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PREVALENCE AND RISK FACTORS OF *ANAPLASMA* INFECTIONS IN EASTERN MOOSE (*ALCES ALCES AMERICANA*) AND WINTER TICKS (*DERMACENTOR ALBIPICTUS*) IN MAINE, USA

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ABSTRACT: Eastern moose (*Alces alces americana*) are heavily parasitized by winter ticks (*Dermacentor albipictus*), the dominant cause of increased calf mortality in the northeastern US. Although much work has focused on the direct negative effects of winter tick on moose, it remains unknown whether diseases transmitted by ticks may also affect moose health or pose a risk to other species. We explored the role that moose and winter ticks play in transmission of the tick-borne bacterial pathogens, *Anaplasma* spp., which cause mild to severe illness in humans and domestic animals. Our objectives were to 1) estimate the prevalence of *Anaplasma* spp. in moose and winter ticks; 2) determine the phylogenetic placement of these strains with respect to those found in other hosts and vectors; and 3) explore risk factors of *Anaplasma* infection in moose. A total of 157 moose (142 calves, 15 adults) were captured in western (n=83) and northern (n=74) Maine in 2017 and 2018. We screened for *Anaplasma* spp. in moose whole blood samples using a genus-specific PCR assay targeting the 16S rRNA gene. Over half (54%) of the moose were infected with *Anaplasma* bacteria, with a greater proportion of moose harboring *Anaplasma*-infections in the western (67%) versus northern study areas (38%). Male moose exhibited a higher prevalence than did females (63% vs. 47%). In contrast, *Anaplasma* spp. prevalence in winter ticks was low (<1%). Sequencing and phylogenetic analysis revealed that the single *Anaplasma* strain in moose was highly divergent from the strain in winter ticks and most closely related to an uncharacterized North American cervid strain. We conclude that winter ticks are unlikely to play a significant role in *Anaplasma* transmission to moose; however, high infection prevalence warrants further investigation into the impacts of *Anaplasma* spp. infection on moose health.

Key words: *Alces alces*, *Anaplasma*, disease transmission, moose, phylogenetics, rickettsia, vector-borne disease, winter tick.

INTRODUCTION

Anaplasma spp. are among several vector-borne pathogens that are emerging in the northeastern US (Dumler et al. 2005) and are of concern to both human and animal health. The genus encompasses multiple obligate intracellular rickettsial species that infect host blood cells, which can cause the disease anaplasmosis. *Anaplasma phagocytophilum*, in particular, is the cause of human granulocytic anaplasmosis (HGA; formerly human granulocytic ehrlichiosis or HGE; Rikihisa 2011) and is recognized as a frequent cause of fever in areas where *Ixodes* spp. ticks are found, including the upper Midwest, New England, northern California, and several regions in Europe (Walker and Dumler 1996). Along with fever, common features associated with HGA include headaches, myalgia, malaise, leukopenia, thrombocytopenia, and mild hepatic injury (Dumler et al. 2005).

Wildlife and domestic animals can also harbor *Anaplasma* spp. For example, *Anaplasma marginale* is globally widespread in the Americas, Asia, Africa, and Europe (Guglielmone 1995; de la Fuente et al. 2005b; Kocan et al. 2010) and is found primarily in cattle, causing a disease (bovine

anaplasmosis) that is characterized by anemia, weight loss, and often death. *Anaplasma marginale* can establish life-long chronic infections in animals, with severe health and economic impacts; however, there is currently no effective, widely available vaccine (Kocan et al. 2003), despite the disease and pathogenic agent having been first characterized over a century ago (Theiler 1910). Other related *Anaplasma* spp. have been known to cause milder infections, with high host specificity, such as with *Anaplasma ovis* in sheep (Splitter et al. 1956). Additionally, multiple genetically distinct *Anaplasma* spp. have been found with unknown pathogenicity in both wildlife and humans (Lobanov et al. 2012; Hailemariam et al. 2017).

Although *Anaplasma* spp. infections are widespread in animals and humans, knowledge regarding the epidemiology of the pathogens, including natural reservoirs and transmission routes, is remarkably scarce (Stuen et al. 2005; Rikihisa 2011). Wildlife hosts for the *A. phagocytophilum* strain responsible for all human cases (HGA signature sequence) have been thoroughly studied, with three mammalian species, the white-footed mouse (*Peromyscus leucopus*), raccoon (*Procyon lotor*), and gray squirrel (*Sciurus carolinensis*) known to be competent reservoirs for this strain (Telford et al. 1996). Serologic and molecular evidence has suggested that numerous other mammals could be reservoirs for *Anaplasma* spp. (Levin et al. 2002); for example, a variant of the HGA signature strain has been found to infect 20.8% of white-tailed deer with no measured adverse effects to their health (Massung et al. 2005). Eurasian moose (*Alces alces alces)* are also known to carry *A. phagocytophilum* at a prevalence as high as 82% in some populations (Malmsten et al. 2019; Stigum et al. 2019), which may have implications for both human and animal health. Likewise, a high *Anaplasma* seroprevalence (80%) was detected in a moose population in New Hampshire, but not investigated further due to a lack of correlation with selected health metrics (Jones 2016). Our study aims to further examine the potential for moose in the northeastern US to harbor *Anaplasma* spp. infections.

