

ABSTRACT

TURNER, MELISSA M. Paternity and Intracranial Abscessation in White-Tailed Deer Under Quality Deer Management. (Under the direction of Drs. Christopher S. DePerno and Richard A. Lancia.)

Mating systems, which can show temporal and spatial plasticity within a given species, may influence inbreeding, effective population size, genetic diversity, reproductive fitness, and possibly even survival. Although observational research on white-tailed deer has indicated dominant males monopolize breeding opportunities, recent molecular work suggests a more complex system. It is possible that population characteristics fostered under management strategies such as Quality Deer Management (QDM) influence the pre-breeding interactions that affect the distribution of mating success. Our objective was to evaluate the white-tailed deer mating system under QDM through paternity analysis. Using polymerase chain reaction at 8 microsatellite loci and tissue samples from hunter-killed deer at Chesapeake Farms in Chestertown, Maryland, we evaluated 731 deer. Paternity was assigned using Cervus 3.0 and Newpat XL. The 3.5+ age class dominated mating at Chesapeake Farms, with 45% of paternity. However, together the 1.5- and 2.5-year-old age classes accounted for more than half of paternity (56%). We did not detect evidence of polyandry at Chesapeake Farms. Our results suggest the interaction between the balanced sex ratio and older male age structure fostered by QDM influence the mating system by facilitating breeding by younger males. A more equitable mating system suggests there is little selective benefit to monopolizing breeding and may lead to a greater lifetime contribution to the next generation per male and greater genetic diversity and population health.

Understanding the distribution of disease in wildlife is key to predicting the establishment of one health concerns, many of which are zoonotic in nature. The widespread distribution of white-tailed deer and the species' close proximity to humans suggest deer management and population health have implications beyond stewardship as animals can serve as reservoirs for emerging infectious diseases. The intracranial abscessation suppurative meningitis (IASM) disease complex can contribute substantially to mortality in deer. Past studies have most often linked IASM with *Arcanobacterium pyogenes*, a commensal organism in livestock that can serve as a primary pathogen or part of mixed infections in numerous species, including humans. Our objective was to understand the role of *A. pyogenes* in deer and what might predispose a population to carrying it. We used basic bacterial culture techniques to assess *A. pyogenes* prevalence around antler pedicles and in nasopharyngeal membranes of hunter-killed male deer across six regions in Maryland. We evaluated *A. pyogenes* prevalence in deer of both sexes and all age classes under Traditional Deer Management (TDM) and Quality Deer Management (QDM). Finally, we tested live-caught neonates. We evaluated the significance of age, site/region, and sex using binary logistic regression. We did not detect *A. pyogenes* on deer in 3 of the 6 regions studied. The Upper Eastern Shore was the only region where *A. pyogenes* was common; 45% and 66% of antler and nasal swabs tested positive, respectively. Overall, 78% of animals sampled on the QDM property and 95% of animals on the TDM property carried *A. pyogenes* regardless of sex, age class or management with the exception of neonates, which did not carry *A. pyogenes*. The prevalence of *A. pyogenes* in one region suggests the bacterium may be endemic to Upper Eastern Shore deer. Because the region is home to little livestock activity, deer may serve as the reservoir for *A. pyogenes*. The high level of *A. pyogenes* on the Upper

Eastern Shore, as well as low-level presence in adjacent regions, suggest an emerging one health concern warranting further study.

Paternity and Intracranial Abscessation in White-Tailed Deer Under Quality Deer Management

by
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DEDICATION

To my husband, Steve, for his love, support, graphical and scientific expertise, and unflagging interest in my research, and to my son Isaac, who helps me see every day why every effort to understand the beautifully complex world around us is worthwhile.

BIOGRAPHY

Melissa Turner was born on March 3, 1978 in Stonington, Maine, to Michael and Kathy Turner. She has one sister, Jenn Turner. Melissa grew up in Maine and spent much of her life exploring the woods and combing through shells on the beach with her family, forming a lifelong appreciation for the outdoors. Her parents' background in science and their ample supply of books on all topics related to marine ecology laid the groundwork for her interest in science, even as she pursued a degree in English at Smith College in Northampton, Massachusetts, and a career as a writer and editor. Eventually she returned to school, first in the Biology program at the University of North Carolina-Greensboro and finally at N.C. State as a master's student in Fisheries, Wildlife, and Conservation Biology.

Melissa is married to Stephen Allen, and they have one son, Isaac Charles Allen, born January 8, 2011. Upon graduation, Melissa hopes in the short term to pursue a career as a freelance science writer and editor. Longer-term, she hopes to continue working in the field of wildlife management and conservation.

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TABLE OF CONTENTS

List of Tables	vi
List of Figures	viii
1. The Genetic Mating System of White-Tailed Deer (<i>Odocoileus virginianus</i>) under Quality Deer Management	1
Introduction	1
Study Area	4
Methods	4
Results	8
Discussion	8
Management Implications	12
Literature Cited	14
2. Habitat, Wildlife and One Health: <i>Arcanobacterium pyogenes</i> in Maryland and Upper Eastern Shore White-Tailed Deer Populations	24
Introduction	24
Methods	28
Results	31
Discussion	33
Literature Cited	39

LIST OF TABLES

THE GENETIC MATING SYSTEM OF WHITE-TAILED DEER (*ODOCOILEUS VIRGINIANUS*) UNDER QUALITY DEER MANAGEMENT

Table 1. Conditions for polymerase chain reaction for 8 loci used to analyze parentage among white-tailed deer at Chesapeake Farms, MD, USA, 2003-2009	22
Table 2. Characteristics of loci in analysis of 8 microsatellites in white-tailed deer from Chesapeake Farms, Maryland, USA, 2003-2009	22
Table 3. Paternity and maternity assignments by program among white-tailed deer at Chesapeake Farms, Maryland, USA, 2003-2009	23
Table 4. Percent of white-tailed deer paternity assignments by age class at Chesapeake Farms, Maryland, USA, 2003-2009	23

HABITAT, WILDLIFE AND ONE HEALTH: *ARCANOBACTERIUM PYOGENES* IN MARYLAND AND UPPER EASTERN SHORE WHITE-TAILED DEER POPULATIONS

Table 1: Percent of white-tailed deer carrying <i>A. pyogenes</i> by region and swab type in Maryland, USA, 2010.....	46
Table 2: Bacteria carried by white-tailed deer (nasal%, head%) by region in Maryland, USA, 2010.....	47
Table 3: Percent of white-tailed deer carrying <i>A. pyogenes</i> by sex and sample site on the Upper Eastern Shore in Maryland, USA, 2010.....	48
Table 4: Bacteria carried by white-tailed deer (nasal%, head%) at two properties in Maryland, USA, 2010.....	49
Table 5: Binary logistic regression for occurrence of <i>A. pyogenes</i> on white-tailed deer on the Upper Eastern Shore of Maryland, USA, 2010.....	50

LIST OF FIGURES

HABITAT, WILDLIFE AND ONE HEALTH: *ARCANOBACTERIUM PYOGENES* IN MARYLAND AND UPPER EASTERN SHORE WHITE-TAILED DEER POPULATIONS

Figure 1: Pitting of bone and fluid-filled nodule inside skull plate of infected white-tailed deer at Chesapeake Farms, Maryland, USA	51
Figure 2: Intracranial abscess in male white-tailed deer at Chesapeake Farms, Maryland, USA.	52
Figure 3: Physiogeographic regions in Maryland, USA.	53
Figure 4: <i>A. pyogenes</i> % by sex, age class and property on the Upper Eastern Shore, Maryland, USA.	54
Figure 5: Livestock density by physiogeographic region in Maryland, USA.	54

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THE GENETIC MATING SYSTEM OF WHITE-TAILED DEER (*Odocoileus virginianus*) UNDER QUALITY DEER MANAGEMENT

Introduction

Recent advances in molecular DNA fingerprinting technologies have enhanced our understanding of mating systems in a variety of species by allowing biologists to test observation-based hypotheses about genetic mating systems and reproductive success in natural populations (Jones et al 1999, Worthington Wilmer et al. 1999, Garnier et al. 2001, Cerchio et al. 2005, Booth et al. 2007, DeYoung et al. 2009). Within a given species, plasticity in the mating system may exist spatially and temporally (Apollonio et al. 1992, Rowe et al. 1994, Mobley and Jones 2007). Such variation may influence levels of inbreeding (Stockley et al. 1993), effective population size (Sugg and Chesson, 1994, Parker and Waite 1997), genetic diversity (Williams 1975), reproductive fitness (Thirgood 1991), and possibly even survival (Beehler and Foster 1988). A clear understanding of mating strategies is therefore essential to the formulation of effective management decisions for species of economic importance or conservation concern (Clutton-Brock 1989, Garnier et al. 2001, Festa-Bianchet 2003).

