while sites below this threshold were considered to be unaffected by disease.

We sampled all deer (males and females of any age) with a sterile, rayon-tipped applicator (Puritan Medical Products, Guilford, ME, USA) along the dermal lining of the

forehead. We placed each sample in an individual 2.5 mL Cryosaver vial (Cryosaver, Hardy Diagnostics, Santa Maria, CA, USA) and then froze them at -20°C until analyzed.

We extracted DNA from swabs using the DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA, USA), per manufacturer's instructions, with the following modifications: extra phosphate-buffered saline (PBS) (400 µl total) and lysis (AL) buffer (400 µl total); an additional five minutes to incubation for lysis; and an additional one minute to the final incubation step. We stored the extracted samples at -20°C until further processing.

Using PCR, we determined the presence of *T. pyogenes*. Previous research shows the *plo* gene to be present in all reported isolates of *T. pyogenes* (Jost and Billington 2005). Thus we used the presence of *plo* to identify swabs containing *T. pyogenes*. Each single PCR amplification occurred in 25-µl reaction mixtures containing 11 µl of DNA-free PCR water (MO BIO Laboratories, Inc., Carlsbad, CA, USA), 0.25 µl of dNTP, 7.75 of GoTaq Flexi DNA Polymerase (Promega Corporation, Madison, WI, USA), 0.5 µl of each primer, and 5 µl volume of DNA sample. Amplifications were performed in an automated thermal cycler (BioRad DNA Engine, Hercules, CA, USA), with the cycling conditions described in Table 4.1. The PCR products were analyzed by electrophoresis on agarose gel (1.5%, with ethidium bromide) at 100 V for 20 minutes. A 100 basepair DNA ladder (Promega Corporation) was used as DNA marker. The gel was visualized using an AlphaImager gel documentation system (ProteinSimple, Santa Clara, CA, USA) to determine presence or absence of the *plo* gene, and thus *T. pyogenes*. Precautions were taken to avoid contamination of samples, including separation of the areas for DNA extraction and PCR preparation; regular changing of gloves; and use of DNA-away (Molecular BioProducts, Inc., San Diego, CA, USA) on equipment. All PCR cycles included negative controls of DNA-free PCR water (MO BIO Laboratories, Inc.) without DNA template

to ensure there was no contamination in the reaction mixture. All cycles included a positive control (JGS 189, JGS 230).

We assessed the presence of known and putative virulence determinants in samples containing *T. pyogenes* by PCR. We utilized the same PCR amplification reaction mixture as previously described for the *plo* gene with modifications to the primer sequence and cycling conditions (Table 4.1). We analyzed the PCR products as previously described, and the result was approved by the correct molecular size of the amplification product.

For our analysis, we collapsed all samples taken into two categories: those taken at affected sites and those taken at unaffected sites. We then assessed differences in virulence determinant occurrence between affected and unaffected sites by utilizing chi-square tests for and set the level of significance at $p < 0.05$. We conducted all analysis in program R version 2.11.1 (R Development Core Team 2010).

RESULTS

Of the 29 sites examined, we detected male deer with cranial abscess disease at 12 sites which were classified as affected sites. On unaffected sites, 88 of 366 (24%) swabs tested positive for *T. pyogenes,* whereas 98 of 280 (35%) swabs tested positive for *T. pyogenes* on affected sites. Occurrence of virulence determinants between affected and unaffected sites was greater at affected sites for *nanH, nanP, fimA, fimC, fimE,* and *fimG* ($p \leq 0.05$; Figure 4.1; Table 4.2). Only the occurrence of *cbpA* did not differ between site classifications ($p = 0.81$).

DISCUSSION

The occurrence of seven virulence determinants of *T. pyogenes* detected from the foreheads of healthy white-tailed deer were greater at sites affected by cranial abscess disease. *CbpA* was the only virulence determinant not detected differently between affected and

unaffected sites. The more frequent occurrence of these virulence determinants creates differences in population-level pathogenicity, and may be responsible for the site-to-site variation seen in this disease (Baumann et al. 2001, Cohen 2014).