Anaplasma spp. transmission among vertebrate hosts is primarily vector-borne and occurs in two main ways: biologically, involving replication of the bacteria within ticks (as occurs with *A. phagocytophilum*), and less frequently mechanically by biting flies or via blood-contaminated fomites (as seen with *A. marginale*). Additionally, transplacental transmission of both *A. marginale* and *A. phagocytophilum* has been reported in livestock (Zaugg 1985; Grau et al. 2013; Reppert et al. 2013). The primary tick vector for *Anaplasma* transmission in the US is *Ixodes scapularis*; however, there is known to be a wide variety of vectors for *Anaplasma*. In Europe, the main vector of pathogenic *Anaplasma* spp. is *Ixodes ricinus*, plus occasionally *Rhipicephalus bursa*, *Dermacentor marginatus*, the deer ked (*Lipoptena cervi*), and tabanid flies (de la Fuente et al. 2005a; Víchová et al. 2011; Stuen et al. 2013). In the northeastern US, none of these potential vectors are present; here the primary ectoparasite of moose is the winter tick (*Dermacentor albipictus*), an abundant species of hard tick with several hosts in its native range of North America (Samuel 2004). Although the winter tick is thought unlikely to act as a disease vector due to its one-host, 1-yr life cycle (Samuel 2004), evidence suggests that transovarial transmission of *A. phagocytophilum* can occur in winter ticks (Baldridge et al. 2009), and winter ticks have been shown experimentally to be competent vectors of *A. marginale* (Stiller et al. 1983).

Winter tick parasitism is thought to be the primary cause of moose calf mortality in the northeastern US (Jones et al. 2019). Much less is known about the prevalence of coinfecting pathogens, such as *Anaplasma* spp. Our study aimed to fill this knowledge gap on tick-borne infections in North American moose by: 1) estimating the prevalence of *Anaplasma* spp. in moose and in winter ticks in Maine; 2) determining the phylogenetic placement of these strains in moose and in winter ticks, with respect to those found in other hosts and vectors; and 3) exploring risk factors for *Anaplasma* spp. infection in moose.

MATERIALS AND METHODS

The study area

Data were collected from moose within wildlife management districts (WMDs) 2 and 8, in Maine (Fig. 1). The WMDs are geographic areas defined by the Maine Department of Inland Fisheries and Wildlife, within which similar biologic, geophysical, and hunting characteristics exist. The western Maine study district (WMD 8) extends north and west of the town of Greenville to the Quebec border. It is approximately 3,154 km^2 and encompasses the same study site used by Jones et al. (2019). This study area is a privately owned, managed commercial timberland where the dominant cover type is northern hardwood forest with some conifer stands (DeGraaf et al. 1992). In contrast, WMD 2 is a smaller area, approximately 1,867 km^2, but has a higher density of moose due to higher-quality habitat (Kantar and Cumberland 2013), which may be due to the abundance of forested areas where there is significant snow cover in the winter, cooler temperatures in the summer, and ample access to ponds and lakes (DeGraaf et al. 1992; Franzmann and Schwartz 1998).

Sample collection

From 2014 through 2018, moose were captured within WMDs 2 and 8 (Fig. 1) during December and January by the Maine Department of Inland Fisheries and Wildlife. Whole blood samples were collected during the 2017 and 2018 captures from 157 moose (15 adults, 142 calves; 57% female) and screened for the presence of *Anaplasma* spp. Calves were approximately 6–8 mo old at the time of capture. Additionally, 82 winter ticks were collected from moose during captures spanning 2014 through 2018. A subset of these ticks (n=55) were collected from 23 moose that were also screened for *Anaplasma* infection. Maine Medical Center Research Institute's Vector-Borne Disease Lab provided an additional 162 winter ticks from hunter-harvested moose, collected between September and October from several unknown locations across Maine. Six blacklegged ticks (*I. scapularis*), collected between May and July in 2017–18, were also obtained opportunistically from the University of Maine Cooperative Extension Tick Identification Lab. Blacklegged ticks are known vectors of *A. phagocytophilum*, so these served as comparative sequences in the phylogenetic reconstruction of *Anaplasma* spp. Whole body tissues from 244 winter ticks (154 nymphs, 88 adults, 2 unknown) were screened for *Anaplasma* spp. infections. Of these, sex was recorded for 138 winter ticks in 71 pools (22 female, 15 male, and 34 mixed pools).

