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Evaluating the Effects of Parasite Infections on Reproductive Ability in Maine Moose

Isabella Costa
University of Maine - Main, isabella.costa@maine.edu

EVALUATING THE EFFECTS OF PARASITE INFECTIONS ON REPRODUCTIVE ABILITY IN MAINE MOOSE

by

Isabella Costa

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Advisory Committee:
Pauline Kamath, Associate Professor of Animal Health, Advisor
Robert Causey, Associate Professor of Animal and Veterinary Sciences
Sue Ishaq, Assistant Professor of Animal and Veterinary Sciences
Margaret Killinger, Professor in Honors

ABSTRACT

Despite conservation efforts, moose have experienced increasing mortality rates. Winter ticks are known to cause anemia and lower reproductive potential in moose. Moreover, a genus of bacteria, *Anaplasma* spp., is known to cause a reduction in reproductive efforts in other animals, such as cattle. Because both winter ticks and *Anaplasma* spp. may affect female reproductive ability, the overall goal of this research was to determine if *Anaplasma* bacterial infections could be impacting female moose reproduction within the state of Maine. To address this, samples and biological data from moose were collected during hunter harvest, in collaboration with hunters and the Maine Department of Inland Fisheries and Wildlife (MDIFW), and molecular analyses were performed to determine *Anaplasma* infection status. Using these data, statistical models were used to examine relationships between *Anaplasma* infections and two reproductive indices: corpus luteum (CL) counts, reflecting the number of ova that have ovulated, and lactation status, reflecting the presence of a calf born in the previous spring. Potential individual factors affecting these reproductive indices were also considered, including age, weight, and location. Out of the female moose (cows) sampled, less than half (13 out of 36; 36%) of the moose tested positive for *Anaplasma* with the use of a PCR-based assay. Moreover, 19% (7 out of 36) of the cows had undetected CL. However, of those with CL, 61% (22 out of 36) of the moose had one CL whereas 19% (7 out of 36) had two CLs. *Anaplasma* infection was not significantly associated with reproductive indices (CL counts or lactation status) in moose cows. Given the variable factors affecting cow reproduction, further research is needed to examine the effects of *Anaplasma* infection on successful pregnancy and calf recruitment into the population.

DEDICATION

I am pleased to dedicate this thesis to my incredible family. Mom, dad, Cristina, Gabby, Ashlynn, and Jordan…None of this would have been possible if it weren't for you. Thank you for always being undoubtedly supportive. I love you guys!

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INTRODUCTION

Despite management efforts across the New England states, the population of the Eastern moose (*Alces alces*) has been experiencing increasing calf mortality (Jones et al., 2019). Factors that play a role in decreased survival include climate change and disease (Timmerman & Rodgers, 2017). Recent studies have proposed that changes in climate are affecting both moose and parasite distributions, increasing transmission and bringing about escalations of disease occurrence in moose (Ditmer et al., 2018; Malmsten et al., 2018; Weiskopf et al., 2019). Moose are valuable contributors to the Maine economy by providing tourism, hunting, and recreational opportunities. Additionally, moose are culturally important animals for the indigenous populations within the New England region; for example, the Wabanaki tribes use moose meat and products for sustenance (DiMatteo-Lepape, 2019). Therefore, the effects of parasites on moose are an impending threat to the livelihoods and well-being of tribal communities.

One parasite, the winter tick (*Dermacentor albipictus*), has been found to significantly contribute to the high mortality rates of the moose population throughout New England (Jones et al., 2019; Ellingwood et al., 2020). Although northern New Hampshire and western Maine house the largest moose population in the contiguous United States (Timmerman & Rodgers, 2015), recent declines in the population have been attributed to increasing levels of winter tick infestations, which are suggested to be the cause of greater than 50% late-winter mortality of 9 to 12-month-old calves (Jones et al., 2019). Models have shown prolonged time periods of high tick loads can cause a significant decline in calf survival (< 50%), full-grown calving (< 60%), and prevalence of twins (< 5%), along with the total loss of one-year-old calves' productivity

(Ellingwood et al., 2020). Furthermore, moose cows will twin as long as conditions are good; thus, if the prevalence of twins is high, this is an indicator of a suitable environment in which moose can thrive. Given this, previous studies have used moose twinning rates as a form of population assessment (Franzmann & Schwarts, 1985). Therefore, observations of recent low twinning prevalence in Maine are a reason for concern, warranting further research to understand the impacts of parasite infections on moose reproduction (Jones et al., 2019).

The winter tick is an external parasite that reproduces and survives by living off the blood of its hosts. This species is a one-host tick meaning they receive consecutive blood meals from one animal throughout the course of their eight-to-nine-month life cycle. For example, if a moose is parasitized by winter ticks, those winter ticks will stay attached to that moose for their larvae, nymph, and adult stage (Addison & McLaughlin, 1988). High infestation may be acquired on a singular moose due to the one-host life cycle of the ticks, with instances reported of approximately 95,000 ticks on a singular moose calf (Jones et al., 2019). Winter ticks are known to cause anemia, a low red blood cell count, and are suspected to lower the reproductive potential of female moose (Musante et al., 2007). Additional negative effects include mortality in calves, extensive hair loss, issues with thermal regulation, and starvation (Addison & McLaughlin, 1986; Addison & McLaughlin, 2014; Ellingwood et al., 2019).