The mating system of white-tailed deer (*Odocoileus virginianus*) is characterized by polygyny, wherein a single male forms a tending bond with a single female, courting, guarding and ultimately mating with her during estrus before moving on to another female

(Hirth 1977, Marchinton and Hirth 1984, Clutton-Brock 1989, Holzenbein and Schwede 1989). Adult males are thought to form dominance-based hierarchies through pre-breeding interactions such as sparring that establish their right to breed. Dominance is believed to be correlated with age, weight, antler size, antler rubbing, marking, scraping behavior, elevated levels of testosterone, and experience (Hirth 1977, Townsend and Bailey 1981, Marchinton and Hirth, 1984, Ozoga and Verme 1985, Miller et al. 1987). Although yearling males have been shown to breed, it is unclear if they display the dominance behaviors (e.g., rubbing, marking, scraping behavior) that are believed to lead to mating success in a mixed-age population (Ozoga and Verme 1985, DeYoung et al. 2006, DeYoung et al. 2009). However, mating systems where solitary males defend a single female against other males (e.g., white-tailed deer) may ultimately facilitate breeding by non-dominant males who have access to unintended females and polyandry if tending males are displaced (Hirth 1977, Clutton-Brock 1989). Given the demands of the tending bond and the spatial dispersal of females, it is unlikely that dominant males would be able to find, court, defend and mate with all receptive females during the rut (DeYoung et al. 2006, Sorin 2004). Additionally, because yearling males apparently continue to spar after breeding begins and chase females when older males are occupied with females or challengers, their lack of clear hierarchical status suggests they may contribute to breeding (Hirth 1977, Ozoga and Verme 1985).

Although observational research on white-tailed deer has indicated dominant males monopolize breeding opportunities (Hirth 1977, Marchinton and Hirth 1984), recent molecular work suggests a more complex system, with younger males successfully siring offspring (Sorin 2004, DeYoung et al. 2006, DeYoung et al. 2009). Although dominance was correlated with breeding success in a captive setting, the oldest individuals were unable

to completely monopolize breeding in free-range populations (DeYoung et al. 2006, DeYoung et al. 2009).

It is possible that population characteristics can further influence the pre-breeding interactions that affect mating and the distribution of mating success. Quality Deer Management (QDM) is an increasingly common management strategy that influences population dynamics through harvest restrictions (Newsom 1984, Woods et al. 1996). QDM is characterized by increased female harvest and restraint in harvesting young males (Hamilton et al. 1995, Shaw 2005) resulting in increased male age structure, a more balanced sex ratio, high estrous synchrony, and a reduced population density. Although the interaction between age structure and sex ratio is unclear and intraspecific mating systems may vary (Clutton-Brock 1989, Thirgood 1990, DeYoung et al 2009), the balanced sex ratio (1:1.25 to 1:1.5 in our study population, M. Conner, Chesapeake Farms, unpublished data) and high estrous synchrony achieved under QDM, in concert with an older male age structure, could impact the mating system of white-tailed deer by lowering reproductive success per individual male and across age classes (Langbein and Thirgood 1989, Festa-Bianchet 2003). Conversely, the mating system of deer may influence the degree to which the goals of QDM, specifically an increase in “quality” deer, are met if it is driven by morphological characteristics (Shaw 2005).

Healthy adult female white-tailed deer produce an average of nearly 2 fawns per pregnancy (Verme 1965, Verme and Ozoga 1981). Although polyandry has been documented in captive white-tailed deer (DeYoung et al. 2002, Sorin 2004, DeYoung et al. 2006), the factors influencing the strategy are unclear. For example, DeYoung et al. (2002) detected no clear relationship between sex ratio and polyandry. However, high estrous

synchrony, as observed in northern latitude free-ranging populations and in those under QDM, may negatively influence polyandry through reduced competition for estrous females.

Conceivably, the sex ratio and male age structure fostered by QDM could impact competition for mates, which in turn may affect mating strategies and influence levels of polyandry. In an effort to clarify the roles of sex ratio and age structure in the mating system of white-tailed deer, our objective was to evaluate parentage in a population managed under QDM. This objective was achieved through the application of microsatellite DNA fingerprinting technology.

Study Area

Chesapeake Farms is a 1,300-ha property on Maryland's Eastern shore comprising 50% forest with non-alluvial swamps, 20% cropland, 13% fallow fields, and 17% composed of impoundments and other managed wildlife habitat (Shaw 2005, Karns 2009). The white-tailed deer population, hunted annually for at least 40 years, was managed under QDM beginning in 1994 employing limited harvest of males restricted to individuals with antler spreads wider than ear tips (i.e. 2.5+ years old). The male:female sex ratio was 1:1.25 for the duration of the study with density of ~30 deer/km² in 2003, ~25 deer/km² in 2004-2007, and ~33 deer/km² for 2008-2009 (M. Conner, unpublished data).

Methods

Sample collection

We collected tongue tissue from male and female deer harvested at Chesapeake Farms between 2002 and 2009. We recorded dressed weight, sex and antler points and spread, and animals were aged using tooth wear and replacement employing a set of known-age jaws

collected onsite for comparison (Severinghaus 1949). We collected ear tissue biopsies from adult males collared for unrelated studies and fawns that were captured each spring as part of routine tagging efforts at Chesapeake Farms as well as tissue samples from fetuses when available in harvested females (e.g., taken in spring). A total of 731 samples were used for parentage analysis: 230 male, 501 female, 12 fetuses and 60 neonates.

DNA Extraction and Microsatellite Genotyping

DNA was extracted from tissue samples following a modification of the PureGene DNA isolation protocol (Gentra Systems, Minneapolis, Minnesota, USA). DNA was resuspended in 1x TE buffer and subsequently tested for quality and concentration on a Nanodrop 1000 spectrophotometer (ThermoScientific, Wilmington, Delaware, USA). All samples were standardized to a concentration of 25 ng/ μ L, and stored at -20°C prior to processing. Samples were genotyped using a panel of eight microsatellite loci (D, K, N, P, Q, R, Cervid 1, and BL 25) previously described by Anderson et al. (2002). Polymerase chain reactions (PCR) were carried out in 5.6 μ l volumes, each containing 1X Bioline 10X NH₄ buffer (160 mM (NH₄)₂SO₄, 670mM Tris-HCl , 0.1% Tween-20), 1.8 - 2 mM MgCl₂ (Table 1), 100 μ M dNTPs, BSA, 50 ng DNA template, 0.5U Taq DNA Polymerase (Bioline USA, Boston, Massachusetts, USA), and ddH₂O to 5.6 μ l. Primer concentration varied between 0.02 pM to 1 pM (Table 1) with the forward primer of each end-labeled with a M13F-29/IRD700 IRDye™ tag (Li-Cor, Lincoln, Nebraska, USA). Annealing temperatures ranged from 58°C to 67°C (Table 1). Due to evidence of high frequency null alleles at the P locus, indicated by significant amplification failure and high levels of homozygosity, primers were redesigned using PRIMER3PLUS (Untergasser et al. 2007) as follows: forward - [GATATACCTGGTCTGACCTGTCAG](#); reverse - [CATGCCAATCAGATGTTAGAC](#).

The redesigned P-primer is hereon referred to as P-2011, in order to avoid confusion with P. Conditions for polymerase chain reaction amplification were altered from those outlined previously by Anderson et al. (2002) and comprised an initial denaturation stage of 5 mins at 94°C, followed by 26 – 35 cycles each consisting of 30 secs at 94°C, 30 secs at the ideal temperature for each primer set, and 1 min at 72°C (see Table 1 for specific primer details). Following PCR, products from up to three loci were combined (Table 1) and 5 µl of sequence stop solution (95% Formamide, 20 mM EDTA, Bromophenol blue) was added. Reactions were subsequently denatured at 90°C for 4 min, and 1 µl was loaded onto 25 cm 6% 1X TBE polyacrylamide gels, run on a Li-Cor 4300 dual-laser automated DNA sequencer, and sized using 50–350 bp IRDye™ standards (Li-Cor, Lincoln, Nebraska, USA). All gels included three positive controls and one negative control. Gels were run at a constant power of 40W at 50°C for up to 2 hours. Results were analyzed using GENEPROFILER™ software (Version 4.05, Scanalytics, Rockville, Maryland, USA).

Genetic Data Analysis

Testing for Hardy-Weinberg Equilibrium (HWE) was conducted in Cervus (Version 3.0, Marshall et al. 1998). MICRO-CHECKER 2.2.3 software (Oosterhout et al. 2004), was used to evaluate the likelihood that null alleles, scoring error, or large allele dropout was present in our dataset. Parentage was assigned by year with candidate files created for potential mothers, fathers and offspring based on age at time of sampling. Aging of deer 1.5 years or younger uses replacement of the third premolar (Severinghaus 1949). Aging of deer 2.5 years and older by tooth wear can be problematic, but all deer in the present study were aged using locally collected known-age deer jaws (Gee 1998, Gee et al. 2002). However, to limit error introduced by aging problems, all deer were assigned to age classes of 0.5, 1.5, 2.5, and

3.5+ for construction of candidate parent files and age-class breakdown of matches (DeYoung et al. 2009).