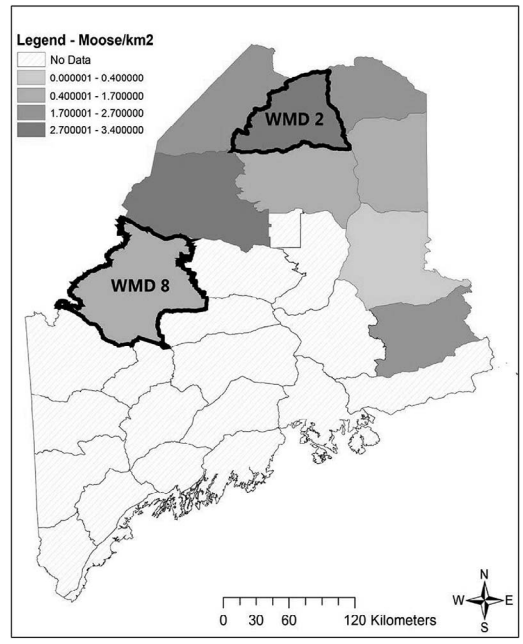

FIGURE 1. Map of the state of Maine, USA showing the western (WMD 8) and northern (WMD 2) wildlife management districts where Eastern moose (*Alces alces americana*) blood samples were collected in the winters of 2017–18 to genetically screen for *Anaplasma* spp. bacterial infections. Estimated moose population density indicated by a gradient and based on data from Kantar and Cumberland (2013).

DNA extraction and sample processing

Genomic DNA was extracted from moose ethylenediaminetetraacetic acid-anticoagulated whole blood and winter tick bodies using the Qiagen DNeasy protocol (Qiagen Inc., Germantown, Maryland, USA), and all extractions were checked for purity based on examination of 260/280-nm ratios. Very few winter ticks from which DNA was extracted were engorged (6/244, 2.5%), which is important because engorged female ticks tend to have high DNA concentrations and inhibitors that can interfere with PCR amplification (Schwartz et al. 1997). All nymphal and adult tick samples originating from the same moose were pooled, with 1–5 ticks per extraction. From the winter ticks tested, there were 113 unique pools and an average of 2.2 ticks/pool. Pooling of ticks was done to 1) increase total DNA concentration prior to PCR, 2) maximize the cost efficiency to increase the sample size of winter ticks screened, 3) increase the probability of detecting low-prevalence infections, and 4) account for correlated infections in winter ticks collected from a single moose. In preparation for downstream processing, all extractions were

TABLE 1. List of oligonucleotide primers in 5′ to 3′ orientation, targeting 16S rRNA and cytochrome c oxidase I (COI) genes. A partial sequence of the 16S rRNA gene was amplified and sequenced for the identification of *Anaplasma* species in Eastern moose (*Alces alces americana*), winter tick (*Dermacentor albipictus*), and blacklegged tick (*Ixodes scapularis*) collected between 2014–18 in Maine, USA. The COI gene locus was targeted to confirm the tick species.

Target	Primer	Amplicon size (base pair)	Sequence 5′→3′	Source
Anaplasma (16S)	EE-1	1,433	-TCCTGGCTCAGAACGAACGCTGGCGGC-	Barlough et al. 1996
	EE-2		-AGTCACTGACCCAACCTTAAATGGCTG-	
	EE-3	925	-GTCGAACGGATTATTCTTTATAGCTTGC-	
	EE-4		-CCCTTCCGTTAAGAAGGATCTAATCTCC-	
Ticks (COI)	LCO1490	658	-GGTCAACAAATCATAAAGATATTGG-	Hebert et al. 2003
	HC02198		-TAAACTTCAGGGTGACCAAAAAATCA-	

standardized to a DNA concentration of <25 ng/μL.

