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A HEADACHE FROM OUR PAST? INTRACRANIAL ABSCESS DISEASE, VIRULENCE FACTORS OF *TRUEPERELLA PYOGENES*, AND A LEGACY OF TRANSLOCATING WHITE-TAILED DEER (*ODOCOILEUS VIRGINIANUS*)

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ABSTRACT: *Trueperella pyogenes*, a bacterial opportunistic pathogen residing along the skin layer of apparently healthy animals, is the etiologic agent of intracranial abscessation-suppurative meningoencephalitis, a cause of mortality for male white-tailed deer (*Odocoileus virginianus*). Occurrence of this disease has been speculated to be influenced by virulence of *T. pyogenes* residing on white-tailed deer in geographically distinct metapopulations. To determine if differences in virulence potential of *T. pyogenes* could affect occurrence of disease across populations, we examined if frequency of seven virulence genes of *T. pyogenes* from forehead swabs of 186 apparently healthy white-tailed deer differed between sites in the state of Georgia, US, where ≥1 male tested positive for a cranial abscess and sites where no individuals tested positive for a cranial abscess. We detected six of seven virulence genes more frequently at sites where we detected ≥1 male with a cranial abscess compared to sites where we did not detect any individuals with a cranial abscess (*nanH*, $P<0.001$; *nanP*, $P=0.007$; *fimA*, $P<0.001$; *fimC*, $P=0.037$; *fimE*, $P<0.009$; *fimG*, $P<0.001$; and *cbpA*, $P=0.872$). Our findings suggest differences in the pathogenic potential of *T. pyogenes* at individual sites may help to explain spatial variability of this disease. Anecdotally, the incidence of cranial abscess disease in Georgia seems to be associated with areas that were restocked with white-tailed deer from a high-fenced property in Wisconsin, US. Given the spatial distribution of this disease, we speculate that these genetic differences in *T. pyogenes* may have arisen from white-tailed deer restocking efforts, and our observations may be a legacy of an introduced disease manifesting itself generations later.

Key words: Intracranial abscessation, *Odocoileus virginianus*, restocking, subcutaneous abscess, *Trueperella pyogenes*, virulence factors.

INTRODUCTION

The intracranial abscessation-suppurative meningoencephalitis disease complex is one of many causes of mortality in white-tailed deer (*Odocoileus virginianus*) populations (Davidson et al. 1990; Baumann et al. 2001), although in some regions, it can account for 35% of annual mortality of mature males (Karns et al. 2009). Risk factors associated with intracranial abscess disease remain speculative but may be related to an individual's sex and age (Karns et al. 2009; Cohen et al. 2015b) and prevalence of the purported etiologic agent, *Trueperella pyogenes* (Belser et al. 2015). *Trueperella pyogenes* is a bacterial opportunistic pathogen commonly residing on the skin layer and mucosal membranes of apparently healthy deer (Turner et al. 2013; Belser et al. 2015, 2016) and induces suppurative infections in the form of abscesses, empyemas, and pyogranulomas (Moore et al. 2010). *Trueperella pyogenes* has been isolated from 111 of 165 published cases of intracranial abscess disease in which culture was attempted (Davidson et al. 1990; Nettles et al. 2002; Karns et al. 2009; Cohen et al. 2015a).

Trueperella pyogenes has several known and purported virulence factors that affect its pathogenic potential (Jost and Billington

2005). Successful adhesion to and colonization of the host is critical for infection and mediated by adhesion factors, such as fimbriae (*fimA*, *fimC*, *fimE*, and *fimG*), neuraminidases (*nanH* and *nanP*), and collagen-binding proteins (*cbpA*; Jost and Billington 2005). These surface-binding proteins can be host and organ specific, and their presence or absence can affect infection rates (Jost and Billington 2005; Zhao et al. 2013; Machado and Bicalho 2014). Thus, occurrence of these virulence factors across strains of *T. pyogenes* directly affects its pathogenicity (Jost and Billington 2005; Bicalho et al. 2012; Machado and Bicalho 2014).