Beyond parasitism by winter ticks, moose are known to be affected by additional co-infecting parasites. For example, *Anaplasma* infections have been documented at a high prevalence rate of 54% in Maine moose (Elliott et al., 2021). *Anaplasma* is a genus of bacteria that can cause disease in both humans and animals. *Anaplasma* bacteria is transmitted to the host via a vector, with common vectors being *Ixodes scapularis*, *Ixodes ricinus*, *Rhiphicephalus*, and *Amblyomma* (Rymaszewska & Grenda, 2008). From there, the *Anaplasma* species will infect the host's blood cells and cause a disease known as anaplasmosis (Rymaszewska & Grenda, 2008). People diagnosed with anaplasmosis, caused by *A. phagocytophilum*, will often experience symptoms including but not limited to high temperature, headache, chills, and muscle pain (Center for Disease Control and Prevention, 2022). Cattle infected with *A. marginale* often display symptoms of high temperatures, abortion, and even death (Zhyldyz et al., 2019). Other symptoms of animals infected with *Anaplasma* species include decreases in body weight and milk production (Rymaszewska & Grenda, 2008).

The *Anaplasma* bacteria infecting moose is a distinct species that shares a common ancestor with *A. marginale*, and the vector remains unknown (Elliott et al., 2021). Researchers are unsure how *Anaplasma* impacts moose health, but there is some evidence that *Anaplasma* infections may reduce calf survival, particularly for those individuals co-infected with winter ticks (Elliott 2019; Woods et al., in preparation). In Europe, moose are known reservoirs of *A. phagocytophilum*. Previous studies have shown that *A. phagocytophilum* in moose are not suspected of causing reproductive failure and have not been linked to decreasing calf rates (Milner & van Beest, 2013); in addition, these infections are not conjectured to cause any negative health impacts. However, in a case study performed in Norway, anaplasmosis (formerly known as ehrlichiosis) was suspected to be the cause of death in a moose calf (Jenkins et al., 2001). Given these contradictory results, more research needs to be conducted to better understand the effects of *Anaplasma* infection on moose health and reproduction.

Moose are seasonally polyestrous breeders, with mating taking place in the fall, typically from September through October (Swartz, 1992). During the female estrus period, when they are fertile, there are typically one or two dominant follicles (in rare circumstances, three) that reach maturity, rupture, and release ovum (i.e., the egg) to complete ovulation. The number of follicles that ovulate may vary over space and time, as well as with a cow's age, nutrition, and body condition (Bergeron et al., 2013; Garel et al., 2009; Malmsten et al., 2014). For example, previous studies have shown that the likelihood of ovulation occurring in moose may be negatively affected by body weight and high stress levels (Malmsten et al., 2014; Spong et al., 2020).

The corpus luteum (CL) is a temporary organ that develops in the ovaries where the dominant follicle used to be, following ovulation. It is a structure in the female reproductive tract that is required to support fetal growth and fertility post ovulation during the luteal phase. The CL will then begin to regress after it has made the uterus a suitable place for the fetus to thrive with the use of progesterone hormone for fetal development (Duncan, 2021). If a mammal does not ovulate, the CL will not form, making it a suitable index for reproductive ability (Hobson & Baker, 1979; Rao & Gibori, 1987). In New Hampshire yearling age class moose, a statistically significant decline of the mean body weight across both sexes, the number of CL, and antler beam diameter were reported between the years of 1988 – 2009. Nevertheless, in 2013, the ovulation rate together with the mean body weight of yearling moose cows in the states of New Hampshire and Vermont existed as being below average (Bergeron et al., 2013).

Lactation is a fundamental process within the growth and development of mammals and has been determined to be the most integrated aspect of the reproductive cycle within females (Ceacero et al., 2016). The start of lactation, termed lactogenesis, involves hormonal management which is poorly understood (Gorewit, 1988). Throughout gestation, the development of the mammary gland secretory apparatus is performed by influences related to rising concentrations of prolactin, placental lactogen, estrogen, and progesterone. Even though prolactin concentrations increase during gestation, only some of the prolactin's efforts can take place at that time because of the increased levels of both estrogen and progesterone that inhibit a number of prolactin's results. On the other hand, after parturition, progesterone and estrogen levels decrease. Consequently, prolactin then has the ability to make use of its results within the mammary glands and launch milk excretion (Heil & Subramanian, 1998). Thus, lactation begins after parturition, allowing it to act as an indicator for past reproductive ability.

The management of moose in Maine by government agencies and other affiliated groups plays a major role in moose population well-being, through consideration of the interlinkages between humans and moose (Ericsson, 2003). In Maine, moose management is primarily overseen by the Maine Department of Inland Fisheries and Wildlife (MDIFW), while the Penobscot Nation Department of Natural Resources manages moose populations on tribal lands. The Wabanaki tribes have the authority to harvest wildlife throughout their lands and this is paramount as moose hunting plays a major role in Wabanaki culture and history (DiMatteo-Lepape, 2019; Elliott, 2019; Prins & McBride, 2007). It has been noted that Wabanaki citizens are worried and have been apprehensive about moose health, including fears that a reduction in moose well-being and moose populace could decrease moose hunting and visual observations (Elliott, 2019). More significantly, Wabanaki citizens have explicitly communicated concerns that a reduction of moose in good health may affect their cultural heritage and sustenance hunting practices (Elliott, 2019).

Little is known about whether *Anaplasma* infection affects reproduction in moose, in part because assessing reproduction directly through observation has been a challenge. Thus, the goal of this study was to determine whether *Anaplasma* infections affect the reproduction of female moose in Maine. To do this, we collected biological samples and associated data from moose during the fall hunter harvest, when cows are expected to have completed ovulation and may also continue to nurse a calf born in the previous spring. Our specific research objectives were to (1) determine whether *Anaplasma* infection influenced CL counts and lactation, as indicators of reproduction, and (2) evaluate other potential predictors (e.g., age, dressed body weight) of CL counts in female moose). We expect these parasites to have other impacts on population fitness (beyond survival) through impacts on reproduction. Furthermore, knowing the drivers of moose recruitment is valuable for informing management and harvest recommendations in order to mitigate moose population declines.