PCR amplification, electrophoresis, and 16S rRNA sequencing

We amplified, through a nested PCR, a partial sequence (928 base pairs [bp]) of the *Anaplasma* 16S rRNA gene, as described by Barlough et al. (1996). All reactions included a negative and an *Anaplasma*-positive control obtained from C. Lubelczyk (Maine Medical Center Research Institute, Vector-Borne Disease Laboratory), which was previously sequenced and identified as *A. phagocytophilum*. The assay used has previously been demonstrated to detect *Anaplasma* DNA derived from as few as 4–7 infected neutrophils in equine blood samples (Barlough et al. 1996). *Anaplasma* PCR assays were carried out in a total volume of 25 μL, which contained 2 μL of template DNA (standardized at <25 ng/μL), 5 μL of 5× PCR buffer (Promega, Madison, Wisconsin, USA), 200 μM deoxynucleotide triphosphates (New England BioLabs, Ipswich, Massachusetts, USA), 0.5U Promega GoTaq DNA polymerase (Promega), and 0.4 μM of each primer (EE-1 and EE-2; Table 1). The second reaction used the same reagents as the first with the exception of the nested primers (EE-3 and EE-4; Table 1) and 2 μL of the amplified product from the first reaction as a template. Thermocycling conditions for the first, outer reaction were as follows: initial denaturation at 94 C for 4 min; 35 cycles of 94 C for 30 s, 50 C for 30 s, and 74 C for 1.5 min; final extension at 74 C for 10 min. Thermocycling conditions for the second, inner reaction were as follows: initial denaturation at 95 C for 2 min; 35 cycles of 94 C for 30 s, 55 C for 30 s, and 72 C for 1 min; final extension at 72 C for 5 min.

For tick species identification, the mitochondrial cytochrome c oxidase I (mtCOI) region was amplified and sequenced as described by Hebert et al. 2003 (Table 1). The PCR amplifications of the mtCOI region used the same reagents and concentrations as described earlier. The following thermocycling conditions were applied: initial denaturation at 94 C for 1 min; 5 cycles of 94 C for 1 min, 45 C for 1.5 min, and 72 C for 1.5 min; 35 cycles of 94 C for 1 min, 50 C for 1.5 min, and 72 C for 1 min; final extension at 72 C for 5 min. All PCRs were performed using an Eppendorf (Hamburg, Germany) or BioRad (Hercules, California, USA) thermocycler. In addition to molecular detection, ticks were morphologically confirmed using physical descriptions from the literature (Samuel 2004; Sonenshine and Roe 2013).

Products of the PCRs were quantified and qualified by gel electrophoresis, using a 1–2% agarose gel in standard 0.5× Tris-borate-ethylenediaminetetraacetic acid buffer. Upon successful amplification of the 16S rRNA gene locus, PCR products were purified using the Illustra ExoProStar (Cytiva, Marlborough, Massachusetts, USA) and sent to the University of Maine Sequencing Facility for sequencing on an ABI 3730 sequencer (Applied Biosystems, Foster City, California, USA). All sequences were manually edited and aligned using the MUSCLE alignment plugin available in the *Geneious* software, version 11 (Edgar 2004; Kearse et al. 2012). Sequence data were compared and aligned against the nucleotide collection in the National Center for Biotechnology Information GenBank (Clark et al. 2016) using the basic local alignment search tool (BLAST) search for taxonomic identification. All sequences used in the phylogenetic analysis are provided in Table 2.

Phylogenetic analyses

Phylogenetic analyses were conducted on the aligned sequence data set using a Bayesian-based Markov Chain Monte Carlo approach, imple-

TABLE 2. Associated metadata for all *Anaplasma* 16S rRNA partial gene sequences used in reconstructing a phylogeny to genetically characterize sequences found in Eastern moose (*Alces alces americana*), winter ticks (*Dermacentor albipictus*), and blacklegged ticks (*Ixodes scapularis*) collected between 2014–18 in Maine, USA. Taxonomic identification, host species, geographic origin, and National Center for Biotechnology Information GenBank (Clark et al. 2016) accession numbers are provided. *Anaplasma* sp. denotes an uncharacterized strain. Sequences identified in this study are indicated with an asterisk (*). For some sequences, data on host species or origin were not available (NA).