Because the virulence factors of *T. pyogenes* can be host and organ specific, discerning the relationship between its pathogenic properties and the infecting strains is important for understanding the epizootiology of *T. pyogenes*–associated diseases (Jost and Billington 2005; Machado and Bicalho 2014; Rzewuska et al. 2016). *Trueperella pyogenes* isolates cultured from subcutaneous abscesses in the cranial region of white-tailed deer typically test positive for genes encoding virulence factors that enhance epithelial attachment, specifically *cbpA*, *nanH*, *nanP*, *fimA*, *fimC*, *fimE*, and *fimG* (Cohen et al. 2015a). Because the presence of these virulence genes influences the pathogenicity of *T. pyogenes*, the prevalence of *T. pyogenes*–associated diseases can be affected by the occurrence of these genes within bacterial colonies (Jost and Billington 2005; Bicalho et al. 2012; Machado and Bicalho 2014). Thus, differences in the frequency of virulence genes of *T. pyogenes* across white-tailed deer populations may help explain the spatial distribution of intracranial abscess disease.

In an attempt to explain the spatial variation of intracranial abscess disease observed in previous studies (Baumann et al. 2001; Turner et al. 2013; Cohen et al. 2015b), we used PCR to determine the frequency of genes coding for virulence factors of *T. pyogenes* found on the foreheads of apparently healthy deer harvested across populations. If these pathogen-related factors drive the occurrence of this disease, we hypothesized the frequency of virulence genes aiding epithelial attachment of *T. pyogenes* (i.e., *cbpA*, *nanH*, *nanP*, *fimA*, *fimC*, *fimE*, and *fimG*) to be greater at sites where we detected $\geq$1 male with a subcutaneous abscess in the cranial region.

MATERIALS AND METHODS

Abscess screening, sampling design, and protocol

From 14 September 2011 to 15 January 2012 and 14 September 2012 to 15 January 2013, we examined hunter-harvested deer from 28 sites across the state of Georgia, US, for signs of subcutaneous abscesses in the cranial region and recorded sex, estimated age (0.5, 1.5, 2.5, 3.5, or $\geq$4.5 yr old; Severinghaus 1949), and harvest site. A site was a property in which all deer harvested within the property boundary were brought to a designated check station. We chose sites to ensure not only a thorough spatial distribution (Fig. 1) but also geographically distinct populations. The size of these sites ranged from 830 ha to 4,372 ha.

We defined a subcutaneous abscess in the cranial region to be any purulent infection occurring below the dermis but above the skull bones, located along the cranium or around the base of the antler pedicles (Davidson et al. 1990). Although we recognize that not all subcutaneous abscesses in the cranial region become intracranial abscesses, we believe there is enough evidence in the literature to demonstrate that subcutaneous abscesses in the cranial region are a strong surrogate measure for intracranial abscesses. Davidson et al. (1990) provides the greatest detail available on the epidemiologic features of intracranial abscess disease, stating that in 15 of 18 cases of intracranial abscess reports in which a possible contributing factor to this disease was identified, the purported factors were "subcutaneous abscess around antler pedicle ... [or] head ..." or trauma to antler pedicle resulting in localized abscesses. Also, Davidson et al. (1990) noted pitting, erosion, and necrosis of the cranial bones on the external surfaces of the parietal and frontal bones usually on or near the antler pedicles as a sign of an intracranial abscess. These erosions often extend into the cranium through sutures (Davidson et al. 1990; Baumann et al. 2001; Campbell and VerCauteren 2011), which is characteristic of an infection first occurring in the subcutaneous region and entering the brain chamber through erosion of the suture lines (Davidson et al. 1990; Campbell and VerCauteren 2011). Finally, Davidson et al. (1990) suggests the direct invasion of an abscess through or between cranial bones is the predominated route of intracranial abscess formation among males.