Besides *plo*, both *nanH* and *fimA* are dominating genes for neuraminidase and fimbriae expression (Zhao et al. 2013). These genes are upregulated at the beginning of infection, and seem critical for promoting initial adhesion to the epithelial linings (Zhao et al. 2013). Meanwhile, nanP seems important for inducing latent infections, and enhancing epithelial attachment and subsequent colonization (Zhao et al. 2013). Furthermore, *nanP* and *fimA* were present in 100% of isolates from active cranial abscesses (Cohen 2014). We found *nanH*, *nanP* and *fimA* occurred 415%, 171% and 276%, respectively, more frequently on affected sites compared to unaffected sites. These differences in occurrence may account for the site-to-site variability.

FimC, *fimE*, and *fimG* seem important for recognition of host specific tissue (Jost et al. 2002, Zhao et al. 2013). Of these three, *fimE* occurred most frequently in affected sites (52%), and was also detected over three times more commonly on affected sites compared to unaffected sites. Further, *fimE* has been present in 98% of isolates from cranial abscesses where virulence determinant occurrence was examined (Cohen 2014). Although all fimbriae subunits C, E, and G could account for some differences in site variability, fimE may to play a more important role in adhesion to the epithelial linings of white-tailed deer and is likely critical for the ability of *T. pyogenes* to cause cranial abscesses.

The reason for the virulence differences between affected and unaffected sites is speculative. *T. pyogenes* is a common microflora resident in livestock (Jost and Billington 2005) and virulent strains could have been introduced by livestock-wildlife interaction or these

differences may be a relic of the reintroduction of white-tailed deer into Georgia during restoration efforts conducted in the 1940-1970's. Georgia was restocked with deer from within Georgia, but also received many deer from Wisconsin and Texas (Blackard 1971). Interestingly, the areas of Georgia in which cranial abscesses occur were restocked with deer from the Sandhill Game Farms (now Sandhill Wildlife Area) of Wisconsin, a property surrounded by a high fence since before restocking took place, and where cranial abscesses do occur (Wayne Hall, *personal communication*). In contrasting, other regions of Georgia were restocked primarily with deer from within Georgia and from Texas (Blackard 1971), where cranial abscesses reportedly do not occur (Baumann et al. 2001). Although the restocking of Georgia with different deer herds might have introduced different strains of *T. pyogenes* into the state, the evidence is anecdotal and the commonality of *T. pyogenes* on other animals makes any inferences difficult.

ACKNOWLEDGEMENTS

Funding for this project was provided by the Georgia Wildlife Resources Division through the Wildlife Restoration Program, which derives monies through an excise tax on sporting arms and ammunition paid by hunters and recreational shooters. We thank all the regional wildlife biologists as well as the technicians who assisted in data collection. We thank all of the private landowners for allowing us access to their properties and for their help with data collection. We thank J. Fischer, the Southeastern Cooperative Wildlife Diseases Study and the Veterinary Diagnostics Laboratory at the University of Georgia for their equipment and assistance. We thank M. MacKenzie for her assistance in the lab.

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Table 4.1. Sequence of PCR primers and cycling conditions used to amplify virulence determinants of *T. pyogenes*.

Target gene	Virulence factor	Oligonucleotide sequence (5' to 3')	Amplicon size (bp)	Reference
plo	Pyolysin	Fw: GGCCCGAATGTCACCGC	270[a]	Jost et al. (2002)
		Rv: AACTCCGCCTCTAGCGC		
nanH	Neuraminidase H	Fw: CGCTAGTGCTGTAGCGTTGTTAAGT	781[b]	Silva et al. (2008)
		Rv: CCGAGGAGTTTTGACTGACTTTGT		
nanP	Neuraminidase P	Fw: TTGAGCGTACGCAGCTCTTC	150[b]	Silva et al. (2008)
		Rv: CCACGAAATCGGCCTTATTG		
cbpA	Collagen-binding protein	Fw: GCAGGGTTGGTGAAAGAGTTTACT	124[b]	Silva et al. (2008)
		Rv: GCTTGATATAACCTTCAGAATTTGCA		
fimA	Subunit of type A fimbriae	Fw: CACTACGCTCACCATTCACAAG	605[c]	Silva et al. (2008)
		Rv: GCTGTAATCCGCTTTGTCTGTG		
fimC	Subunit of type C fimbriae	Fw: TGTCGAAGGTGACGTTCTTCG	843[b]	Silva et al. (2008)
		Rv: CAAGGTCACCGAGACTGCTGG		
fimE	Subunit of type E fimbriae	Fw: GCCCAGGACCGAGAGCGAGGGC	775[d]	Silva et al. (2008)
		Rv: GCCTTCACAAATAACAGCAACC		
fimG	Subunit of type G fimbriae	Fw: ACGCTTCAGAAGGTCACCAGG	929[c]	Silva et al. (2008)
		Rv: ATCTTGATCTGCCCCCATGCG		

