



## Tick development on sexually-active bull moose is more advanced compared to that of cow moose in the winter tick, *Dermacentor albipictus*

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### ABSTRACT

We performed a complete survey of ticks on 100 cm<sup>2</sup> skin samples collected from 30 moose (*Alces alces*) harvested in 2017 in central and northern Maine, U.S.A. The samples were collected from 15 bulls, 13 cows, and 2 calves in mid-October when moose are breeding and winter ticks (*Dermacentor albipictus*) quest for a host. We identified only winter ticks with 99.2% in a juvenile stage; 3 adult ticks were found. Unfed nymphs were most common on bulls, whereas most ticks were fed larvae on cows and calves. The mean total count on bull samples was 21 ± 4.4 (range = 0–55) and higher than on cows (6 ± 0.5; range = 2–8). Unlike previous surveys, tick abundance was lowest on calves. Tick abundance was independent of age or weight of adult moose. The higher abundance and more rapid development of winter ticks on adult bulls likely reflects the seasonal influence of increased movements and hormonal cycles associated with reproduction.

### 1. Introduction

The winter tick, *Dermacentor albipictus*, uses the same individual moose, *Alces alces*, to complete all blood-feeding (parasitic stages) associated with its developmental stages (larva, nymph, and adult), molts, ecdysis, and mating (Samuel, 2004). These developmental events occur with the tick lodged deeply within the dense hair-coat at the base of hair shafts at the surface of the skin. Using 13 tethered moose, Addison and McLaughlin (1988) found that 60–80% of larval ticks developed into nymphs within 14 days post-infestation during September–November; larvae are the sole infestation stage. Nymphs initiate feeding in late December–February, and after developing into adults, concentrate feeding in March–April. After mating, the engorged female drops to the ground seeking moisture-rich shelter for oviposition.

Winter tick larvae hatch ~4 weeks post-oviposition and remain inactive during summer (summer quiescence; Yoder et al., 2016). They become active during early autumn (mid-September) when they climb vegetation, quest for a host, and infest moose. Questing ends when snow cover or cold temperatures are persistent in November–December. The tick abundance on calf moose is typically higher than that

of adults, presumably because they spend more time foraging (Addison et al., 1979; Welch and Samuel, 1989; Mooring and Samuel, 1998; Sine et al., 2009; Bergeron and Pekins, 2014). Due to increased activity and movements associated with breeding in October, adult bulls typically have higher tick loads than cow moose (Samuel, 2004; Bergeron and Pekins, 2014).

In October 2017 during the middle of the larval questing period, we had the opportunity to collect and examine hide samples (skin and fur) from 30 harvested moose from central-northern Maine. The purpose of this study was to detect infestations of *D. albipictus* and to ascertain if it was the only tick species present on these moose. Further, the abundance and feeding status of parasitic stages were measured on the hide samples and compared among bulls, cows, and calves to determine possible differences between ages and sexes of moose. This is the first study to compare tick stages on bulls, cows, and calf moose as an outcome of natural tick infestation during the moose breeding season.

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## 2. Materials and methods

### 2.1. Hide collection

Skin samples were collected from 30 moose ( $n = 15$  bulls, 13 cows, 2 calves) harvested legally during the annual moose hunt in Maine; the small calf sample reflects the restricted antlerless harvest in most areas. Sampling occurred in mid-October 2017 within a 2 week period that coincided with moose breeding and larval questing by winter ticks (Samuel, 2004). Each moose received an individual tag and seal number to identify the sex, age, and harvest location (Seal#). Collection permits are held by P. J. Pekins and L. Kantar.

For consistency, a single skin sample was cut at the barrel behind the shoulder of each moose. This sampling area on the moose was selected as the best site for collection based on previous studies (Yoder et al., 2019) and was the only area approved by permits. To make comparison with previous literature on tick counts, the area of this skin sample corresponded with “section F” on the moose in Addison et al. (1979).