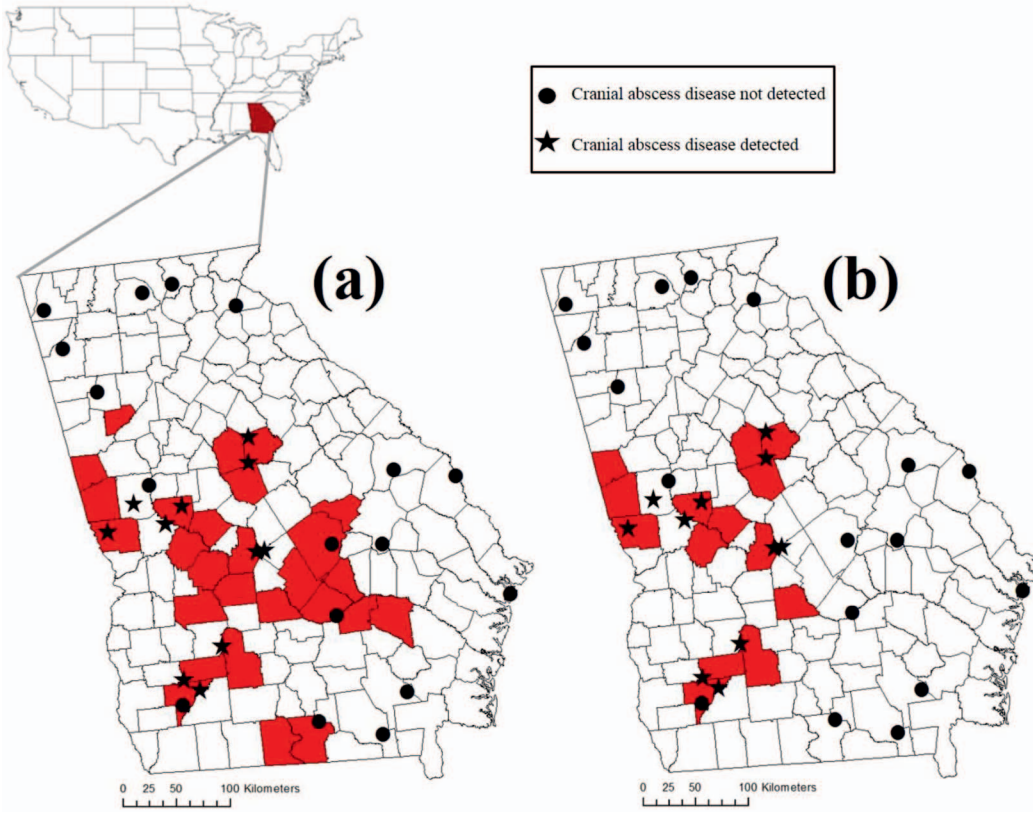

FIGURE 1. Location of 28 sites in Georgia, USA, where we quantified the presence of *Trueperella pyogenes* and seven associated virulence genes from swabs taken from the forehead of hunter-harvested, apparently healthy adult (≥1.5-yr-old) white-tailed deer (*Odocoileus virginianus*). From 14 September 2011 to 15 January 2012 and 14 September 2012 to 15 January 2013, all white-tailed deer harvested at these sites were also examined for the presence a cranial abscess. We considered sites in which an adult (≥1.5 years old) male deer was detected with a cranial abscess to be positive for this disease. Counties colored in red represent locations where historic records demonstrate (a) deer from the Sandhill Game Farms in Wisconsin, USA, were released and (b) and only counties in which greater than 20 deer were released (Blackard 1971). Scale bar is representative of Georgia.

Because subcutaneous abscesses in the cranial region, which we refer to as cranial abscesses (Belser et al. 2015; Cohen et al. 2015a, 2015b.) are typically the predecessor of intracranial abscesses and are grossly observable in the field, we used these cranial abscesses as a surrogate measure of the potential occurrence of intracranial abscesses. We inspected the cranium of each deer, with 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 (Davidson et al. 1990). In addition, we palpated the skin around the base of the pedicle to express pus and detect a subcutaneous infection. These gross lesions typically consisted of scabbing around the pedicle base, with purulent discharge around or under the base, and an engorged suppurative infection. To ensure the cranial abscesses we detected were bacterial abscesses, we took samples of suppurative infections along the cranium with a sterile rayon-tipped cotton swab (Puritan Medical Products, Guilford, Maine, USA) when we could sample within 1 h of death, then placed the swabs in a 2.5-mL Cryosaver vial containing a brucella-glycerol solution (Hardy Diagnostics, Santa Maria, California, USA), and froze them at –20 C until processing. After thawing, we streaked each sample onto Columbia agar supplemented with 5% defibrinated sheep blood (bioMérieux, Craponne, France) for 48 h at 37 C. Colonies were isolated on the basis of gross and microscopic morphology and postincubation hemolysis. Iden-