We assigned parentage using two programs, Cervus 3.0 and NEWPAT XL (Marshall et al. 1998, Worthington Wilmer et al. 1999, Cerchio et al. 2005, Shaw 2005). Cervus uses a maximum-likelihood method by comparing the LOD scores, a measure of linkage, of the two most likely candidate fathers to calculate a delta statistic. The critical values of the delta statistic to assign parentage at 80% and 95% confidence, respectively, were derived from parentage simulations based on population sampling parameters and allele frequencies. NEWPAT employs a randomization approach by comparing genotypes directly and calculating the likelihood that a match would occur at random. Because it is possible for true parent-offspring pairs to contain genetic mismatches due to PCR problems, scoring error, mutation or null alleles (Queller et al. 1993, Marshall et al. 1998, Dakin and Avise 2004, Hoffman and Amos 2005), both programs allow for some mismatching between genotypes. We specified a 1% typing error rate in Cervus (Sorin 2004, DeYoung et al. 2009). Typing error could include PCR error, scoring error or null alleles. Following published studies, we accepted Cervus assignments at $\geq 80\%$ confidence (Marshall et al. 1998, Slate et al. 2000, DeYoung 2009). Also, due to previously published evidence of null alleles at some loci, we allowed a maximum of one null match (i.e., both individuals are homozygous for different alleles and presumably share a null allele at a given locus) in NEWPAT (DeYoung et al. 2003, Shaw 2005). We did not allow for scoring error in NEWPAT because a screened subset of individuals ($N = 51$) contained less than 1% scoring error. Also in NEWPAT, we restricted results to a range of relatedness values derived from our known matches (i.e., 0.32-0.71). Relationships assigned by both programs meeting all criteria were considered positive parentage assignments. Matches were also screened manually and any

individuals whose age was uncertain at time of parentage (i.e., they were assigned an age range when harvested).

Results

An average of 716 individuals were scored per locus (range 708 – 730, Table 2). Significant deviations from Hardy-Weinberg equilibrium were detected at four loci, Cervid 1, BL25, N and D. Where deviations occurred, they resulted from an excess of homozygotes. MICRO-CHECKER identified these loci as potential candidates for exhibiting null alleles, with population null allele frequencies estimated to range from 0.048 to 0.083 following Brookfield (1996). Across loci, allelic diversity ranged from to 2 to 15, with a mean value 8.75 per locus. Mean expected heterozygosity was 0.656 (range 0.072 – 0.870), whereas mean observed heterozygosity was 0.596 (range 0.073 to 0.862). Combined non-exclusion probability (i.e., the probability that a non-parent is considered as a candidate parent) across all loci was 0.0147.

A total of 445 offspring were considered in parentage analysis (Table 3). Paternity was assigned following combined analysis in *Cervus* and NEWPAT to 52 deer, representing 37 sires; sires represented all age classes (Table 4), but younger deer (i.e., 1.5 and 2.5) collectively accounted for 56% of all sires. Average lifetime offspring detected was 1.49 (range 1-4). We assigned maternity to 53 deer, representing 49 dams. Average lifetime offspring detected was 1.14 (range 1-2). Although we detected siblings likely born in the same year, we were unable to confirm multiple paternity.

Discussion

Our results indicate males of all age classes breed under QDM. Our results are contrary to what one might expect under a system that includes high numbers of the older-

age-class males typically associated with mating success (Hirth 1977, Marchinton and Hirth 1984). Our findings are supported by genetic studies of non-QDM populations, wherein breeding is distributed among all age classes (Sorin 2004, DeYoung et al. 2009). Although the 3.5+ age class is responsible for more breeding (i.e., 45%) than any other age class at Chesapeake Farms, the 1.5- and 2.5-year-old age classes together comprise more than half (i.e., 26% and 30%, respectively) of successful matings. Breeding by the youngest sexually mature males (i.e., the 1.5-year-old age class) is particularly surprising under QDM because of the abundance of 3.5+ males, which are estimated to comprise more than half the male population at Chesapeake Farms (M. Conner, unpublished data). However, the balanced sex ratio of Chesapeake Farms could facilitate breeding by subordinate males through several possible mechanisms. Most does at Chesapeake Farms are bred within a single initial rut, followed by a small secondary rut, restricting opportunities for any one male to mate with multiple females (Miller et al. 1995). Additionally, males of any age occupied by a tending bond are unable to monopolize other females, allowing competing males the opportunity to mate regardless of their hierarchical status (Hirth 1977, Jones et al. 2011). Finally, it is possible the abundance of older adult males shifts the social dynamics of that age class. Typically, dominant males establish hierarchy ahead of the rut, but an abundance of physically mature males may mean dominance is less clearly defined as breeding begins due to increased competition (Hirth 1977, Ozoga and Verme 1985).

We did not detect evidence of polyandry at Chesapeake Farms, possibly a result of the low level of parentage assigned. It is possible that under QDM, polyandry may be minimal because the tending bond likely limits the total number of females with which any single male can mate (Hirth 1977). Nevertheless, levels of parentage assignment are limited

in genetic studies that use harvested animals because once an animal is sampled it is removed from the population and no longer contributing to future generations (DeYoung 2009).

Working with harvested deer in a QDM population further limits the number of males sampled because harvest of males is restricted. Extensive efforts to tag and collect DNA from fawns on a QDM site would improve parentage assignment and analyses by providing a known-age animals and leaving sampled animals in the population.

Breeding under QDM has potential to shed light on deer behavior in general. Surreptitious mating elevating the reproductive success of younger-age-class males, and the 1.5-year-old age class in particular, clearly is a different strategy than that used by dominant males. Studies in other animals suggest a variety of strategies can lead to breeding success within a species (e.g., Thirgood 1990, Shuster and Wade 1991, Coltman et al. 1999, Gemmell et al. 2001). In populations where the oldest age class is relatively small, logic would suggest there is little barrier to breeding by younger males. However, the abundance of younger males breeding at Chesapeake Farms raises the question of why the oldest age class, present in significant numbers, is unable to dominate breeding. There may be little selective benefit to monopolizing breeding among deer. The spatial distribution of females and the effort required to protect them from other males means considerable effort and energy must be expended to find, court, and defend large numbers of females, particularly in a short time span (DeYoung et al. 2009). Each additional mating leads to greater energy expense for males who are left depleted by the rut as winter approaches (Gavin et al. 1984, Ditchkoff et al. 2001). A smaller number of matings per year may increase individual fitness by resulting in a larger lifetime contribution to the next generation.

Microsatellite DNA loci are widely recognized for their application in addressing questions at both the individual and the population level. Analysis and the interpretation of results, however, may be complicated by genotyping errors. Although the incidence of factors such as allelic dropout can be minimized through the use of adequate concentrations of high-quality DNA template (Wandeler et al. 2003), errors resulting from null alleles are more difficult to address. In the absence of species-specific markers, investigators often screen primers developed for closely related species. In many cases these may prove ideal, however in some instances, amplification failures and/or an excess of homozygotes result from mutations at one or both priming sites (Dakin and Avise 2004). At the population level, loci exhibiting genotyping errors, including null alleles, often result in a deviation from Hardy-Weinberg equilibrium. While observed allelic and genotypic frequencies can be adjusted if null alleles are detected, thus permitting further population-level analyses (Oosterhout et al. 2004), at the individual level in studies of parentage, this adjustment is not possible. Fortunately, statistical software programs for parentage analysis can accommodate null alleles, allowing the user to define a given number of mismatches (Worthington et al. 1999).

Of the 4 problematic loci screened in this study, 3 were developed in species other than white-tailed deer (i.e., N and D were developed in mule deer [*Odocoileus hemionus*], as was P, which we replaced with P-2011, and BL25 was developed in cattle [*Bos primigenius*]). Additionally, of our known fetal-maternal pairs, we detected a mismatch at the N locus between dam and fawn, wherein both animals were homozygous for different alleles; presumably the pair shared a null allele. However, our relatively low number of unscored individuals at problematic loci leads us to believe that while null alleles are present

within the population screened, bias introduced for parentage analysis is likely minimal due to the over-inflation of homozygotes or non-amplifications (Dakin and Avise 2004). The most likely outcome of null alleles in analysis involves false exclusion of the true parent (Dakin and Avise 2004), leading to lower levels of parentage assignment. Although lower levels of parentage assignment would lower estimates of individual mating success, overall estimates of relative mating success should be unaffected as long as there is no bias associated with age. Tissue samples were analyzed blindly with respect to age and sex, and unless closely linked with an expressed gene [i.e., genetic hitchhiking (Barton 2000)], microsatellites and their null alleles are not subject to the effects of natural selection (DeWoody 2005). To overcome the possibility that Cervus, which assumes HWE, would misassign parentage, we used NEWPAT to screen our results. NEWPAT allows mismatches at a specified number of loci and screens for such problems as scoring error. Only parentage assignments from both programs meeting our conservative criteria were considered positive relationships. We believe the resulting parentage assignments are conservative, accurate, and help explain the white-tailed deer mating system under QDM.