METHODS

Hunter Harvest Data Collection

Whole blood samples (for molecular *Anaplasma* infection assays) and biological data from moose were collected over three years (2021 – 2023) during the fall hunter harvest in Maine, in collaboration with hunters and the MDIFW (Figure 1). Throughout the months of September to November, hunters were sent a sampling kit through the mail and were instructed to collect whole blood samples in ethylenediaminetetraacetic acid (EDTA)-anticoagulated, purple top blood tubes at the site of harvest in the field. Briefly, whole blood was collected at the site of the bullet wound as soon as possible after the animal had been harvested. Once the tubes were approximately ¾ full of blood, the caps were secured, and the individual tubes were inverted 15 to 20 times, ensuring that the blood samples were mixed with the preservative. Additionally, hunters brought harvested animals into registration stations where additional samples (i.e., ovaries for estimation of CL in females, a tooth for approximation of age) were collected and the lactation status was assessed in cows. In addition, individual moose were weighed to estimate the dressed body weight (i.e., the weight of the animal after being harvested). It is important to note that the dressed weight can vary widely between individual animals due to differences in how hunters process the carcasses.

The moose age was assessed with the use of a canine or incisor tooth sample that was extracted at the harvest station. From there, the tooth was cut horizontally, and the annuli in the cementum of the root tips were counted (Boerjte et al., 2015). CL counts were recorded by cross sectioning the ovaries to look for a follicle visually. Lactation status was assessed by means of determining if the moose were producing and releasing milk from the mammary glands at the time of harvest.

Molecular Diagnostics of *Anaplasma* Infection in Moose

Previously published and validated methodologies for molecular detection of *Anaplasma* from whole blood were used (Elliott et al., 2021). Briefly, DNA was extracted using the Qiagen DNeasy protocol (Valencia, CA). Purification of DNA was accomplished through an AMPure XP (Beckman Coulter) magnetic bead protocol, and DNA concentrations were quantified as well as quality assessed via a Nanodrop One Spectrophotometer (Thermo Fisher Scientific, Waltham, MA). Purified extractions were standardized to a DNA concentration that was between 10 and 20 ng/uL. Then, a polymerase chain reaction (PCR) assay was utilized to determine *Anaplasma* infection status. This allowed for the identification of *Anaplasma* DNA (if any) with two sets of formerly used and publicized primers (Barlough et al., 1996; Elliott et al., 2021). The nested PCR first amplified the 16S rRNA gene and the second reaction then amplified the segment that is distinct to the *Anaplasma* genus.

Anaplasma PCRs were run in a total volume of 10 uL, which included 2 uL of template DNA (standardized to ~ 10 ng/uL), 5 uL of 5x PCR buffer (Promega, Madison, WI), 200 uM deoxynucleotide triphosphates (dNTPs, New England BioLabs, Ipswich, MA), 0.5 U Promega GoTaq DNA polymerase (Applied Biosystems, Foster City, CA), and 0.4 uM of each primer (EE-1 and EE-2). The second reaction in the nested PCR applied the same reagents as mentioned above, but instead used the primers EE-3 and EE-4, and 1 uL of the amplified product from the original reaction. Thermocycling environments for the initial, external reactions were as follows: a starting 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 environments for the subsequent, internal reaction were: a starting 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 (Elliott et al., 2021). Every PCR test ran contained a negative control, an extraction blank, as well as a known positive to warrant that contamination was not present and the reaction amplified the intended target (Elliott et al., 2021). We ran two PCR assays for each sample to validate PCR testing outcomes. The outcomes of the PCR tests were determined through gel electrophoresis and 1.5% agarose gel.

Statistical Analyses

We used the R programming language for conducting statistical analyses as well as describing our raw data. We plotted and evaluated the distribution of the raw data (i.e., corpus luteum (CL), age, *Anaplasma* infection status, dressed weight and lactation status. Additionally, a Chi-Squared test was run to determine if CL counts varied by *Anaplasma* infection status. A second Chi-Squared test was run to assess whether lactation status (i.e., yes, no, or unknown) varied by *Anaplasma* infection status.

A one-way ANOVA test was run to examine potential differences in infection prevalence among CL count groups (i.e., 0, 1, or 2), using CL counts as a categorical variable. A Tukey's test of honest significance was completed with the ANOVA to determine if the mean infection prevalence significantly differed among CL groups. Similarly, another ANOVA test and Tukey's test of honest significance were completed to determine if *Anaplasma* infection prevalence (i.e., positive, or negative) varied by lactation status (i.e., yes, no, or unknown).

Single Poisson generalized linear models (GLM) were run to examine whether *Anaplasma* infection status, age, and dressed weight predicted either CL counts (discrete variable reflecting reproductive potential). Distributions of animal age and dressed weight were assessed and determined to be relatively normally distributed. Due to a limited sample size of paired blood and ovaries (n = 36), the GLM models were run with only a single independent predictor variable (i.e., age, infection status or dressed weight) to examine relationships with reproduction, using CL counts as the response variable. The statistical analyses resulted in the examination of a total of six different models for the purpose of identifying predictors of reproduction in moose. For all models run, we ensured all assumptions were met by visualizing the, "Residuals vs. Fitted, Q-Q Residuals, Scale-Location, and Residuals vs. Leverage," plots.

RESULTS

Biological samples were collected from 36 harvested female moose from seven wildlife management districts (WMDs) in Maine (Figure 1). These moose varied in weight, age, lactation status, and CL count. Of the 32 animals with recorded ages, the minimum age was 2.5 years whereas the maximum age was 12.5 years old with the average overall age being 6.8 years old at the time of harvest (Figure 2, Table 1). Of the 22 individuals that had their dressed weight recorded, the minimum weight was 362 pounds while the maximum weight was 740 pounds, with the average weight being 581 pounds (Figure 3, Table 1). Of the 20 cows that had their lactation status recorded, 9 (45%) were lactating at the time of harvest, and 11 (55%) were not lactating. Additionally, more than three quarters of the cows (29 out of 36; 81%) had at least one CL. Of these, 61% (22 out of 36) of the cows had one CL, whereas 19% (7 out of 36) had 2 CLs. (Figure 4, Table 1). Less than half (13 out of 36; 36%) of the moose tested positive for *Anaplasma* based on the results of the PCR-based assay.