tification of *T. pyogenes* was confirmed with a BBL Crystal Gram-Positive test strip (BD, Franklin Lakes, New Jersey, USA). Identification of other bacteria was based on a panel of classic biochemical tests (i.e., catalase, coagulase, maltose, and lactose; Cohen et al. 2015a).

Forehead swabbing and screening for *T. pyogenes*

Because we were interested in assessing if occurrence of genes coding for virulence factors of *T. pyogenes* residing on white-tailed deer contributed to the occurrence of cranial abscesses, we first swabbed apparently healthy white-tailed deer of any age with a sterile, rayon-tipped applicator (Puritan) along the skin surface of the forehead. We had no reason to expect differences in the adhesion of *T. pyogenes* to the skin of male or female deer (Turner et al. 2013; Belser et al. 2015), so we swabbed individuals of either sex. We then extracted DNA from swabs by using the DNeasy Blood and Tissue Kit (Qiagen, Valencia, California, USA), per the manufacturer's instructions, with the following modifications: extra phosphate-buffered saline (400 µL total) and lysis (AL) buffer (400 µL total), an additional 5 min to incubation for lysis, and an additional 1 min to the final incubation step. These modifications to the DNA extraction protocol were done to compensate for the glycerol in the Cryosaver vials and were undertaken on the basis of consultations with Qiagen technical support. We stored extracted samples at −20 C until we used PCR techniques to identify the presence of *T. pyogenes*.

We conducted PCR in 25-µL reactions by using GoTaq Flexi DNA Polymerase and GoTaq PCR reagents (Promega Corporation, Madison, Wisconsin, USA) per the manufacturer's recommendations. We targeted the *plo* gene, which is specific to *T. pyogenes* and has been reported in all strains (Jost and Billington 2005; Cohen et al. 2015a), by using primers F (5′-GGCCCGAA TGTCACCGC) and R (5′-AACTCCGCCTCT AGCGC; Billington et al. 1997). We performed amplifications in an automated thermal cycler (Bio-Rad DNA Engine, Hercules, California, USA), with the following cycling conditions: 35 cycles, each consisting of a 1-min denaturation at 94 C; a 1-min annealing at 55 C and a 1-min extension at 72 C; and a final 5-min extension at 72 C. We electrophoresed the PCR products in ethidium bromide–stained 1.5% agarose gels and used a 100 base-pair DNA ladder (Promega) as the DNA marker. We visualized the gel by using an AlphaImager gel documentation system (ProteinSimple, Santa Clara, California, USA) to determine the presence or absence of the *plo* gene and thus *T. pyogenes*. We took precautions to avoid contamination of samples, including separating the areas for DNA extraction and PCR preparation, regularly changing gloves, and using DNA-Away (Molecular BioProducts, Inc., San Diego, California, USA) for surface and equipment sterilization between batches. All PCR cycles included negative controls to ensure there was no contamination in the reaction mixture. We used *T. pyogenes* reference strains (JGS 189 and JGS 230) as positive controls for every cycle.

To ensure the reliability of our PCR process, we extracted a subset of bands of 24 positive samples by using the QIAquick Gel Extraction Kit (Qiagen) per the manufacturer's instructions. We then sent these samples for sequencing at the Georgia Genomics Facility (University of Georgia, Athens, Georgia, USA). We used BLAST (National Center for Biotechnology Information 2017) to search the resulting sequences for identification (Hall 1999). Nineteen genetically sequenced samples were determined to be *T. pyogenes*. Five results were inconclusive.