Management implications

The most important question relative to any management strategy is whether it is achieving its goals. The goals of QDM are multifaceted, but they include an effort to produce “quality” deer, characterized in part by good health and large body size. A mating system where all age classes breed has numerous possible effects on that effort, including increased fitness due to greater genetic diversity, and, to the extent that “quality” is associated with breeding success, possibly a greater lifetime contribution to future

generations by the highest-quality males. Although genetics play a role in characteristics such as body size, antler development and other factors, tenets of QDM such as ecosystem management and harvest levels are likely to have a much more tangible, immediate effect on the quality of the deer population.

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TABLES

Table 1: Conditions for polymerase chain reaction for 8 loci used to analyze parentage among white-tailed deer at Chesapeake Farms, MD, USA, 2003-2009. T_A, annealing temperature.

Locus	T _A	MgCl ₂ (mM)	BSA	Primer (pmol/μL)	Cycles	Electrophoresis group ¹
Cervid 1	64	1.8	0.1	0.2	26	A
BL25	67	2	0.1	0.2	35	A
P-2011	58	2	N/A	1	35	B
D	58	2	N/A	0.3	35	C
Q	59	2	N/A	0.08	32	D
R	59	2	N/A	0.06	35	C
K	64	2	0.1	0.04	35	C
N	61	2	N/A	0.02	32	D

¹ PCR product were combined, designated by letter, following individual PCR reactions

Table 2. Characteristics of loci in analysis of 8 microsatellites in white-tailed deer from Chesapeake Farms, Maryland, USA, 2003-2009. N, sample size; H_O, observed heterozygosity; H_E, expected heterozygosity; HWE, Hardy-Weinberg equilibrium - * = P = <0.001, NS = non-significant; Nf, null allele frequency estimate based on Brookfield (1996).**

Locus	Alleles	N	H _O	H _E	HWE	Nf
Cervid 1	13	721	0.782	0.875	***	0.048
BL25	4	714	0.625	0.717	***	0.052
N	15	709	0.717	0.870	***	0.081
D	10	712	0.628	0.777	***	0.083
Q	15	715	0.862	0.867	NS	0
R	2	722	0.238	0.241	NS	0
K	3	730	0.073	0.072	NS	0
P-2011	8	708	0.845	0.826	NS	0
Mean	8.75	716.38	0.596	0.656		

Table 3. Paternity and maternity assignments by program among white-tailed deer at Chesapeake Farms, Maryland, USA, 2003-2009.

	Candidate parents	Candidate offspring	Cervus	NEWPAT	Both
Paternity	155	475	87	174	52
Maternity	440	475	71	269	53

Table 4. Percent of white-tailed deer paternity assignments by age class at Chesapeake Farms, Maryland, USA, 2003-2009.

Share of breeding	Age class		
	1.5	2.5	3.5+
	26% (<i>n</i> = 12)	30% (<i>n</i> = 14)	45% (<i>n</i> = 21)

HABITAT, WILDLIFE AND ONE HEALTH: *ARCANOBACTERIUM PYOGENES* IN MARYLAND AND UPPER EASTERN SHORE
WHITE-TAILED DEER POPULATIONS

Introduction

The “one health” concept is enjoying a resurgence as physicians, scientists, and veterinarians develop greater appreciation for the health implications of the complex interactions between the environment, humans, and domestic and wild animals (Kahn et al. 2007, Kaplan et al. 2009, Zinsstag et al. in press). Wildlife species serve important roles in one health processes as key players in disease interactions involving feral and domestic livestock, and through direct impacts on human health. The majority of emerging infectious diseases in humans are zoonotic (Taylor et al 2001), and most of these are believed to originate from wildlife populations or to be amplified through interactions between wildlife and domestic and feral livestock (Jones et al 2008, Rhyan and Spraker 2010). Understanding the spatiotemporal distributions of disease in wildlife is key to identifying the dynamics and predicting the establishment of emerging infectious diseases (Cutler et al 2010).

The white-tailed deer (*Odocoileus virginianus*) is one of the most abundant and widely distributed large ruminant mammal species in North America (Baker 1994, Nowak 1999, Wilson and Reeder 2005). U.S. white-tailed deer populations have expanded vastly since the early 20th century through management focused on species recovery (Waller and Alverson 1997, Russell et al. 2001). Human-deer and deer-livestock interactions have increased and evolved as well with the expansion of the interface boundary between the species, though more quantitative data on this phenomenon are needed (Zang et al. 2008,

Baker 2010). Approaches to management of burgeoning deer populations have implications beyond resource stewardship, including the potential for increased deer-human conflict (Messmer 2009) and for deer populations to serve as reservoirs for infectious diseases (Rhyan and Spraker 2010). Similarly, environmental impacts on pathogen viability in different physiogeographic environments can alter the overall impact of potential emerging one health concerns.

The intracranial abscessation-suppurative meningitis disease complex (IASM) is generally considered a minor cause of population-wide mortality among white-tailed deer (Davidson et al. 1990, Baumann et al. 2001). Infection prevalence differs regionally, but the disease complex can contribute substantially to deer mortality. In some deer populations IASM has been associated with nearly 35% of annual mortality among mature males (Karns et al. 2009). Skulls of animals affected by IASM are characterized by erosion and pitting of bones, and often fluid-filled nodules beneath the antler pedicle, lesions that are not readily appreciated in living deer (Figure 1). Clinically, antlers may be disfigured, and the antler pedicle is typically surrounded by inflamed tissue and extravasated viscous fluid (Figure 2). The untreated clinical disease is considered fatal in white-tailed deer. Clinical signs of IASM in white-tailed deer mimic other key zoonoses and include incoordination, lack of fear, blindness, weakness, emaciation, and circling (Davidson et al. 1990, Davidson and Nettles 1997).

The precise etiology of IASM in white-tailed deer is not definitively established, but abscesses are most frequently associated with the gram-positive, non-motile, non-spore-forming, short, rod-shaped bacterium *Arcanobacterium pyogenes* (formerly *Corynebacterium, Actinomyces* 1982) (Davidson et al. 1990, Baumann et al. 2001). A.

A. pyogenes is generally considered a commensal organism and an opportunistic pathogen of domestic livestock, particularly cattle (*Bos primigenius*) and swine (*Sus scrofa*). It is a common inhabitant of the mucous membranes of both cattle and swine and can be routinely 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). The organism has also been isolated from clinical infections in a wide range of domesticated and wild ungulates, including domestic sheep (*Ovis aries*), blackbuck (*Antilope cervicapra*) and fallow deer (*Dama dama*) (Griner et al. 1956, Andrews and Ingram 1982, Narayanan et al. 1998, Jost et al. 2002, Lavin et al. 2004, Billington and Jost 2005, Ertas et al. 2005, Portas and Bryant 2005). *A. pyogenes* has been associated with a variety of disease conditions ranging from abortion to osteomyelitis (Timoney et al. 1988, Lewis 1997).

A. pyogenes expresses several known and suspected virulence factors, which may explain its ability to colonize many different host tissues and cause a diverse range of diseases (Jost and Billington 2005). *A. pyogenes* is not considered part of the normal human flora (Jost and Billington 2005), and it is an under-recognized and frequently misdiagnosed emerging human pathogen with the potential to serve as a primary pathogen, though it is more commonly isolated as part of a mixed infection (Kavitha et al 2010, Gahrn-Hansen and Fredricksen 1992). Under reporting of *A. pyogenes* infections is likely because the organism's biochemical profile is very similar to that of *A. hemolyticus* (Vega and Gavan 1970, Gahrn-Hansen and Fredricksen 1992). Many but not all human cases of *A. pyogenes*-related disease reported in the literature have been associated with underlying health problems including diabetes and cancer (Levy et al 2009), but the organism can express a

wide range of virulence factors, and the pathogenesis of infection by *A. pyogenes* is not well characterized (Jost and Billington 2005). The broad array of human disease conditions reported due to *A. pyogenes* includes abdominal abscessation, otitis media, cystitis, mastoiditis, septicemia, sigmoiditis, appendicitis, cholecystitis, peritonitis, endocarditis, meningitis, arthritis, empyema, and pneumonia (Ballard et al. 1947, Chlostka et al., 1970, Vega and Gavan 1970, Jootar et al. 1978, Norenberg et al. 1978, Lipton and Isalska 1983, Gahrn-Hansen and Fredricksen 1992). Though some authors feel the predominant risk factors associated with human disease include close contact with animals, that contact is often not recognized in the history or signalment of the specific cases (Gahrn-Hansen and Fredricksen 1992).