Taxa ID	Host species	Origin	Accession no.
Anaplasma bovis	*Bos taurus*	India	MH244925
Anaplasma bovis	*Lepus sylvaticus*	Massachusetts	AY144729
Anaplasma centrale	*Bos taurus*	Southern Italy	EF520690
Anaplasma marginale	NA	Florida	AF309867
Anaplasma ovis	*Ovis aries*	China	AY262124
Anaplasma phagocytophilum (1)	*Alces alces alces*	Norway	KT070819
Anaplasma phagocytophilum (2)	*Alces alces alces*	Norway	KT070822
Anaplasma phagocytophilum (1)	*Alces alces alces*	Sweden	KC800983
Anaplasma phagocytophilum (2)	*Alces alces alces*	Sweden	KC800985
*Anaplasma phagocytophilum**	*Dermacentor albipictus*	Maine	MW899038
Anaplasma phagocytophilum	*Homo sapiens*	Connecticut	KT454992
Anaplasma phagocytophilum	*Ixodes ricinus*	Warsaw, Poland	MH122891
Anaplasma phagocytophilum	*Ixodes scapularis*	Connecticut	EF123258
Anaplasma phagocytophilum (1)*	*Ixodes scapularis*	Maine	MW899039
Anaplasma phagocytophilum (2)*	*Ixodes scapularis*	Maine	MW899040
Anaplasma phagocytophilum	*Ixodes scapularis*	Saskatchewan, Canada	HG916767
Anaplasma platys	*Canis familiaris*	Southern Italy	EU439943
Anaplasma platys	*Canis familiaris*	India	KT982643
Anaplasma sp. (1)*	*Alces alces americana*	Maine	MW899041
Anaplasma sp. (2)*	*Alces alces americana*	Maine	MW899042
Anaplasma sp.	*Odocoileus heminous*	British Columbia, Canada	JN673772
Anaplasma sp.	*Odocoileus virginianus*	British Columbia, Canada	JN673768
Anaplasma sp. Dedessa	*Bos taurus*	Illubabor zone, Ethiopia	KY924886
Anaplasma sp. Saso	*Bos taurus*	Illubabor zone, Ethiopia	KY924885
Ehrlichia chaffeensis	NA	NA	M73222
Ehrlichia ewingii	NA	NA	U96436
Neorickettsia sennetsu	NA	NA	M73225
Rickettsia rickettsii	NA	NA	L36217

mented in MrBayes version 3.2.6 (Huelsenbeck and Ronquist 2001) via the *Geneious* software, version 11 (Kearse et al. 2012). *Rickettsia rickettsii* was used as the outgroup to root the tree (Lobanov et al. 2012). Before running the model, the best-fit nucleotide substitution model was selected by examining likelihood scores calculated for 24 hierarchical substitution models and applying the Bayes information criteria in jModelTest version 2 (Posada 2008; Darriba et al. 2012). Phylogenetic reconstruction was carried out by performing two independent runs, using four heated chains per run. Each analysis ran for 1,100,000 generations, sampling every 200 generations, and a burn-in of 110,000 generations was used. Convergence and stationarity of runs was assessed using Tracer version 1.7 (Rambaut et al. 2018), by examining trace outputs, standard deviations of the split frequencies between runs, potential scale reduction factors, and effective sample size for the estimated parameters.

Statistical analyses

Contingency analyses (chi-square test) were performed using program R-3.5.1 (R Core Team 2019) to test for differences in infection prevalence by sex, age, and study area. We defined a calf as a moose less than 1 yr of age, whereas an adult was defined as greater than 1 yr. The Wilson

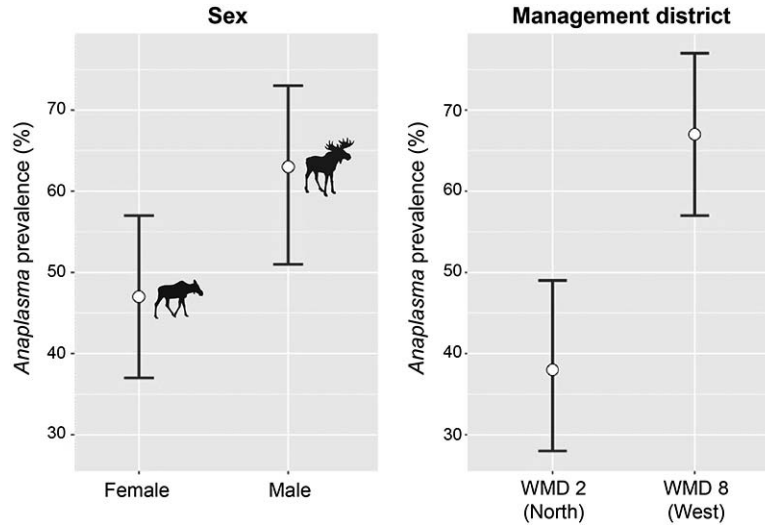

FIGURE 2. *Anaplasma* prevalence and Wilson interval scores, by (A) sex and (B) wildlife management district (WMD) in 157 blood samples collected from Eastern moose (*Alces alces americana*) between 2017–18 in Maine, USA.

score interval (Wilson 1927) was calculated to provide confidence limits for the proportion of infected moose overall and within each sex and district for a specified 95% confidence level. For winter ticks, maximum likelihood methods were used to estimate the prevalence of *Anaplasma* spp. in the pooled samples as described by Williams and Moffitt (2005). Both the Wilson score interval and the pooled prevalence for the variable winter tick pool sizes were calculated using the Epitools epidemiological calculators (Sergeant 2018).