Detection of virulence genes and site-level differences in potential pathogenicity

After we identified which forehead swabs contained *T. pyogenes*, we then assessed the presence of known and putative virulence genes in these same samples by using PCR. We identified seven virulence factors that have been directly shown to increase pathogenicity of *T. pyogenes*. These virulence factors all promote adherence and subsequent colonization of *T. pyogenes* and are the following: fimbriae subunits A, C, E, and G, which are coded by genes *fimA*, *fimC*, *fimE*, and *fimG*, and aid attachment to epithelial cells (Jost et al. 2001, 2002; Jost and Billington 2005); neuraminidase subunits H and P, which are coded by genes *nanH* and *nanP*, and increase attachment to the extracellular matrix (Jost et al. 2001, 2002; Jost and Billington 2005); and finally, collagen-binding protein A, which is coded by gene *cbpA*, and aids in attaching to the collagen-rich tissue (Esmay et al. 2003; Pietrocola et al. 2007). We prepared the PCR amplification reaction mixture as previously described for *plo* with modifications to the primer sequence and cycling conditions (Table 1). We analyzed the PCR products as previously described and confirmed presence of each virulence factor gene by correct molecular size of the amplification product.

We first sought to determine if there were any differences in occurrence of any of the seven virulence genes of *T. pyogenes* found between male and female deer. We used a chi-square test and set the level of significance at $P \leq 0.05$. Because we were interested in determining if the potential pathogenicity of *T. pyogenes* residing

TABLE 1. Sequence of PCR primers and cycling conditions used to amplify virulence genes of *Trueperella pyogenes*.[a]

Target gene	Virulence factor	Oligonucleotide sequence (5′ to 3′)	Amplicon size (base pair)
plo	Pyolysin	F: GGCCCGAATGTCACCGC	270[b]
		R: AACTCCGCCTCTAGCGC	
nanH	Neuraminidase H	F: CGCTAGTGCTGTAGCGTTGTTAAGT	781[c]
		R: CCGAGGAGTTTTGACTGACTTTGT	
nanP	Neuraminidase P	F: TTGAGCGTACGCAGCTCTTC	150[c]
		R: CCACGAAATCGGCCTTATTG	
cbpA	Collagen-binding protein	F: GCAGGGTTGGTGAAAGAGTTTACT	124[c]
		R: GCTTGATATAACCTTCAGAATTTGCA	
fimA	Subunit of type A fimbriae	F: CACTACGCTCACCATTCACAAG	605[d]
		R: GCTGTAATCCGCTTTGTCTGTG	
fimC	Subunit of type C fimbriae	F: TGTCGAAGGTGACGTTCTTCG	843[c]
		R: CAAGGTCACCGAGACTGCTGG	
fimE	Subunit of type E fimbriae	F: GCCCAGGACCGAGAGCGAGGGC	775[e]
		R: GCCTTCACAAATAACAGCAACC	
fimG	Subunit of type G fimbriae	F: ACGCTTCAGAAGGTCACCAGG	929[d]
		R: ATCTTGATCTGCCCCCATGCG	

[a] The primers and cycling conditions for the target genes were from Silva et al. (2008), except for the gene *plo*, which were taken from Jost et al. (2002). F = forward; R = reverse.

[b] Cycling conditions: 35 cycles of 94 C (60 s); 55 C (60 s); 72 C (60 s); and final extension 72 C (300 s).

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

[d] Cycling conditions: 94 C (180 s); 35 cycles of 94 C (60 s); 57 C (60 s); 72 C (60 s); and final extension 72 C (420 s).

[e] Cycling conditions: 94 C (180 s); 35 cycles of 94 C (60 s); 55 C (60 s); 72 C (60 s); and final extension 72 C (420 s).

on the skin affected occurrence of cranial abscess disease within deer populations, we analyzed differences in occurrence of these seven virulence genes between sites where we detected ≥1 male with a cranial abscess and sites where we did not detect any individuals with a cranial abscess. Again, we used a chi-square test to compare occurrence of these genes between sites where deer were found with cranial abscesses to sites where no deer were found to have cranial abscesses, and we set the level of significance at $P \leq 0.05$. We conducted analyses in program R v.3.1.1 (R Development Core Team 2013).