[a] Cycling conditions: 35 cycles of 94°C (60 s), 55°C (60 s), 72°C (60 s); final extension 72° C (300 s)

[b] Cycling conditions: 94°C (180 s), 35 cycles of 94°C (60 s), 60°C (60 s), 72°C (60 s); final extension 72° C (420 s)

[c] Cycling conditions: 94°C (180 s), 35 cycles of 94°C (60 s), 57°C (60 s), 72°C (60 s); final extension 72° C (420 s)

[d] Cycling conditions: 94°C (180 s), 35 cycles of 94°C (60 s), 55°C (60 s), 72°C (60 s); final extension 72° C (420 s)

Table 4.2. Presence of known and putative virulence determinant genes of *T. pyogenes* from the forehead of healthy white-tailed deer at sites affected or unaffected by cranial abscess disease. Male white-tailed deer harvested at 29 sites during 2011 and 2012 were examined for signs of cranial abscesses. Sites where no cranial abscesses were found were considered unaffected sites, whereas sites where cranial abscesses were found were considered affected sites. A chi-square test was used to determine if there were differences in occurrence of these genes between these sites (*df* = 1 for each test). Affected sites had significantly higher occurrences of all virulence determinants except *cbpA*.

Virulence factor	Number of detections		X^2	*P-value*
	At affected site (n = 98)	At unaffected sites (n = 88)		
nanH	37	8	19.24	< 0.01
nanP	61	32	7.08	< 0.01
cbpA	22	22	0.06	0.82
fimA	43	14	15.78	< 0.01
fimC	41	24	3.71	0.05
fimE	51	23	11.93	< 0.01
fimG	39	18	7.28	< 0.01