Using a Pearson's Chi-Squared test, it was determined that *Anaplasma* infection prevalence did not differ significantly among cows with different numbers of CLs (p = 0.406). Based on an ANOVA that examined the average infection prevalence by CL count groups, we also found that the probability of infection did not differ by CL count (i.e., 0, 1, or 2), (Figure 5). Similarly, using an ANOVA to examine average infection prevalence by lactation status (i.e., yes, or no), we found that there was no significant correlation between infection status and lactation (Figure 6). Assuming a 95% confidence level, based on an ANOVA test, we found no significance between infection status and CL counts, even when considering the number of CL (i.e., 0, 1, or 2). Similarly, when considering an additional ANOVA test run, there is a 95% confidence level that there is no significant correlation between infection status and lactation when considering the status of lactation (i.e., yes, or no).

DISCUSSION

In this study, we show that *Anaplasma* infection status does not appear to affect reproductive ability, assessed by both corpus luteum counts (commonly utilized as an indicator of ovulation) and lactation status, in the Eastern moose. Pearson's Chi-squared analysis indicated no significant difference in the number of corpora lutea in moose infected versus uninfected by *Anaplasma* bacteria. Similarly, Pearson's Chi-squared analysis resulted in no significant difference in the probability of *Anaplasma* infection in harvested moose that happened to be lactating. Moreover, as determined via univariate Poisson generalized linear models, biological factors such as age and dressed body weight, also did not significantly affect the reproductive ability (CL counts or lactation status) of moose cows.

There are many other abiotic and biotic factors that could be impacting reproductive success, including environmental factors (e.g., rainfall, temperatures), other parasites (e.g., winter tick), genetics, abnormalities, nutritional deficiencies, and stress (Malmsten & Dalin, 2014). Parasites have been found to affect moose fitness, in terms of both survival and reproduction. For instance, winter tick infestations on calves may lead to increased mortality when exposed to harsh winter weather and have also been shown to potentially reduce reproductive ability for adult moose, which together may contribute to the observed regional population declines (Musante et al., 2007; Ellingwood et al., 2020; Rosenblatt et al., 2021).

It is probable that the predictor variables being evaluated in this study, namely, CL counts and lactation, were not adequate measures of reproductive potential. For instance, the number of CL (if any) produced by a moose cow is one of the main determinants to verify if that individual animal is ovulating, which reflects the potential for conception (Rao & Gibori, 1987). However, CL counts are not necessarily representative of the individual animal's ability to support a healthy fetus and carry it to full term, as well as a successful birth and survival of the calf. On the other hand, lactation may be a better measure for indicating past reproduction and survival of the calf through the fall at minimum when the samples were collected. However, it only reflects the success of the previous year's reproductive cycle, and the animal could have become infected with *Anaplasma* after the birth of the calf over the period of the summer. Briefly, lactation serves as an indication that the individual female has previously been pregnant and birthed a calf in the prior spring that she was supporting through the fall. Thus, if a cow is lactating, this serves as an indicator that their calf survived throughout the fall. This is due to the fact that the production of milk normally occurs after the female has completed parturition (Bruckmaier & Zinn, 2023) for the primary purpose of providing nourishment to the offspring.

More importantly, a previous study performed in Sweden from 2007 to 2011 communicated that the inconsistency between ovulation rates and early embryonic formation/flourishing conveys that ovulation rates exist as being representative but are not by definition adequate approximations of the moose reproductive ability (Malmsten & Dalin, 2014). To accomplish this, the researchers utilized reproductive tracts (ovarian structures, corpora lutea, and uteri) collected from 213 hunter-harvested moose cows to approximate reproductive potential through both ovulation rates and pregnancy rates (Malmsten & Dalin, 2014). Moose that were confirmed pregnant had their embryos further analyzed to establish if they were viable as well as determining if the ovum was unfertilized. Furthermore, the embryonic mortality together with the prevalence of endometriosis in moose were assessed (Malmsten & Dalin, 2014). Ovulation rates in moose were reported to be notably greater than the proportion of generated embryos within the uterus, suggesting both a high rate of embryonic death and unfertilized oocytes, which can make assessing moose reproductive ability by CL counts difficult (Malmsten & Dalin, 2014). In conclusion, the authors warned against the exclusive use of CL counts as an index of reproduction could give rise to overestimates of moose productivity (Malmsten & Dalin, 2014).

A previous study in moose collected multiple sample types to estimate a female's reproductive status more holistically (Malmsten et al., 2014). This study took place between 2008 and 2011, which incorporated 250 samples of reproductive organs, namely the uterus (consisting of the caruncles, mucosa, embryos, and fetuses), the ovaries (specifically, the CL, follicles, and corpus albicans), as well as the cervix. By doing so, females were successfully categorized into a total of nine varying classifications based on the report of their reproductive status (Malmsten et al., 2014). Rather than possessing only one piece of information related to the reproduction of individual animals, having multiple details about different structures may help to guide knowledge related to a moose cow's ability to reproduce.