RESULTS

We examined 5,659 deer across 28 sites for signs of a cranial abscess. We did not detect cranial abscesses on any of the inspected 2,106 females and 340 males <1 yr old. We examined 3,213 male deer ≥1.5 yr old for cranial abscesses, of which 1,266 were 1.5 yr old, 908 were 2.5 yr old, 585 were 3.5 yr old, and 434 were ≥4.5 yr old (Table 2). We were unable to determine the age for 40 individuals. The number of individuals we examined varied by property but ranged from as small as 15 at one site to as large as 541 at another site (Table 2). We detected cranial abscesses in 2.5% (80/3,213) male deer ≥1.5 yr old we examined.

Of the 80 males that tested positive for a cranial abscess, we subsampled 65 to ensure these were bacterial infections. All samples isolated from subcutaneous abscesses (65/65) had growth of bacterial species, confirming the abscesses we detected were bacterial. *Trueperella pyogenes* was the predominant bacterium and was isolated from 71% (46/65) of samples. Other bacteria isolated included *Staphylococcus aureus*, *Enterococcus* spp., *Corynebacterium* sp., *Serratia proteamaculans*, *Ralstonia pickettii*, and *Enterobacter* sp. (Cohen et al. 2015a).

Of 28 sites surveyed, we detected ≥1 male with a cranial abscess at 39% (11/28) sites (Fig. 1). Therefore, we determined frequency of virulence genes on the forehead swabs at 11 sites, where ≥1 male tested positive for a cranial abscess and 17 sites where no individ-

TABLE 2. Total number of hunter-harvested male white-tailed deer (*Odocoileus virginianus*) examined for the presence of a subcutaneous abscess in the cranial region (termed "cranial abscess") by age class (0.5, 1.5, 2.5, 3.5, 4.5+ yr old and unknown [U]; Severinghaus 1949) and site (county in Georgia, USA). Total number of individuals that were observed to have an abscess at each site in Georgia from 14 September 2011 to 15 January 2012 and 14 September 2012 to 15 January 2013.

Site	Total examined (*n*)	Cranial abscesses (*n*)	Males by age class (*n*)					
			0.5	1.5	2.5	3.5	4.5+	U
Baker	55	0	5	5	21	11	13	0
Burke	98	0	3	34	36	23	2	0
Chatham	144	0	28	32	29	29	26	0
Dougherty	57	2	1	25	11	8	12	0
Fannin	111	0	9	44	23	14	21	0
Floyd	330	0	52	100	85	61	32	0
Gilmer	58	0	2	24	12	10	7	3
Habersham	235	0	10	103	46	42	34	0
Harris	23	4	0	1	5	10	7	0
Houston	139	5	9	70	32	18	10	0
Laurens	68	0	5	36	18	6	3	0
Lee	107	7	20	35	14	18	20	0
Lowndes	55	0	11	12	17	12	13	0
Meriwether 1	37	2	2	0	6	18	11	0
Meriwether 2	34	4	0	15	7	5	7	0
Paulding	135	0	20	50	27	25	11	2
Putnam 1	80	6	10	1	4	34	31	0
Putnam 2	541	33	40	250	132	69	50	0
Screven	268	0	29	112	66	16	10	35
Sumter	19	0	4	1	7	4	3	0
Talbot	83	1	13	2	17	31	20	0
Telfair	29	0	3	14	9	2	1	0
Toombs	56	0	4	28	10	11	3	0
Twiggs	209	10	12	115	54	19	9	0
Walker	314	0	19	70	161	30	34	0
Ware 1	152	0	11	49	33	38	21	0
Ware 2	76	0	10	28	15	12	11	0
Worth	50	6	8	10	11	9	12	0
Total	3563	80	340	1266	908	585	434	40

uals tested positive. Of the 646 apparently healthy white-tailed deer we swabbed on the forehead for *T. pyogenes*, 449 were males, and 196 were females.