The *A. pyogenes*-associated IASM syndrome in white-tailed deer presents unique opportunities to examine factors that may play a role in the dynamics of what may be an under-recognized emerging one health problem. Adult male white-tailed deer appear to be particularly susceptible to IASM (Davidson et al. 1990, Karns et al. 2009), which could be an important mortality factor in deer populations operated under management strategies designed to foster a balanced sex ratio and an older age structure among male deer such as Quality Deer Management (QDM) (DeYoung 1989). If strategies such as QDM contribute to IASM, management decisions may have broader impacts on deer population health than previously recognized and may impact the health of feral and domestic livestock as well as humans. Infections in male deer are more frequently recognized, but female deer and fawns (> 6 months old) can acquire IASM and pulmonary, mammary, and disseminated systemic infections caused by *A. pyogenes* (Turnquist and Fales 1998, Baumann et al. 2001, Dyer et al. 2004). These health outcomes suggest deer management strategies might not be the only

determinant of the prevalence of the disease complex. Other factors such as environmental characteristics may play a role in the impact of the bacterium. Though *Arcanobacterium* infections and cervid IASM occur across much of North America (Baumann et al. 2001), there is evidence that *A. pyogenes* may not thrive under certain environmental conditions, particularly in arid climates (Baumann et al. 2001, Karns et al. 2009).

We used basic bacterial culture techniques to assess *A. pyogenes* prevalence across deer populations in different physiogeographic regions and under different deer management strategies to evaluate the impacts of these factors on a potential emerging one health concern.

Methods

All procedures followed guidelines set by the North Carolina State University Institutional Animal Care and Use Committee (09-065-O), and all animal handling methods used followed guidelines approved by the American Society of Mammalogists (Gannon et al. 2007). Three related studies were conducted. In all studies, white-tailed deer were sexed by manual palpation and visual examination and assigned to age classes (i.e., 0.5, 1.5 and 2.5+) using tooth replacement patterns (Severinghaus 1949). Animals were weighed when possible and examined physically for external visible signs of disease or injury. Following the methods of Karns et al (2009), we collected head and nasal swabs from each animal using Remel Bacti-Swab transport swabs (Thermo Fisher Scientific, Waltham, Massachusetts, USA). For head samples, we swabbed around antler pedicles for males, and residue was collected from the top of the head where pedicles would be if present for females, or the whole dorsal frontal area of the head for neonates. We collected nasal samples by swabbing the nasopharyngeal membranes by inserting the sterile swab deep into the nasal cavity of one

nostril taking care not to contact the external nares, and rotating the swab gently before removing it. All samples were kept on ice, immediately refrigerated upon leaving the field, and transported to the Salisbury Animal Health Diagnostic Lab (Salisbury, Maryland, USA) or the Frederick Animal Health Diagnostic Lab (Frederick, Maryland, USA) for aerobic bacterial culture on blood agar plates. A gram stain and catalase test were conducted for all colonies growing. Aerobic bacteria were identified to genus and speciated based on morphology, staining characteristics and biochemical characteristics based on standard American Society for Microbiology techniques (Lennette et al. 1985, Holt et al. 1994).

Statewide Study

To establish prevalence of *A. pyogenes* relative to habitat characteristics we sampled from 6 regions across Maryland (Figure 3). Regions approximately follow physiogeographic provinces specified by the Maryland Geological Survey (MGS), but sampled animals could only be identified to county of origin. When county boundaries overlapped provinces, the entire county was assigned to the region that included the majority of the county land mass. Similarly, we divided the MGS-designated Blue Ridge province into the adjacent Ridge and Valley province and Piedmont Plateau province because of sampling difficulties. Cecil County, in northeastern Maryland and part of the Upper Eastern Shore, was not sampled. The westernmost Appalachian province was characterized by gently folded bedrock of shale, siltstone and sandstone. The second westernmost Ridge and Valley province was characterized by poor soils and deeply folded sedimentary, shale or sandstone bedrock. The central Piedmont Plateau province was largely comprised of eroded rocks of volcanic origin. The Coastal Plain regions of the east, the Western Shore and the Upper Eastern and Lower

Eastern shores, were characterized by fertile soils and abundant ground water (Maryland Geological Survey 2011).

Data for this study were collected from 18 October to 12 December, 2010. In each of the 6 regions, head and nasal swabs were collected from a total of 234 hunter-killed male deer (Appalachian N = 50; Ridge and Valley N = 21; Piedmont N = 50; Western Shore N = 33; Lower Eastern Shore N = 32; Upper Eastern Shore N = 48). The antlered:antlerless ratio (fawn males are included in antlerless numbers) in the statewide harvest was 1:1.96 in 2010, respectively.

White-tailed Deer Management Study

To determine the impact of deer management practices on prevalence of *A. pyogenes* on the Upper Eastern Shore region of Maryland, we collected head and nasal swabs from (N = 113) hunter-killed male and female deer data from two properties with similar habitat characteristics. The QDM property was 1,300 ha on Maryland's Eastern shore comprising 50% forest with nonalluvial swamps, 20% cropland, 13% fallow fields, with the remaining 17% impoundments and other managed wildlife habitat (Shaw 2005, Karns 2009). The QDM property is managed under Quality Deer Management (QDM) since 1994, which is designed to foster a balanced sex ratio and an older age structure among male deer (Hamilton et al. 1995). On this property white-tailed deer had been hunted annually with the harvest of males limited to individuals with antler spreads wider than ear tips (i.e., 2.5+ years old). Harvest male:female sex ratio was 1:3.4 and 1:2.5 for 2009 and 2010, respectively.

The TDM property was 925 ha comprising 37.6% marshland, 37.3% forest, 17.6% cropland, with the remainder grassland, moist soil, water, and development located approximately 12.4 km from Property A on the same shoreline. The white-tailed deer

population had been hunted annually with no age or sex restrictions. Male:female sex ratios in recent harvests on the TDM property were 1.3:1 and 2.25:1 for 2009 and 2010, respectively.

Neonate Study

To examine the hypothesis of early (in utero or immediately postpartum) bacterial colonization of deer, head and nasal swabs from 11 neonates at the QDM property were collected 5-8 June, 2009. The neonates were restrained manually for collection of head and nasal cultures.

Data Analysis

For the statewide study, we evaluated the effect of region on whether deer carried *A. pyogenes* using Pearson's chi-square test with Yates' continuity correction conducted in Program R (Version 2.9.1, <http://cran.r-project.org>, accessed 25 April, 2009). For the white-tailed deer management study, we tested whether site, age class and sex predisposed animals to carrying *A. pyogenes* using binary logistic regression with presence of *A. pyogenes* as the dependent variable, and site, age class and sex as independent variables. All analyses were conducted in SYSTAT 13 (Systat Software, Chicago, Illinois, USA), and alpha was set at $P \leq 0.05$.

Results

Statewide Study

Prevalences of *A. pyogenes* and other bacteria across Maryland physiogeographic regions are summarized in Tables 1 and 2. Physiogeographic region was a significant predictor of *A. pyogenes* presence for nasal samples (chi-square = 111.684, df = 1, $p <$

0.001) and head samples (chi-square = 74.932, df = 1, p < 0.001). We did not detect *A. pyogenes* on deer in 3 of the 6 physiogeographic regions studied. On the Lower Eastern Shore, we cultured *A. pyogenes* from only one (3%) nasal swab of 32 deer sampled and none of the head swabs. Similarly, on the Western Shore only one (3%) head swab and no nasal swab cultures included *A. pyogenes* out of 33 animals sampled. The Upper Eastern Shore was the only region where *A. pyogenes* was common; 45% (22/48) and 66% (32/48) of the antler and nasal swabs tested positive, respectively.

White-tailed Deer Management Study

We cultured bacteria from 55 animals (33 females, 22 males) at the QDM property and 58 animals (21 females, 37 males) at the TDM property. The mean number of bacterial species isolated from the QDM property was 2.6 per nasal culture (range 1-4) and 3.0 per antler culture (range 1-5). Overall, 78% of animals sampled on the QDM property carried *A. pyogenes* on at least 1 swab; 54% of head swabs contained *A. pyogenes*, and 79% of nasal swabs carried *A. pyogenes*. The mean number of bacterial species isolated from the TDM property swabs was 3.6 per nasal culture (range 1-7) and 4.0 per head culture (range 1-8, Tables 3, 4). Overall, 95% of the TDM property animals were positive for *A. pyogenes* on at least 1 swab; 84% of nasal swab cultures contained *A. pyogenes*, and 65% of head swab cultures contained *A. pyogenes*.