Each sample was placed in a freezer bag (SC Johnson, Racine, WI) and stored at 5–15 °C in either a 10 L cooler containing cold packs (Koolit; FDC Packaging, Medfield, MA) or a frost-free refrigerator (Fisher Scientific, Pittsburgh, PA). Tick counts were made ~48 h post-collection/harvest. All harvested moose were considered of normal health based on corresponding age and weight data; see Yoder et al. (2019).

Ticks are unlikely to leave the host once they have procured a host for feeding, and the one-host nature of this tick species keeps it associated with the host (Sonenshine, 1991). The hide samples were taken on the day that the moose was shot. Hunters brought the moose to harvest check stations. Researchers collected the hide samples once the moose arrived at the check station and placed the sample immediately into bags for storage in the cooler. The researchers followed a consistent collection protocol. Ticks were nestled and relatively immobile at the surface of the skin, and sequestered at, around, and between the bases of hair shafts. Few, if any were observed crawling off skins, tip ends of hairs, within storage bags, or on gloves while handling the skin samples.

### 2.2. Tick collection

Methods for collecting ticks conform to standard practice (Welch and Samuel, 1989; Sine et al., 2009; Mosallanejad et al., 2011; Bergeron and Pekins, 2014; Thomas et al., 2016). We also compared our counts with similar data collected in a previous study in Maine (Sine et al., 2009) that was the foundational research used to establish the current protocol to estimate tick abundance from transect counts on harvested moose. Sine et al. (2009) performed both a total count and counts along 4-spaced transects on 10 × 10 cm skin patches collected from harvested moose (as in this study), and found that the combined transect count, on average, equaled 42% of the total count. We used this relationship to convert our total counts to equivalent transect counts in order to compare our tick abundance data with those summarized by Dunfey-Ball (2017), who found a predictive relationship between tick abundance (transect counts from 2–10 × 10 cm patches) and occurrence of regional epizootic events in Maine. To calculate tick abundance, we multiplied the total count from our 1–10 × 10 cm patch by 0.42 (Sine et al., 2009) and doubled that number to compare with the Dunfey-Ball (2017) estimates.

Briefly, laboratory work was conducted wearing powder-free gloves (Microflex Co., Reno, NV) within a laminar flow hood that was sterilized daily (Cole-Palmer, Vernon Hills, IL). Collection of ticks from hides utilized the combined picking-float approach (Proctor, 2001), a common method in acarological studies for extracting small mites from a wide variety of substrates. Fine and soft forceps (DR Instruments, Bridgeview, IL), pipette tips (Fisher) and flea combs (H&H Pet Co., Salt

Lake City, UT) were used to collect and handle ticks. Collections were made by hand from a 10 cm × 10 cm quadrat of moose skin (sample) sectioned off with gridlines, under 10–40x light microscopy. Each sample was combed entirely through to the skin in 10, 1 cm rows, three times each. Each sample was surveyed three times by three different researchers. To collect any remaining ticks, hides were then treated by immersion in heptane (Fisher), with periodic agitation for several hours, and filtration (Whatman No. 3 filter paper, Hillsboro, OR) as described by Walter et al. (1987) and Kethley (1991). Ticks from each individual moose were placed into 6 dram glass vials of 70% ethanol (Fisher).

Identification to species and stage was based on keys by Clifford et al. (1961), Brinton et al. (1965), and Lindquist et al. (2016). Live, unfed larvae (larvae have six legs) were brown and fed larvae were whitish; the bodies were distinctly oblong. Live, unfed nymphs (nymphs have eight legs) were brown and fed nymphs were tan to light brown. Live, unfed adults (adults have eight legs) were brown and fed adults were grayish to tan.

### 2.3. Statistical analysis

Counts were expressed by animal and combined into sex and age classes for comparisons. An analysis of covariance (ANCOVA - Tukey's test;  $P = 0.05$ ) was used for these comparisons, followed by a log-it transformation in the case of percentages (JMP, SAS Institute, Cary, NC).

## 3. Results

### 3.1. Tick collection

*Dermacentor albipictus* was the only tick species identified on all skin samples. A total of 394 ticks were collected with 99.2% in the juvenile stage - 308 from bulls, 71 from cows, and 15 from calves (Table 1). Three adult ticks were collected from 2 adult bulls. All tick specimens were archived in ethanol at the Acarology Laboratory, The Ohio State University, Columbus, OH, U.S.A. (voucher numbers OSAL 0129762–0129780).