At sites where we did not detect any individuals with a cranial abscess, 24% (88/366) swabs tested positive for *T. pyogenes*, whereas 35% (96/280) swabs tested positive for *T. pyogenes* at sites where we detected ≥1 male with a cranial abscess. This left us with 88 swabs from sites where ≥1 male tested positive for a cranial abscess and 96 swabs from sites where no individuals tested positive for a cranial abscess to test for presence of virulence genes. Virulence genes occurred equally for *T. pyogenes* found on male or female deer (*nanH*, $P=0.611$; nanP, $P=0.793$; *fimA*, $P=0.821$; *fimC*, $P=0.703$; *fimE*, $P=0.771$; *fimG*, $P=0.475$; and *cbpA*, $P= 0.821$). The occurrence of six of seven virulence genes of *T. pyogenes* (*nanH*, *nanP*, *fimA*, *fimC*, *fimE*, and *fimG*) detected from the foreheads of apparently healthy deer was greater at sites where deer were positive for cranial abscesses (*nanH*, $P<0.001$; *nanP*, $P=0.007$; *fimA*, $P<0.001$; *fimC*, $P=0.037$; *fimE*, $P<0.009$; and

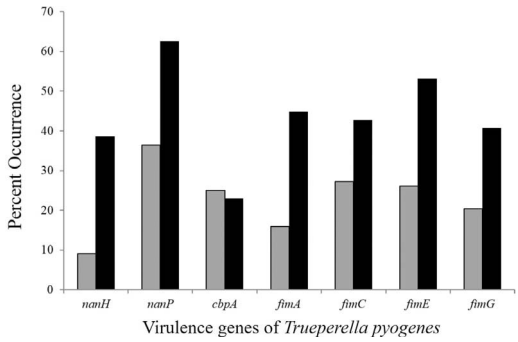

FIGURE 2. Percentage of occurrence of virulence genes of *Trueperella pyogenes* from the forehead of 184 harvested, apparently healthy white-tailed deer (*Odocoileus virginianus*) from 28 sites in Georgia, USA. From 14 September 2011 to 15 January 2012 and 14 September 2012 to 15 January 2013, all white-tailed deer at these sites were also examined for the presence a cranial abscess. Gray-colored bars represent the percent occurrence of virulence genes of *T. pyogenes* from sites where no individuals were detected to have a cranial abscess, whereas black bars represent the percent occurrence of virulence genes of *T. pyogenes* from sites where ≥1 male tested positive for a cranial abscess. Results of a chi-square test suggested significantly higher frequency of virulence genes at sites where individuals tested positive for a cranial abscess (*nanH*, $P<0.001$; nanP, $P=0.007$; *fimA*, $P<0.001$; *fimC*, $P=0.037$; *fimE*, $P<0.009$; and *fimG*, $P<0.001$), excluding *cbpA* ($P=0.872$).

fimG, $P<0.001$); Fig. 2 and Table 3). For example, we found *nanH*, *nanP,* and *fimA* occurred 415, 171, and 276%, respectively, and more frequently on sites where ≥1 male tested positive. The *cbpA* gene was the only virulence gene found at the same frequency between sites where ≥1 male tested positive for a cranial abscess and sites where no individuals tested positive ($P=0.872$).

DISCUSSION

Our findings suggest the occurrence of genes coding virulence factors creates differences in population-level pathogenicity of *T. pyogenes* and helps explain site-to-site variation in the presence of subcutaneous abscesses in the cranial region and, likely, intracranial abscess disease (Baumann et al. 2001; Cohen et al. 2015b). The occurrence of the genes differs across sites but does not differ between

TABLE 3. Frequency of known and putative virulence genes of *Trueperella pyogenes* from the forehead of 184 harvested, apparently healthy white-tailed deer (*Odocoileus virginianus*) at sites in Georgia, USA, where ≥1 male tested positive for a cranial abscess and at sites where no individuals tested positive for a cranial abscess. A chi-square test was used to determine differences in frequency of these genes between these sites (df=1 for each test). All virulence genes except *cbpA* occurred at significantly higher frequency at sites where individuals tested positive for a cranial abscess.