RESULTS

Prevalence of *Anaplasma* species in moose

Over half (84 out of 157; 54%) of the moose tested positive for *Anaplasma* based on the PCR assay. There was a significant difference between the proportions of *Anaplasma*-infected moose in WMD 8 (67%) versus WMD 2 (38%; $P<0.001$), and male moose also exhibited a higher prevalence of infection than did females (63% vs. 47%, $P=0.055$; Fig. 2). Calves had a higher prevalence of infection (80/142, 56%) compared with adults (4/15, 27%), but the difference observed was not significant ($P>0.10$). From the 84 *Anaplasma*-infected moose, we found only two unique 16S rRNA gene sequences (851 bp), which differed by 2 bp. One sequence (GenBank accession no. MW899041) was found in the majority of moose ($n=83$), whereas the second sequence was found in only a single moose (no. MW899042).

All the winter ticks used in this study were confirmed as *D. albipictus*, based on a BLAST search that revealed >99% sequence identity of the amplified mtCOI locus to previously published *D. albipictus* sequences. Of the 55 winter ticks (or 23 unique pools) with paired moose data, 34 (13 unique pools) were from *Anaplasma*-positive and 21 (10 unique pools) were from *Anaplasma*-negative moose. No winter ticks from *Anaplasma*-positive moose were positive, even though 13 out of 23 (57%) of moose with paired tick samples were infected with *Anaplasma*. Only one pooled sample out of 113 unique pools of winter tick (<1%) tested positive for *Anaplasma* (no. MW899038). Specifically, the estimated prevalence for the variable pool size was 0.41% (95% confidence interval, 0.02–1.8%). The pooled sample that tested positive represented two adult winter ticks (1 male, 1 female) collected in January 2017 in WMD 2 (northern study area) from a female moose calf with a reported heavy winter tick load; however, that same female moose tested negative for