Hunter harvest data that includes the reproductive tract of the moose would aid in acting as a primary, prerequisite source by contributing what is known about moose reproduction and population productivity. If carried out sufficiently, more evidence would be provided to aid in the determination of the potential low reproductive rates of the Eastern moose throughout Maine. Furthermore, this study only focused primarily on moose cows. Considering this, the possible implications of reproductive status in bull moose should be reviewed. To do this, the reproductive system of bull moose has the possibility to be examined closely at harvest registration stations, comparable to what was executed in this study. From there, future research could aim to further analyze the sperm production, as well as other indices of reproductive potential, together with *Anaplasma* infection status within bull moose in Maine.

For instance, a study executed from the years 2008 to 2011 aimed to investigate the reproductive characteristics of male moose (Malmsten et al., 2015). The researchers felt this was necessary, as most studies pertaining to reproductive ability within moose focus primarily on the cows, resulting in a small number of studies regarding bull moose (Malmsten et al., 2015). Results showed that out of a sample size of 143 male moose between the ages of 1.5 to 11.5 years old, the quantity of normal spermatozoa decreased temporally and was positively correlated with corpse and testes mass. Both body and testes weight possessed a positive effect on the number of normal spermatozoa notwithstanding age. Overall, for the success of reproduction within moose, a high body mass in males is beneficial, like that of a balanced sex proportion.

In this study, we were working with a limited sample size (n = 36) from cows, due to the difficulty in collecting both ovaries and paired infection data from harvested moose. Our sample size of 36 individual moose cows was quite small and, thus, we did not have enough statistical power to model the relationships between individual factors (i.e., age, dressed weight, *Anaplasma* infection) and reproductive indices (CL counts, lactation status) within a multivariate GLM analysis. Given this, we were only able to assess these relationships with single variable models. However, with a larger sample size, a multivariate GLM analysis would also allow for these relationships between infection and reproduction to be assessed, while also accounting for variation explained by other factors previously known to affect reproduction.

In addition to this, individual moose cows used in this study originated from multiple wildlife management districts (WMDs #1 – 6 and #8) across their range in Maine (Figure 1). Considering this, it may be useful to try to attain additional samples from hunter harvested moose residing within other WMDs. There are 20 different WMDs across the state of Maine that allow moose hunting, of which only 7 were included in this study. Harvest occurs within specific weeks throughout the months of September, October, and November though it is important to note that the precise date differs across different districts. Also, the timing of sampling could affect the reproductive indices measure with regards to how sampling aligns with the female reproductive cycle and weaning.

CONCLUSION

Biological samples were collected from 36 adult female moose from seven WMDs. *Anaplasma* infection status, age, and dressed body weight did not significantly affect CL counts or lactation status (Table 1, Figures 5 and 6). Additionally, 36% (13 out of 36) of the moose tested positive for *Anaplasma*, whereas 81% of the moose had at least one CL and 19% had two CLs. It is important to note that *Anaplasma* infection may not influence reproductive ability (reflected by CL counts and lactation status) of moose cows. Further research is needed to understand whether *Anaplasma* infections impact successful pregnancy and reproduction, since CL counts only reflect ovulation and potential for conception. In addition to this, the timing of the lactation may not have aligned with the sampling. Considering this, there may be other variables not included in this study (e.g., winter ticks, climate, habitat, stress, immune factors, genetics, etc.) that should be evaluated as potential predictors of reproductive ability in moose. Future research aimed at identifying factors that limit reproduction and survival are undoubtedly important and essential for informing management of the Maine moose population.

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APPENDICES

Appendix A: Map of Maine & Number of Samples Collected

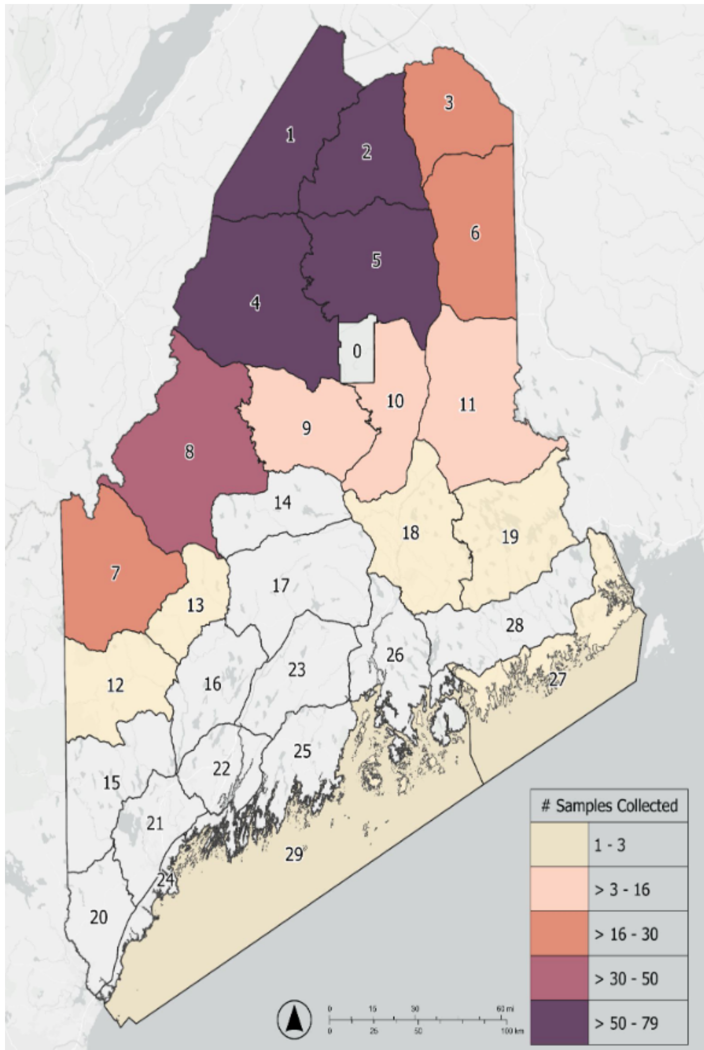

Figure 1. Map showing the numbers of moose sampled during the 2021-2023 fall hunter harvest. Blood, ovaries, CL samples, and associated data were collected from WMDs 1 through 6, and 8.