Virulence factor	No. of detections: At positive sites ($n=96$)	No. of detections: At negative sites ($n=88$)	χ^2	P value
nanH	37	8	19.99	<0.001
nanP	60	32	7.11	0.007
cbpA	22	22	0.02	0.872
fimA	43	14	16.59	<0.001
fimC	41	24	4.14	0.037
fimE	51	23	12.81	0.009
fimG	39	18	7.82	<0.001

T. pyogenes found on males or females of the same site. As a commensal bacterium, *T. pyogenes* can act as a primary pathogen of intracranial abscesses when virulence genes aiding in epithelial attachment are present in its genome (Cohen et al. 2015a). Collectively, this suggests that virulence potential of *T. pyogenes* commensally residing on the foreheads of white-tailed deer is linked to intracranial abscess disease occurrence, but the sex-skewed bias of this disease is likely behaviorally mediated. Males engage more frequently in antagonistic behaviors (DeYoung and Miller 2011), resulting in abrasions and cuts along the cranial epidermis and allowing a gateway for *T. pyogenes* to invade.

The potential for *T. pyogenes* to cause intracranial abscess disease seems directly tied to its ability to successfully adhere to host cells. This may suggest intracranial abscesses are often autogenous and preceded by direct physical trauma that allow *T. pyogenes* to penetrate past the outer epithelial membranes (Jost and Billington 2005; Moore et al. 2010). 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 promote initial adhesion to the epithelial linings (Zhao et al. 2013). Meanwhile, *nanP* seems important for inducing latent infections, enhancing epithelial attachment, and subsequent colonization (Zhao et al. 2013). Furthermore, *nanP* and *fimA* were present in 100% of isolates from cranial abscesses (Cohen et al. 2015a). Collectively, these virulence factors seem critical for *T. pyogenes* to successfully invade and replicate during beginning stages of cranial abscess disease.

The adherence factors, *fimC*, *fimE*, and *fimG* are important for recognition of host-specific tissue (Jost et al. 2002; Zhao et al. 2013). Of these three, *fimE* occurred most frequently (52%) and was detected over three times more commonly on sites where $\geq$1 male tested positive for a cranial abscess. Further, *fimE* has been present in 98% of isolates from cranial abscesses where virulence gene occurrence was examined (Cohen et al. 2015a). Although all fimbriae subunits could account for differences in site variability, *fimE* may play an important role in adhesion to the epithelial linings of deer, thereby increasing the pathogenicity of *T. pyogenes* and its ability to cause cranial abscess disease. The pathogenesis of intracranial abscess disease is poorly understood, but our data suggest virulence potential, particularly for genes affecting cellular adhesion, are important for disease presence. We believe future studies should investigate a more detailed mechanism of these relationships.

The differences in virulence potential among sites raises the question of how these differences originated. We recognize *T. pyogenes* is a common microflora resident in livestock (Jost and Billington 2005), and virulent strains could have been introduced by livestock-wildlife interactions. However, anecdotally, 9 of 11 sites where deer tested positive for cranial abscesses were in counties restocked with deer from one area of Wisconsin. The other two sites were located in counties neighboring areas restocked with deer from the same Wisconsin location. These counties were restocked with deer during the 1960s as part of population restoration efforts from the Sandhill Game Farm (now Sandhill Wildlife Area) of Wisconsin (Fig. 2; Blackard 1971; Cohen et al. 2015b). Sandhill Wildlife Area has been surrounded by a 2.4-m deer-tight fence since before restocking took place and is located in an area where intracranial abscesses are a source of natural mortality for adult male deer (Baumann et al. 2001). In contrast, other regions of Georgia were restocked primarily with deer from within Georgia and from Texas, US (Blackard 1971), where cranial abscesses reportedly do not occur (Baumann et al. 2001; Cohen et al. 2015b). Although the evidence is anecdotal and the commonality of *T. pyogenes* on other animals makes any inferences difficult, we believe the pattern between sites affected by cranial abscesses and the restocking of deer from Wisconsin is suggestive and warrants more research. Future research should elucidate the genetic similarity between strains of *T. pyogenes* residing on deer across Georgia and within Sandhill Wildlife Area.

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