Prevalence of *A. pyogenes* on hunter-killed deer were similar between the QDM and TDM properties for head ($P = 0.35$) and nasal samples ($P = 0.34$, Table 5). Similarly, the prevalence of *A. pyogenes*-positive cultures was similar between the sexes for head ($P =$

0.13) and nasal samples ($P = 0.14$) and across age classes for head ($P = 0.20$) and nasal samples ($P = 0.99$, Table 5, Figure 4).

Neonate Study

Eleven neonates were sampled on the QDM Property. *A. pyogenes* was not cultured from nasal or head swabs of any of these animals.

Discussion

There is a tendency for health research to focus on highly communicable zoonotic diseases with devastating impacts on human patients (ebola, anthrax, bovine spongiform encephalopathy, rabies), and in recent times, a particular emphasis is placed on what are termed emerging diseases. Less dramatic and more common zoonoses, however, can have serious economic and environmental impacts. Endemic, chronic infections with a range of disease manifestations can impact one health in ways that may go unrecognized, despite the potential for control through relatively basic means. The concept of “emerging” is particularly complex because recognition of a disease can be affected by observer and diagnostic effort with increasing prevalence simply reflecting greater awareness by the health community. *A. pyogenes* fits the basic definition of zoonosis from *Stedman’s Medical Dictionary* as “an infection or infestation shared in nature by humans and other animals.” It is carried by economically important wild and domestic animal species with high potential for close human contact and can cause disease in humans (Gahrn-Hansen and Frederiksen 1992, Baumann et al. 2001, Ertas et al. 2005). The long recognition of *A. pyogenes* as a zoonoses argues against assigning it status as an emerging disease on the basis of definitions

focused on recent identification of the pathogen, but because it is a zoonotic pathogen that has been under reported and misdiagnosed in humans, the term is not entirely unwarranted. The geographic differences in *A. pyogenes* prevalence detected in our study suggest the disease may be considered potentially “emerging” in the sense of locality and regional environmental conditions.

It is challenging to separate environmental and host factors in the epidemiology of disease occurrence. Our environmental study, limited to 6 regions in Maryland, showed that site was an important factor in predicting whether *A. pyogenes* was carried by deer. Our chi-square estimates may be inaccurate due to observed values of less than 5, but it is clear region plays a role in distribution of *A. pyogenes* in deer. A broader variety of habitat types or a more fine-scale approach could have shown more profound differences. Our results suggest environment plays a role in whether *A. pyogenes* is present. *A. pyogenes* is considered commensal in domestic livestock species present across the state. However, our results suggest the bacterium is not routinely carried by deer in most of Maryland.

Although the constellation of pathology compatible with IASM has been observed in regions of Maryland where we did not recover *A. pyogenes* (B. Eyler, Maryland Department of Natural Resources, unpublished data), the clinical and gross pathological definitions of IASM are not sufficiently developed to reliably distinguish gross lesions associated with any given bacterium. It is therefore important that studies of IASM include careful bacteriological sampling to identify which bacteria are associated with the lesions. Other bacterial genera (i.e., *Staphylococcus*, *Pseudomonas*) that have been isolated from pyogenic cerebral lesions in white-tailed deer (Baumann et al. 2001) are abundant and were recovered from more than 50% of animals sampled in every region of Maryland.

Presence of *A. pyogenes* in a white-tailed deer is not sufficient for IASM to develop. Interestingly, we detected similar prevalences of *A. pyogenes* on the QDM and TDM properties, but IASM has not been documented at the TDM Property, while IASM has become increasingly common at the QDM Property, although QDM managers may be more likely to detect the infection (Karns et al. 2009). The differences between the properties suggest bacterial presence, sex ratio, and age structure may interact to help drive IASM prevalence on the Upper Eastern Shore. Therefore, deer management appears to play an important role in the extent to which IASM develops in populations where *A. pyogenes* is present at high levels. The implications for wildlife managers employing QDM or trophy management in areas where *A. pyogenes* is endemic are clear. IASM is likely to contribute to deer mortality, and harvest strategies should be adjusted accordingly, possibly further limiting harvest of males. Clearly, more research is needed to clarify the role of environment in prevalences of *A. pyogenes* and IASM, particularly at sites where deer are managed under QDM in areas where we did not detect *A. pyogenes*.

Our Upper Eastern Shore data indicate that *A. pyogenes* is endemic to that region and may even play a commensal role in some white-tailed deer populations. Although IASM is typically associated with adult male deer, the majority of deer sampled at Property A and Property B carried *A. pyogenes* in nasal passages, on heads, or both, regardless of sex or age. Management approach did not affect prevalence of *A. pyogenes*, providing further evidence the bacterium could play a commensal role in deer populations on the Upper Eastern Shore and possibly similar habitats. If *A. pyogenes* is endemic to the Upper Eastern Shore and carried by a majority of deer, the question of maintenance of the pathogen remains open to further study.

We did not detect *A. pyogenes* in Upper Eastern Shore neonates, suggesting that neonates acquire the bacteria sometime after birth, presumably either through contact with their dams or environmental exposure sometime after the perinatal period examined in our study. Many of the neonates in our study were sampled within hours of birth, although some had been alive for several days. Even though these older fawns presumably could have been exposed to *A. pyogenes* carried by their dams through grooming interactions or in the environment, cultures were negative. In contrast, most fawns several months old (defined as the 0.5-year-old age class) sampled on the Upper Eastern Shore during the fall harvest tested positive for *A. pyogenes*. This result indicates neonates eventually acquire *A. pyogenes* sometime during their first six months of life. A longer-duration study of fawn flora would help to clarify the role of deer social interactions, cross-species interactions, and environmental exposure in the acquisition of *A. pyogenes*.

If *A. pyogenes* is endemic to the Upper Eastern Shore of Maryland, it is worth considering the effect deer dispersal may have on distribution of the bacteria. It remains unclear how deer acquire *A. pyogenes* and to what extent carrying the bacteria predisposes them to developing IASM, but if intraspecific interactions are involved, dispersal may be an important factor. In our statewide survey, the only other regions where we detected *A. pyogenes*, the Lower Eastern Shore and the Western Shore, are adjacent to the Upper Eastern Shore. Future work should continue to monitor prevalence of *A. pyogenes* in deer and the occurrence of IASM in these areas while expanding surveillance to adjacent sites in Delaware and Pennsylvania.

The long-accepted view of *A. pyogenes* as a normal commensal organism associated with domestic production animals suggests the possibility that feral and/or production

livestock could serve as the reservoirs in endemic areas. Further, the environmental contamination with *A. pyogenes* by livestock, and/or livestock/deer interactions should be important in the maintenance of the disease in an accommodating environment. Figure 5 presents relative livestock abundance in each of the 6 regions based on annual Maryland livestock inventory data for 2011 (U.S. Department of Agriculture National Agriculture Statistics Service 2007). The agriculture profile of the Upper Eastern Shore is primarily large-scale crop farming characterized by low livestock concentrations. The region ranks 4th of the 6 regions we sampled in cattle and sheep density and last in swine and goat density (U.S. Department of Agriculture National Agriculture Statistics Service 2007). This profile suggests the presence of farm production animals alone may not explain the high prevalence of *A. pyogenes* in the Upper Eastern Shore. It may be important to examine the potential for white-tailed deer or other wildlife species to play key roles in the maintenance of endemic *A. pyogenes*. The low prevalence of IASM coinciding with a high prevalence of *A. pyogenes* at the TDM Property supports the potential role of white tailed deer under traditional management to serve as a potential maintenance reservoir of *A. pyogenes*.

The framework of one health is useful for considering the broader implications of white-tailed deer, *A. pyogenes*, and IASM. Humans are not as far removed from white tailed deer as they may perceive themselves to be. The seasonal close contact experienced by active deer hunters is a key point of one health intersection. Hunters should be educated about the identification of IASM lesions and the importance of proper sanitation and hygiene when handling a deer carcass. Even in populations where IASM has not been documented, *A. pyogenes* may be present and capable of impacting human and animal health. Many other intersections occur, primarily because the white-tailed deer thrives in the presence of humans

and is not generally considered a threatening wildlife presence. White-tailed deer frequent camping and recreational areas and use greenways and wildlife corridors to occupy suburban and even urban human communities. The crepuscular, edge-occupying habits of white tailed deer, and their perception as being non-aggressive, can cause the general public to underestimate their proximity and contact with these animals. The inaccurate perception of exposure risk by the public extends to their perception of the risk for their companion and production domestic animals. Providing information about *A. pyogenes* and possible clinical presentations and therapeutic options to medical and veterinary professionals in areas where high prevalence of *A. pyogenes* is detected in deer should improve disease recognition and outcome. The identification of a highly endemic area by our study offers the opportunity to better understand the actual health risk parameters, transmission routes, and environmental perturbations of disease occurrence, as well as potential of control measures in wildlife, humans and domestic animals.