<u>Appendix B: Histograms of Data Collected</u>

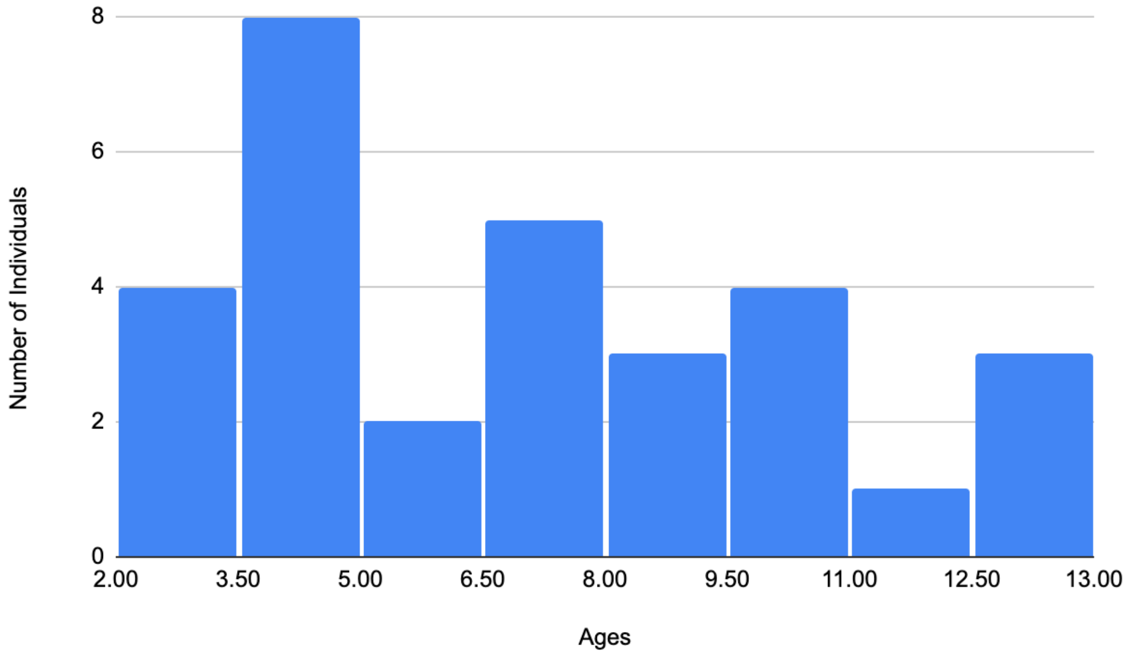

Figure 2. A histogram depicting the estimated age distribution in the samples collected from moose cows during the 2021-2023 fall hunter harvest in Maine. Ages were approximated in years for 32 individuals, with the average age calculated as 6.8 years of age at the time of harvest.

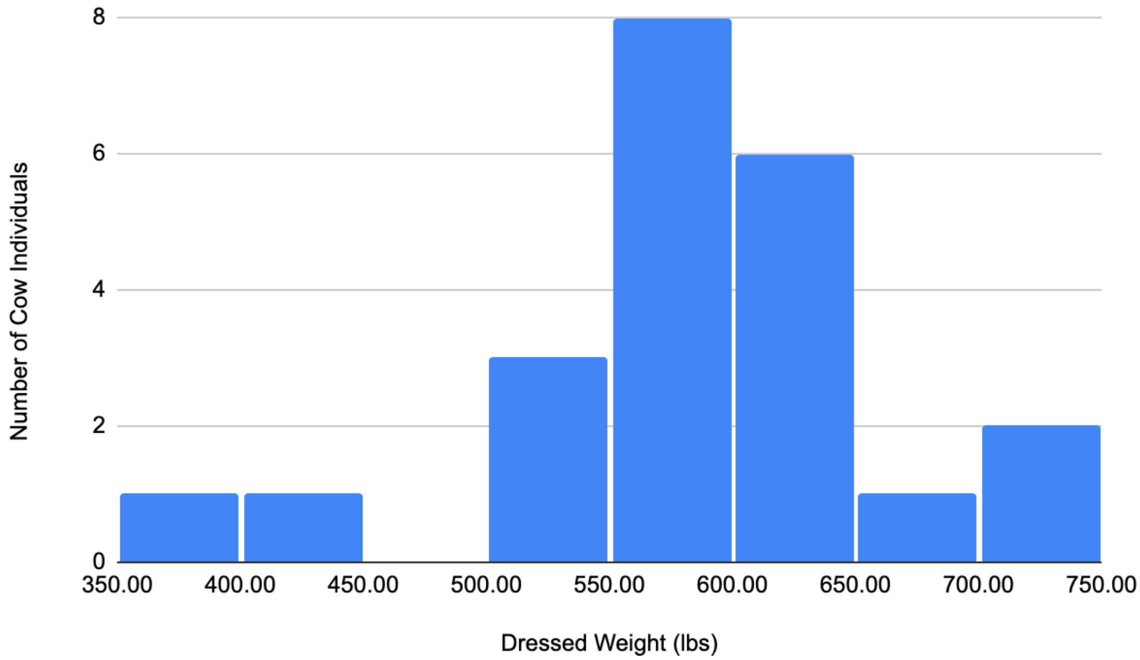

Figure 3. A histogram depicting the dressed weight distribution in the samples collected from moose cows during the 2021-2023 fall hunter harvest in Maine. Weight in pounds was recorded for 22 individuals included in this study, with the average dressed weight calculated as 581 pounds.