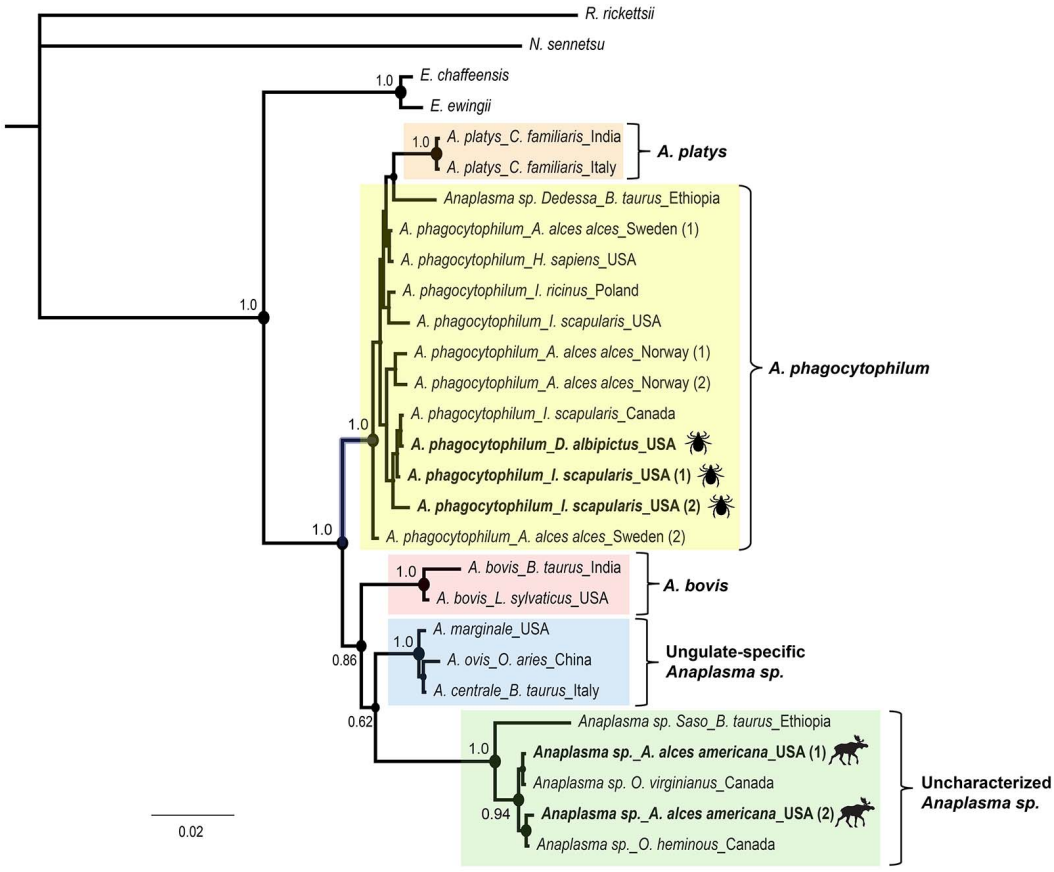

FIGURE 3. *Anaplasma* consensus phylogeny of 16S rRNA partial gene sequences identified in Eastern moose (*Alces alces americana*), winter tick (*Dermacentor albipictus*), and blacklegged tick (*Ixodes scapularis*) samples collected between 2014–18 in Maine, as well as select *Anaplasma* 16S rRNA sequences from GenBank (Clark et al. 2016), as listed in Table 2. Sequence names were designated as follows: *taxonomic identification_host species* (if applicable) *_geographic origin*. A taxonomic identification of *Anaplasma* sp. denotes an uncharacterized strain. Sequences identified in this study are indicated in bold font. Phylogenetic analyses were conducted in MrBayes version 3.2.6 (Huelsenbeck and Ronquist 2001).

Anaplasma and survived the following winter. Furthermore, two blacklegged ticks were positive with two different strains (nos. MW899039 and MW899040), one of which was identical to the strain we found in winter tick.

Phylogenetic analysis

Based on the Bayes information criteria model selection results, the Hasegawa-Kishino-Yano model (Hasegawa et al. 1985) was identified as the best-fit model and included as a prior for nucleotide substitution. Topology and convergence statistics were consistent across the two independent runs. The resulting phylogeny revealed four to five divergent *Anaplasma* clades (Fig. 3). Four clades had high support (posterior probability [PP]=1); however, the ancestral node representing the common ancestor of all *A. platys* and *A. phagocytophilum* sequences indicated weak support (PP=0.64), suggesting that while the two taxa are divergent, the relationships and placement of *A. phagocytophilum* taxa within this cluster could not be resolved.

The phylogenetic model placed the moose *Anaplasma* strains into a clade (PP=1) with other uncharacterized *Anaplasma* spp. (Fig. 3). Notably, these strains were most closely

related to strains identified in other wild cervids; a BLAST search revealed 100% sequence identity of moose *Anaplasma* sequences 1 and 2 to those previously found in white-tailed deer (*Odocoileus virginianus*; no. JN673768) and mule deer (*Odocoileus hemionus*; no. JN673772), respectively, located in British Columbia, Canada (Lobanov et al. 2012). These uncharacterized wildlife strains share a most-recent common ancestor with *Anaplasma* sp. Saso (no. KY924885), which was found in cattle (*Bos taurus*) from the Illubabor zone, Ethiopia (Hailemariam et al. 2017). Our phylogenetic model further suggests that the clade of uncharacterized *Anaplasma* spp. (green in online version only; Fig. 3) share a more distant common ancestor with *A. marginale*, *A. centrale*, and *A. ovis*. The *Anaplasma* sp. identified in winter tick (no. MW899038) had the highest similarity (100%) to an uncharacterized *A. phagocytophilum* (Ap-variant-1) 16S rRNA sequence amplified from *I. scapularis* in Canada (no. HG916767). This winter tick strain also clustered with all *A. phagocytophilum* sequences, including strains sourced from humans (no. KT454992), European moose (nos. KT070819, KT070822, KC800983, KC800985), and blacklegged ticks from Maine (nos. MW899039, MW899040, this study).

DISCUSSION

We found evidence that the majority (54%) of moose in Maine are infected with an uncharacterized strain of *Anaplasma* bacteria. In contrast, only a single pooled sample (<1%) of winter tick tested positive. The *Anaplasma* phylogeny revealed that strains found in moose were highly divergent from those identified in both winter and blacklegged ticks, and most closely related to North American strains derived from other cervids. Together, these data suggest that winter ticks are unlikely to be a vector for *Anaplasma* in Eastern moose. In addition, the observed high *Anaplasma* prevalence in moose highlights a need to further evaluate the transmission dynamics and potential impacts of the bacteria on individual- and population-level health.

Male moose had a higher infection prevalence than did females, although the difference was only marginally significant. Differences in habitat preference, movement, sociality, physiology, and reproductive behavior are potential factors that could drive differences in exposure, and therefore prevalence, between the sexes (Cross et al. 2009). Moose tend to cluster during calving, rutting, and in the late winter (Van Ballenberghe and Peek 1971; Phillips et al. 1973), which would affect transmission. *Anaplasma* species are typically vector-borne (Stuen 2007), so it is also possible that greater movement over larger ranges would increase the frequency of encounters with a competent vector. Males typically have larger home ranges than do females (Goddard 1970; Cederlund and Sand 1994), and differences in movement behavior could drive the observed difference in *Anaplasma* spp. prevalence between the sexes.