### 3.2. Tick counts

The mean total count of ticks was higher on bulls than cows ( $P < 0.05$ ; Table 1), whereas the mean total count was similar on cows and calves ( $P > 0.05$ ). The predominant life stage on bulls was unfed nymphs ( $P < 0.05$  in each pairwise comparison; Table 1). Fed larvae were the dominant stage on cows and calves ( $P < 0.05$  in each pairwise comparison). There was no linear relationship between tick abundance and age or weight of adult moose ( $R \leq 0.2$  in bulls and cows).

## 4. Discussion

Herein we have described for the first time differences in developmental stages on bulls, cows, and calves during the breeding season. This study also reveals that during this time, in terms of developmental stage, post-larvals (nonfed nymphs, fed nymphs, a few adults) dominate on bulls, whereas unfed and fed larvae dominate on cows and calves; thus, ticks feeding on cows and calves experience a developmental lag.

That juveniles (larvae and nymphs) represented > 99% of the documented ticks was not surprising given that the mid-October sampling period corresponded with that of peak larval questing (Samuel, 2004). However, the difference between the predominant life stage found on bulls (unfed nymphs) versus cows and calves (fed larvae) was unexpected. The white color and immobility of fed larvae indicated that they were actually pharate nymphs (i.e., “molters”); indeed, dissections of fed larvae revealed nymphs within. Although development to the

**Table 1**

Survey of *Dermacentor albipictus* collected from moose (Maine, U.S.A.) from 1 to 100 cm<sup>2</sup> area of skin behind front shoulder. Seal#, moose identification number; fed, (detached) or in various stages of engorgement (attached). Calf seal# 24462 is female, and calf seal# 28783 is male. Tick abundance = 2 (total count x 0.42) as indicated in **Materials and methods**, 2.2. *Tick collection*.

Seal#	Larva		Nymph		Male Nonfed	Female Fed	Total count	Tick abundance
	Unfed	Fed	Nonfed	Fed				
<b>Bulls</b>								
24336	0	0	4	2	0	0	6	5
24337	0	0	6	7	0	0	13	11
24338	1	8	19	3	0	0	31	26
24339	0	0	41	12	0	2	55	46
24340	1	2	10	1	1	0	15	13
24341	0	2	0	0	0	0	2	2
24342	0	3	3	5	0	0	11	9
24346	0	0	0	0	0	0	0	0
24347	1	4	2	0	0	0	7	6
27596	1	13	13	6	0	0	33	28
27597	0	4	5	8	0	0	17	14
28358	1	8	18	15	0	0	42	35
28734	0	6	0	3	0	0	9	8
28735	0	28	13	5	0	0	46	39
28736	3	9	2	7	0	0	21	18
<b>Mean</b>	<b>0.5</b>	<b>5.8</b>	<b>9.1</b>	<b>4.9</b>	<b>0.1</b>	<b>0.1</b>	<b>20.5</b>	<b>17.3</b>
<b>S.E.</b>	<b>0.2</b>	<b>1.9</b>	<b>2.8</b>	<b>1.1</b>	<b>0.1</b>	<b>0.1</b>	<b>4.4</b>	<b>3.7</b>
<b>Cows</b>								
24450	1	3	0	0	0	0	4	3
24463	1	6	1	0	0	0	8	7
24466	0	4	1	0	0	0	5	4
24470	0	2	0	0	0	0	2	2
24473	3	2	1	0	0	0	6	5
24475	0	1	1	2	0	0	4	3
24476	1	3	0	0	0	0	4	3
24478	1	5	1	0	0	0	7	6
24479	0	3	2	0	0	0	5	4
28775	1	6	1	0	0	0	8	7
28778	1	2	2	2	0	0	7	6
28781	1	4	1	1	0	0	7	6
28784	2	1	-	1	0	0	4	3
<b>Mean</b>	<b>0.9</b>	<b>3.2</b>	<b>0.8</b>	<b>0.5</b>	-	-	<b>5.5</b>	<b>4.5</b>
<b>S.E.</b>	<b>0.2</b>	<b>0.5</b>	<b>0.2</b>	<b>0.2</b>	-	-	<b>0.5</b>	<b>0.5</b>
<b>Calves</b>								
24462	0	9	0	0	0	0	9	8
28783	2	3	1	0	0	0	6	5
<b>Mean</b>	<b>1.0</b>	<b>6.0</b>	<b>0.5</b>	-	-	-	<b>7.5</b>	<b>6.5</b>
<b>S.E.</b>	<b>1.0</b>	<b>3.0</b>	<b>0.5</b>	-	-	-	<b>1.5</b>	<b>1.5</b>