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TABLES

Table 1. Percent of white-tailed deer carrying *A. pyogenes* by region and swab type in Maryland, USA, 2010.

Region	<i>n</i>	Head		Nasal	
		Mean isolates (range)	<i>A. pyogenes</i> %	<i>n</i>	Mean isolates (range)
Appalachian	47	2.30 (1-6)	0	48	2.35 (1-4)
Ridge & Valley	18	1.33 (1-3)	0	19	1.95 (1-3)
Piedmont	49	3.06 (1-9)	0	48	3.17 (1-8)
Western Shore	24	1.35 (1-3)	0	23	1.48 (1-6)
Lower Eastern Shore	33	2.60 (1-5)	3	24	2.27 (1-4)
Upper Eastern Shore	44	4.05 (2-8)	45	41	3.80 (1-7)

Table 2. Bacteria carried by white-tailed deer (nasal%, head%) by region in Maryland, USA, 2010.

Genus	Appalachian	Ridge & Valley	Piedmont	Western Shore	Lower Eastern Shore	Upper Eastern Shore
<i>Acinetobacter</i>	13, 26	11, 22	19, 37	0, 0	13, 30	2, 7
<i>Aerococcus</i>	0, 0	5, 0	0, 0	0, 0	0, 0	0, 0
<i>Aeromonas</i>	0, 0	5, 0	8, 0	0, 0	0, 0	0, 0
<i>Arcanobacterium</i>	0, 0	0, 0	0, 0	4, 0	0, 3	66, 45
<i>Bacillus</i>	25, 4	21, 0	56, 14	13, 8	13, 12	51, 61
<i>Chryseobacterium</i>	0, 0	16, 11	0, 0	0, 0	0, 0	0, 0
<i>Chryseomonas</i>	0, 0	0, 0	0, 0	0, 0	0, 0	24, 16
<i>Corynebacterium</i>	0, 0	0, 0	0, 0	0, 0	3, 4	5, 5
<i>Enterobacter</i>	0, 0	5, 6	0, 0	0, 0	0, 0	0, 2
<i>Enterococcus</i>	13, 4	32, 0	33, 12	9, 0	46, 18	2, 7
<i>Escherichia</i>	10, 0	6, 0	4, 2	0, 8	0, 0	32, 27
<i>Klebsiella</i>	2, 2	5, 0	0, 0	4, 0	0, 0	0, 0
<i>Kocuria</i>	0, 0	5, 0	0, 0	0, 0	0, 0	0, 0
<i>Mannheimia</i>	0, 0	0, 0	2, 0	0, 0	0, 0	0, 0
<i>Micrococcus</i>	0, 0	0, 0	0, 0	0, 0	0, 0	10, 0
<i>Moraxella</i>	15, 23	5, 0	44, 47	0, 0	38, 42	0, 2
<i>Pantoea</i>	17, 36	11, 17	48, 65	0, 4	17, 55	27, 30
<i>Pasteurella</i>	0, 0	0, 0	0, 0	0, 0	0, 0	7, 9
<i>Pectobacterium</i>	0, 0	47, 17	0, 0	0, 0	0, 0	0, 0
<i>Providencia</i>	0, 0	0, 0	0, 0	0, 0	0, 0	2, 0
<i>Pseudomonas</i>	42, 40	11, 6	50, 50	26, 29	54, 42	10, 9
<i>Serratia</i>	0, 0	0, 0	0, 0	0, 0	0, 0	5, 7
<i>Staphylococcus</i>	63, 64	11, 67	25, 35	52, 71	38, 61	66, 91
<i>Streptococcus</i>	10, 0	0, 0	0, 0	17, 0	4, 3	0, 0

Table 3. Percent of white-tailed deer carrying *A. pyogenes* by sex and sample site on the Upper Eastern Shore in Maryland, USA, 2010.

Site	Sex	<i>A. pyogenes</i> %	Nasal % only	Head % only	Head and nasal %
Property A	F	77	26	0	52
Property A	M	82	27	9	45
	Total	78			
Property B	F	100	19	5	76
Property B	M	92	40	19	35
	Total	95			

Table 4. Bacteria carried by white-tailed deer (nasal%, head%) at two properties in Maryland, USA, 2010.

Genus	TDM Property	QDM Property
<i>Escherichia</i>	30, 23	17, 37
<i>Staphylococcus</i>	66, 81	37, 48
<i>Bacillus</i>	57, 75	23, 19
<i>Pantoea</i>	27, 25	40, 38
<i>Pseudomonas</i>	7, 4	25, 27
<i>Streptococcus</i>	0, 0	2, 0
<i>Proteus</i>	4, 0	0, 2
<i>Arcanobacterium</i>	84, 65	79, 56
<i>Corynebacterium</i>	4, 9	2, 12
<i>Chryseomonas</i>	23, 0	15, 0
<i>Micrococcus</i>	9, 0	0, 0
<i>Serratia</i>	4, 7	2, 4
<i>Penicillium</i>	9, 2	0, 0
<i>Providencia</i>	2, 0	0, 0
<i>Pasteurella</i>	11, 7	0, 2
<i>Mucor</i>	0, 2	0, 2
<i>Flavimonas</i>	2, 4	2, 2
<i>Enterobacter</i>	0, 2	4, 0
<i>Edwardsiella</i>	0, 0	2, 2
<i>Klebsiella</i>	2, 0	0, 2
<i>Acintobacter</i>	0, 2	0, 0
<i>Enterococcus</i>	0, 5	0, 19

Table 5. Binary logistic regression for occurrence of *A. pyogenes* on white-tailed deer on the Upper Eastern Shore of Maryland, USA, 2010

Sample	Parameter	Estimate	Standard error	Z	P	95% confidence interval	
						Lower	Upper
Head	Site	-0.404	0.430	-0.939	0.348	-1.246	0.439
	Age	0.386	0.303	1.273	0.203	-0.209	0.981
	Sex	0.632	0.420	1.505	0.132	-0.191	1.455
Nasal	Site	0.514	0.540	0.953	0.340	-0.543	1.572
	Age	0.003	0.366	0.009	0.993	-0.713	0.720
	Sex	0.785	0.533	1.473	0.141	-0.259	1.829