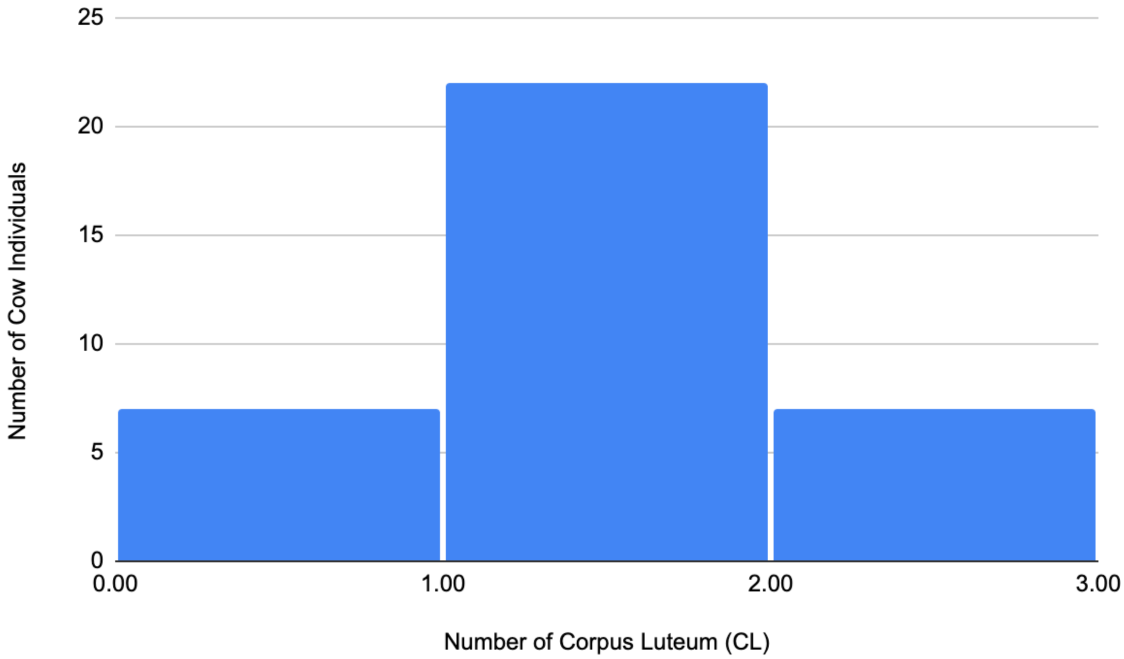

Figure 4. A histogram depicting the corpus luteum counts (i.e., 0, 1, or 2) of the 22 individuals included within this study.

<u>Appendix C: Graphs Depicting Main Findings</u>

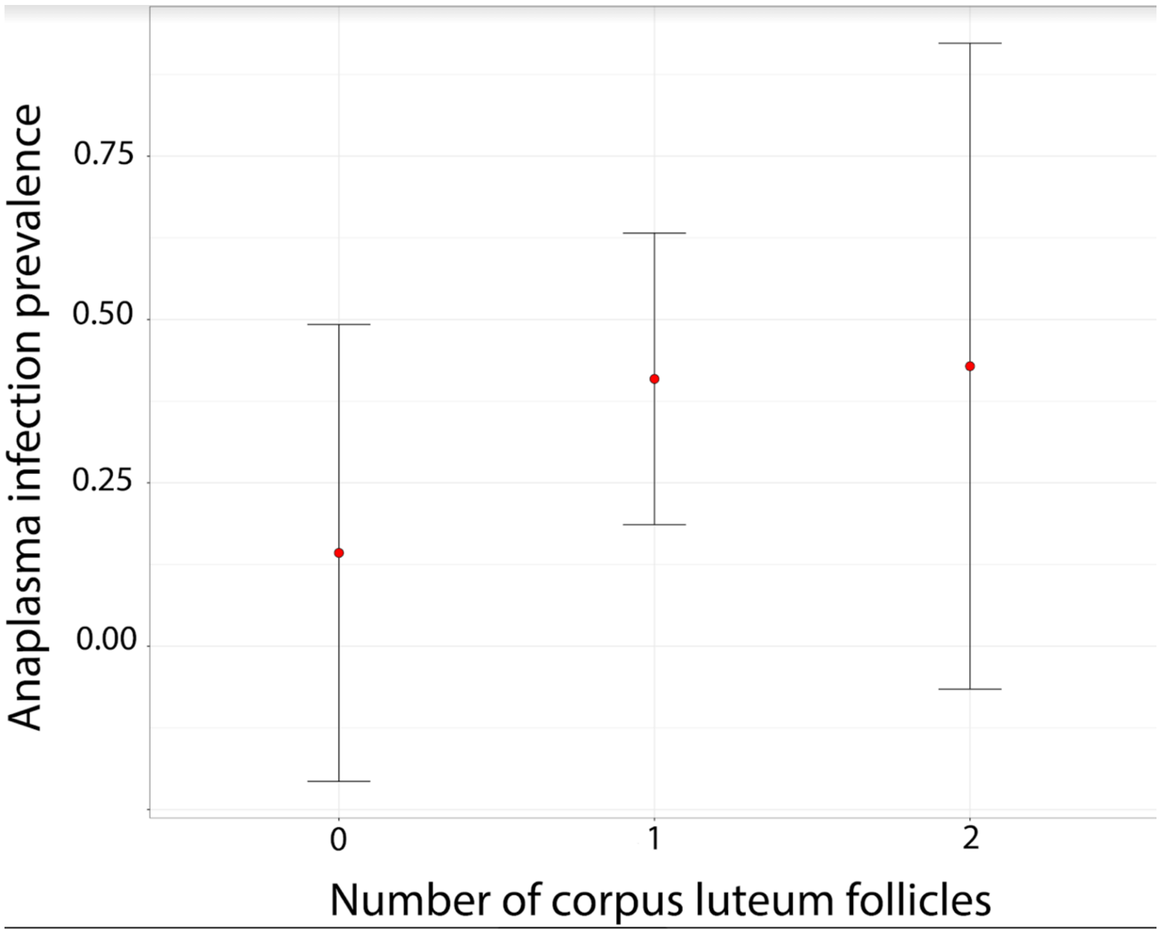

Figure 5. Graph depicting *Anaplasma* infection prevalence by the number of corpus luteum follicles present in moose cows sampled during the 2021-2023 fall hunter harvest in Maine.