nymphal stage on cows and calves is likely not weeks behind that on bulls, a definitive lag time was evident.

Only 3 adult ticks were documented, all originating from bulls; adult ticks are occasionally observed at harvest check stations in Maine (L. Kantar, pers. observ.). Although these could be unmated adults from the previous spring, no evidence exists that adult ticks persist on moose throughout summer. More likely, these adults were rapid developers as none were found on cows, nymphs were predominant on bulls indicating earlier development, and unfed larva can develop to adults in ~1 month in milder, warmer weather (bovine data; Osburn, 1981). The higher tick abundance on bulls than cows was similar to that reported in previous studies (Welch and Samuel, 1989; Sine et al., 2009; Bergeron and Pekins, 2014). Although our calf sample was limited (n = 2), tick abundance on calves was lower than on bulls; typically, it is the highest of all sex-age classes (Welch and Samuel, 1989; Sine et al., 2009; Bergeron and Pekins, 2014).

One possibility is that the greater abundance of ticks and later life stages on bulls could be associated with greater testosterone levels and behavioral differences between male and female moose. Testosterone level in bulls peaked 3–4 weeks prior to the collection of the hide samples (Bubenik, 1998). This seasonal increase is associated with late growth and development of antlers, eventual shedding of antler velvet

in mid-September, and heightened travel and activity associated with breeding (Bubenik, 1998) that increase the relative exposure of bulls to questing larval ticks (Drew and Samuel, 1989; Samuel, 2004). Testosterone appears to act as a phagostimulant in certain ticks, promoting attachment and subsequent development via increased blood feeding (Hughes and Randolph, 2001). The advanced development of larvae to the nymphal stage on bulls presumably reflects this phagostimulant influence. Differences in grooming behavior may also partly account for differences in tick abundance (Samuel, 1991; Mooring and Samuel, 1999). Another potential factor to consider may be differences between bulls and cows in effective immune responses to ticks due to testosterone levels (Hughes and Randolph, 2001). It is unlikely that this earlier development has any influence on survival of adult bulls that rarely experience mortality associated with winter ticks in Maine (Jones et al., 2018) or elsewhere (Samuel, 2004).

As with numerous studies in North America, we found only *Dermacentor albipictus* on moose in Maine (Hoeve et al., 1988; Welch and Samuel, 1989; Samuel, 2004; Sine et al., 2009). However, moose can host other tick species, namely *Ixodes*. For example, *I. ricinus* and *D. albipictus* readily infest moose in Norway (Kjelland et al., 2011), and annual surveys at moose harvest check stations in Maine and adjacent New Hampshire have documented *I. scapularis* on moose (author observations). Relative to co-occurrence, *D. albipictus* is commonly found with *I. scapularis* on white-tailed deer, *Odocoileus virginianus* (Cortinas and Kitron, 2006; Baer-Lehman et al., 2012), a sympatric cervid along the southern range border of moose in North America, including Maine. However, *I. scapularis* typically exists at low frequency in most moose habitat because deer density is usually low-moderate and environmental conditions are more severe than where *I. scapularis* is most common. Further, *I. scapularis* prefers a moisture-rich, humid and milder environment, whereas *D. albipictus* is more xerotolerant and cold-tolerant (Yoder and Spielman, 1992; Holmes et al., 2018).

**Conflicts of interest**

We have no conflict of interest.

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