FIGURES

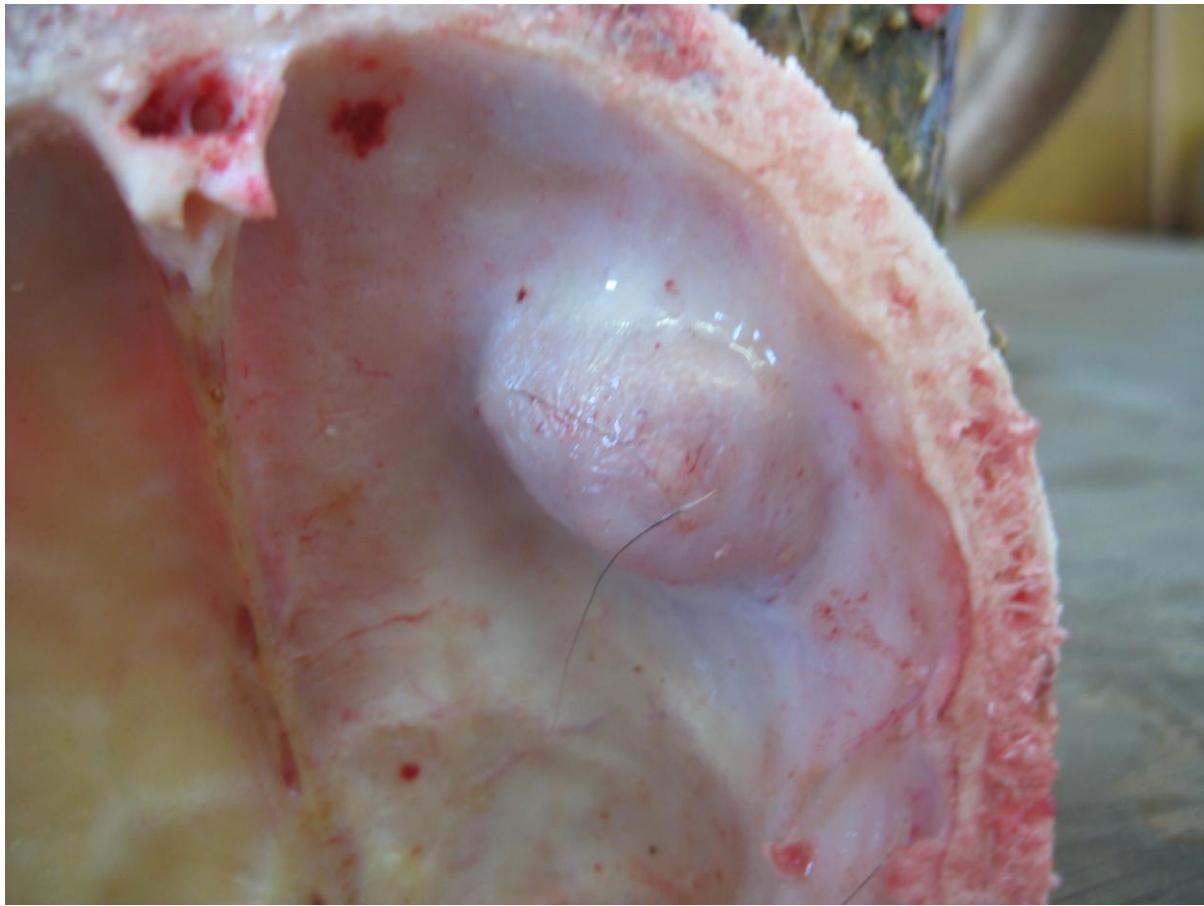


Figure 1: Figure 1. Pitting of bone and fluid-filled nodule inside skull plate of infected white-tailed deer at Chesapeake Farms, Maryland, USA



Figure 2. Intracranial abscess in white-tailed deer at Chesapeake Farms, Maryland, USA

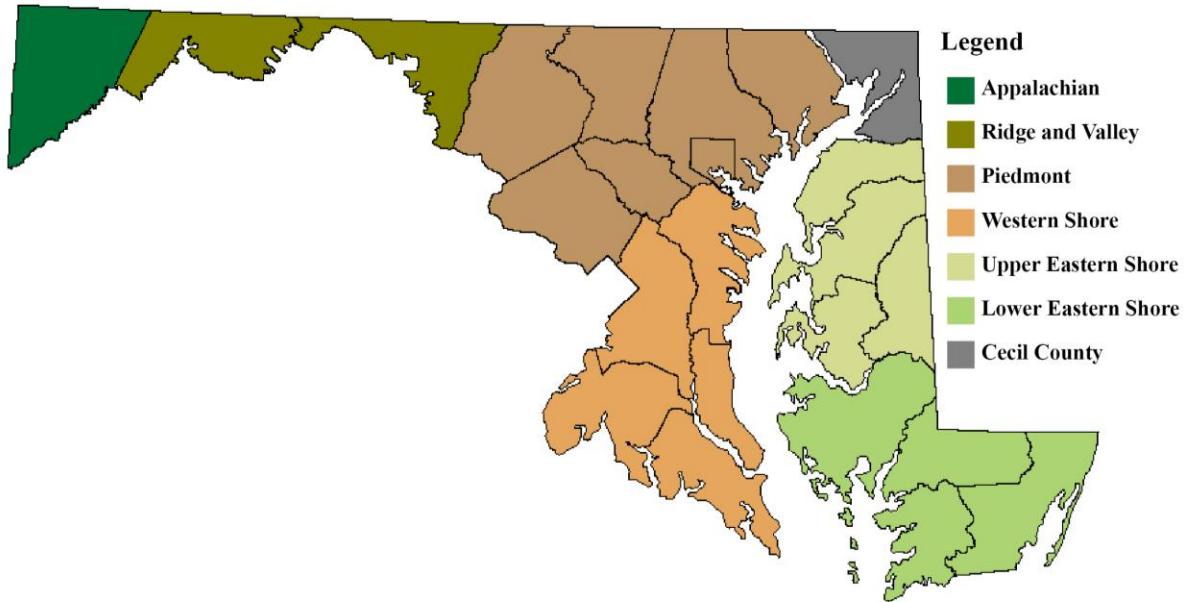


Figure 3. Physiogeographic regions in Maryland, USA

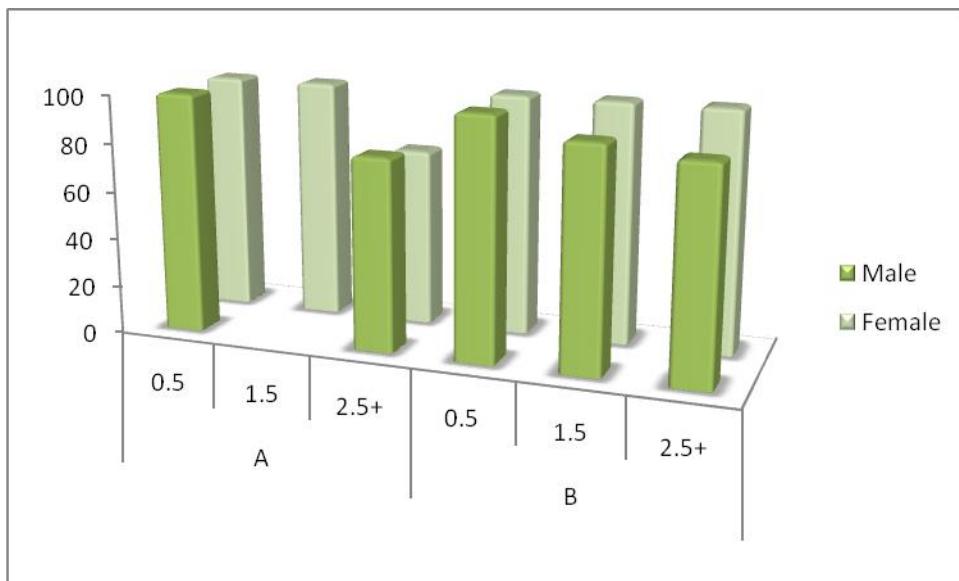


Figure 4. *A. pyogenes* % by sex, age class and site, Upper Eastern Shore, Maryland, USA

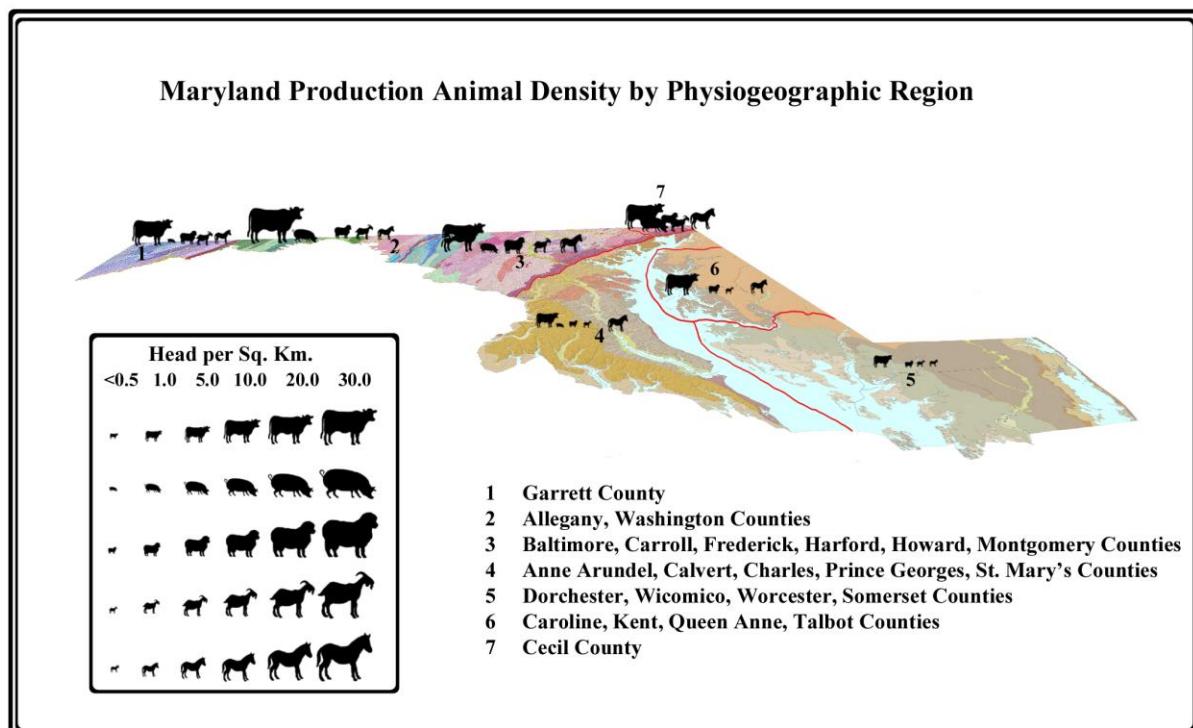


Figure 5. Livestock density by physiogeographic region in Maryland, USA