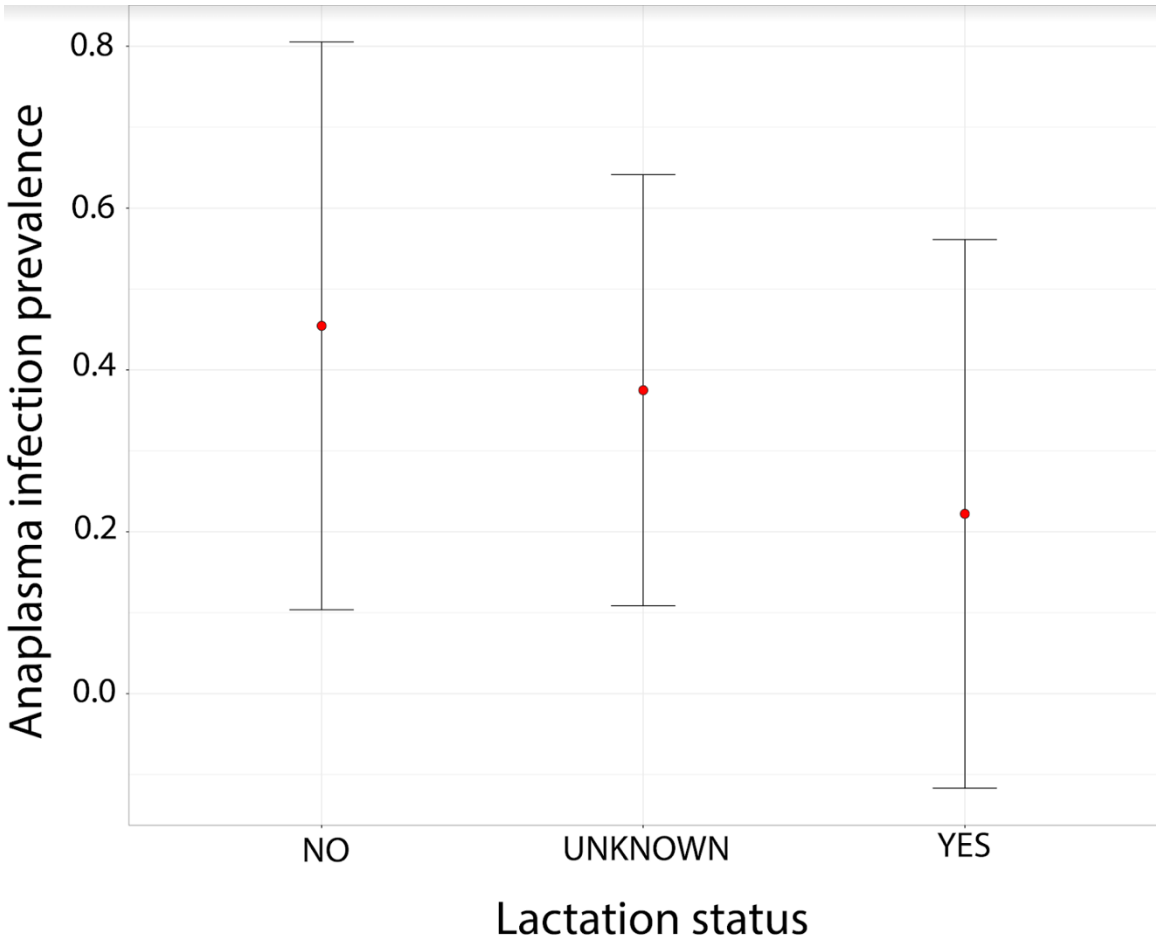

Figure 6. Graph depicting *Anaplasma* infection by lactation status in moose cows sampled during the 2021-2023 fall hunter harvest in Maine.

Appendix D: Tables Relaying Data Collection & Statistical Results

Table 1. Summary of data collection results on CL counts, age, and dressed weight for moose cows sampled during the 2021-2023 fall hunter harvest in Maine.

Variable	Average	Minimum	Maximum
Corpus Luteum (CL) (i.e., 0, 1, or 2)	1	0	2
Age (in years)	6.8	2.5	12.5
Dressed Weight (in lbs)	581	362	740

Table 2. Pearson's Chi-squared test results to determine whether CL counts differed in *Anaplasma* infected vs. uninfected individuals.

Result	Value
X-squared	1.8029
df	2
p-value	0.406

AUTHOR'S BIOGRAPHY

Isabella Costa was born in Brighton, Massachusetts and was raised in Taunton, Massachusetts. She graduated with a major in Large Animal Science at Bristol County Agricultural High School in 2020 and then went on to pursue a Bachelor of Science degree at the University of Maine Orono. While there, Isabella studied Animal & Veterinary Science with a Pre-Veterinary concentration and held many leaderships positions and was an active member in variety of clubs including: Pre-Veterinary Club, Order of Omega, Phi Kappa Phi, Sophomore Eagles, and Delta Delta Delta.

In her free time, Isabella enjoys camping, watching sunsets, going to the beach, and hanging out with her friends and family. After graduating from the University of Maine, Isabella will be attending the prestigious University of Pennsylvania where she will be attending Veterinary School with hopes of becoming a specialist veterinarian.