Despite having lower population density (Kantar and Cumberland 2013), moose in the western study area (WMD 8) had a significantly higher *Anaplasma* prevalence than moose in the northern study area (WMD 2). Lower moose density in WMD 8 is believed to be driven by the lower-quality habitat in the district (Kantar and Cumberland 2013), which highlights the need for further investigation into the relationships between habitat quality, body condition, and parasite infections. It is also important to note that if the unidentified *Anaplasma* strain in moose is vector-borne, then transmission may be frequency- rather than density-dependent (Thrall et al. 1993); if so, prevalence may not be explained by differences in moose population density, but alternatively attributed to variation in vector abundance and distribution between the regions. In addition, because WMD 2 has significantly more snow cover in the winter and cooler temperatures all throughout the year (DeGraaf et al. 1992; Franzmann and Schwartz 1998), we hypothesize that climate could reduce vector abundance, thereby decreasing the risk of *Anaplasma* infections

in Maine moose, but this line of inquiry requires further study.

Although we observed a greater proportion of calves with *Anaplasma* infections (80/142, 56%) than adults (4/15, 27%), this difference was not significant, and an increase in the adult sample size would be necessary to conclude whether infection varies by age. Nonetheless, a possible discrepancy in infection status between calves and adults could be due to adults having more time to clear an infection and gain immunity, which may enable resistance to future *Anaplasma* infections. However, there is also the potential for a persistent, chronic infection into adulthood, and further work is needed to evaluate the relationship between age and infection.

In contrast to the high proportion of moose infected, prevalence of *Anaplasma* in winter tick was extremely low (<1%) and the strain most closely resembled *A. phagocytophilum*, the agent responsible for HGA (Stuen et al. 2013). The strain was also closely related to that detected in blacklegged ticks from moose. Given the difference in strains found in ticks and moose, winter and blacklegged tick are unlikely to be vectors for the cervid-specific *Anaplasma* spp. identified in this study. Although the most common mode of *Anaplasma* spp. transmission involves the replication of the bacteria within ticks, alternative vectors may include blood-sucking or biting insects (Scoles et al. 2005). Mosquitoes, keds, tabanids, and muscid flies are common blood-sucking flies that feed on moose (Burger and Anderson 1974; Samuel et al. 2012; Moon 2019) and could be involved in *Anaplasma* transmission. In addition, vertical transmission between individual moose should not be ruled out as a possible route because transplacental transmission of *A. marginale* to the fetus has been reported in beef cattle (Zaugg 1985; Grau et al. 2013). Further genetic investigation of *Anaplasma* strains from parent-offspring pairs would be needed to evaluate the potential for the bacteria to be vertically transmitted in moose.

We found no evidence that moose act as hosts of *A. phagocytophilum* in Maine. Therefore, it is not likely that *A. phagocytophilum* poses a threat to moose health, nor is it likely that moose could be a source of zoonotic infection to humans. Our results are in contrast to what has been observed in European moose populations in which *A. phagocytophilum* was identified in a large proportion of individuals and shared a >99% identity with the pathogenic strain responsible for HGA in humans (Pūraitė et al. 2015; Malmsten et al. 2019). More research is warranted, however, to determine the potential of winter ticks to transmit *Anaplasma* and the subsequent risk these ticks may pose to other susceptible hosts.

While the findings from this study are compelling, some limitations should be acknowledged. First, the pooling of ticks from the same moose affects our ability to calculate precise proportions of infected ticks; however, due to the low number of *Anaplasma*-positive ticks, this was not deemed a significant limitation. Second, the geographic scope of our sampling was limited and does not allow us to generalize estimates of infection prevalence to other moose populations in Maine as well as in adjacent states and provinces. Many of these jurisdictions are experiencing moose population declines due to intensive harvest and high winter tick infestations (Timmermann and Rodgers 2017; Jones et al. 2019); therefore, further examination of parasite infection prevalence and health consequences in these areas may be prudent. Third, while the *Anaplasma* sp. described in this study is clearly divergent from other strains, the *Anaplasma* genus has a complex lineage (Uilenberg et al. 2004), and more comprehensive genetic data will be required to characterize the evolutionary relationships and phylogenetic placement of the *Anaplasma* strains found in moose.

In conclusion, our study reports the presence of a prevalent, novel *Anaplasma* sp. circulating in Maine's moose population. We found no evidence to support a role of winter tick or blacklegged tick in transmission of this bacteria in moose. These results warrant further research to: 1) obtain additional genetic data to better characterize the vertebrate host range of *Anaplasma* spp., 2)

evaluate possible vectors and alternative transmission modes for the uncharacterized *Anaplasma* sp. found in Eastern moose, 3) determine the geographic extent at which the infection persists in moose, and 4) identify potential effects of *Anaplasma* infections on moose health and long-term population viability. Together, these data could have significant implications for moose management in the northeastern US.

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