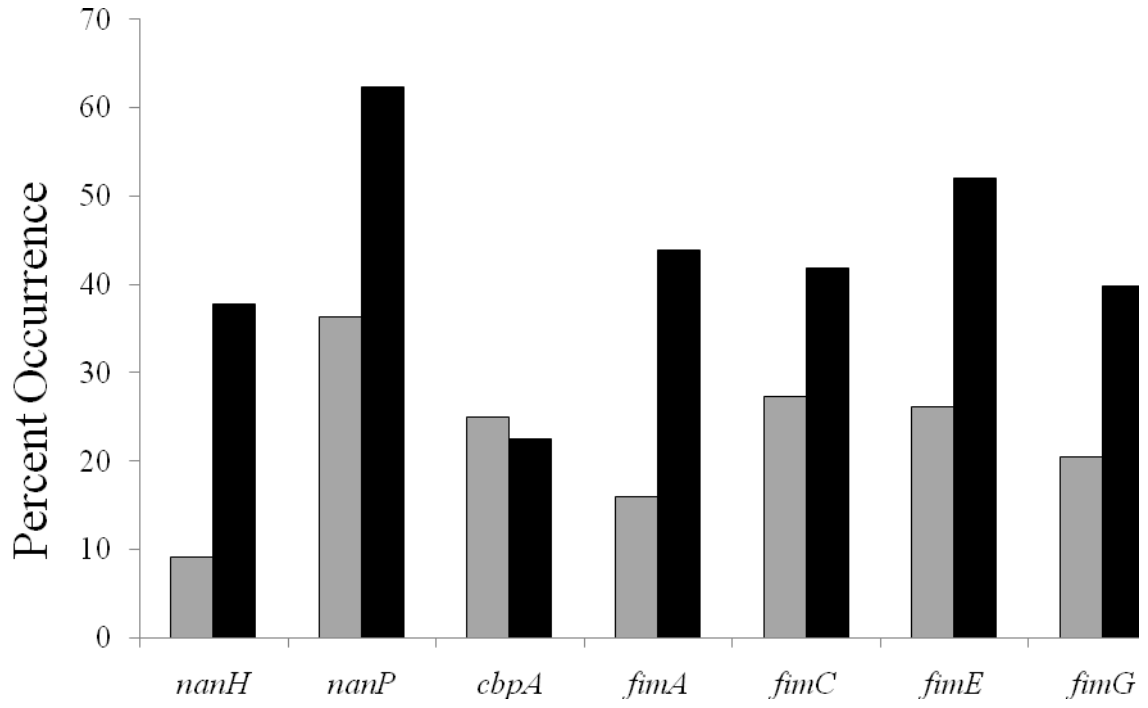

Figure 4.1. Percent occurrence of virulence determinants of *T. pyogenes* from the forehead of white-tailed deer. Grey colored bars represent the percent occurrence from sites where no cranial abscesses were found (17 unaffected sites; n = 88 samples), whereas black bars represent the percent occurrence from sites where cranial abscesses were found (12 affected sites; n = 98 samples). Results of a chi-square test for each virulence determinant showed significantly higher occurrences of virulence determinants in affected sites, excluding *cbpA*.

CHAPTER 5

SUMMARY AND CONCLUSIONS

Cranial abscess disease is a source of morbidity and mortality for male white-tailed deer. Until now, little was known about the prevalence of this disease and its associated risk factors, although some have speculated that both environmental and demographic factors contribute to its apparently patchy distribution. Furthermore, the role of *T. pyogenes,* a resident bacterium along the epidermal surfaces of white-tailed deer, as an etiological agent was speculative, although it had been isolated from many of the reported cases of intracranial abscesses. I designed a series of three studies to examine the role *T. pyogenes* may play as an etiological agent, the risk factors associated with the disease, and what caused the spatial clustering in prevalence seen in the previous study.

First, I examined the virulence potential of *T. pyogenes* isolated from active cranial infections by screening the presence of eight genes which encode for virulence factors. The isolates from these infections commonly tested positive for seven of these eight genes, suggesting that these isolates have high virulence potential and are likely the main causative bacterium of cranial abscesses.

An examination of 7,545 deer across 60 sites demonstrated that the disease mainly affects males. Subsequent analysis of land cover and soil types indicated that these factors did not contribute to the incidence of this disease. Similarly, deer density was not an important risk

factor. The increasing age of the male deer and if it resided at a site with a high buck-to-doe ratio presented the highest risk of it testing positive for the disease. One of the most interesting results was the spatial distribution of sites where cranial abscess disease was detected, which tended to be clustered in the mid-western and central parts of Georgia. Despite having measured a number of factors at the site and individual level, there were still large amounts of variance that were unexplained at the site level, suggesting I had not measured at least one variable that was important to the incidence of this disease.

To determine if the patchy distribution of this disease could be related to the virulence potential of *T. pyogenes*, I examined the presence of seven genes encoding virulence factors from swabs taken from the forehead of healthy deer. From this study, I determined that the *T. pyogenes* residing on the foreheads of healthy deer on sites affected by cranial abscess disease more commonly tests positive for six of these seven genes when compared to unaffected sites. Thus, it is the virulence potential of the *T. pyogenes* residing on the deer which most contributes to the risk of this disease, and there are differences in this potential across Georgia's landscape.

The differences in virulence potential among sites raises the question of how these differences originated. Although speculative, anecdotal evidence suggests it could be related to the restocking of white-tailed deer from Wisconsin into Georgia. I believe future studies should examine if the interstate transport of white-tailed deer brought potentially pathogenic strains of *T. pyogenes* into the state, and if so, what legacies could be created if interstate transportation is permitted in the future.