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The winter tick, Dermacentor albipictus (Packard, 1869): its life history, development at constant temperatures and physiological effects on moose, <u>Alces alces</u> L.

University — Université

University of Alberta

Degree for which thesis was presented - Grade pour lequel cette thèse fut présentée

Sc

1983

Year this degree conferred - Année d'obtention de ce grade

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### THE UNIVERSITY OF ALBERTA

THE WINTER TICK, DERMACENTOR ALBIPICTUS (PACKARD, 1869): ITS LIFE HISTORY, DEVELOPMENT AT CONSTANT TEMPERATURES AND PHYSIOLOGICAL EFFECTS ON MOOSE, ALCES ALCES L.

> by MARGARETTA VICTORIA GLINES

## A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE

DEPARTMENT OF ZOOLOGY

EDMONTON, ALBERTA Spring, 1983 THE UNIVERSITY OF ALBERTA RELEASE FORM

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TITLE OF THESIS

THE WINTER TICK, DERMACENTOR ALBIPICTUS (PACKARD, 1869): ITS LIFE HISTORY, DEVELOPMENT AT CONSTANT TEMPERATURES AND PHYSIOLOGICAL EFFECTS ON MOOSE, ALCES ALCES L.

DEGREE FOR WHICH THESIS WAS PRESENTED MASTER OF SCIENCE YEAR THIS DEGREE GRANTED Spring, 1983

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Date 15 Febru

ABSTRACT

Controversy in the literature and among wildlife managers over importance of the inter tick, *Dermacentor albipictus*, as a mortality factor of moose, *Alces alces*, led me to investigate the life histor development at constant temperatures and physiological effects of winter ticks on moose.

Field investigations were conduct into the life history of the winter tick. Examinatic sector modes hides collected in Elk Island National Park (E.I.N.P.) Alberta during autumn, winter and spring of 1979-80 and 1980-81 showed that larval stages of this one-host tick were present on moose from September to December, but nymphs predominated as early as October. Most nymphs went into diapause and did not take blood from the host until January-February. Unengonged adujts began to appear in February. Females: engorged and dropped off from late March through early May and no ticks were found on hides collected in late May-June. Aerial and ground surveys of moose in E.I.N.P. during winter and spring of 1980 revealed that mobse lost hair during the period engorged nymphs and females were found on the hides.

Development during nonparasitic portions of the life cycle was investigated by incubating engorged femate ticks at 19°, 25° and 30°C (>85% RH) and monitoring reproductive output, preoviposition and egg incubation periods. Total number of eggs produced per female correlated with engonged weight of the female at 19° and 25° but not at 30°C. Maximum reproductive efficiency (no. eggs/g of female) was at 19°C. A large negative correlation between development time and temperature was found for both preoviposition and egg incubation periods. The lower threshold of oviposition was calculated as 15:1°C. Having maximum reproduction at temperatures near the lower threshold of oviposition is likely an adaptation to cold climates.

Physiological effects of D. albipictus on moose were investigated by monitoring blood composition, weight gain, food intake and change in the hair coat of 4 moose calves experimentally infested with ticks and 8 uninfested controls. One tick-infested moose died early in the experiment. All surviving animals lost much hair because of the intense grooming they did in response to irritation from engorging nymphal and female ticks. Control moose did not lose hair. The infested animal with the least amount of hair loss suffered weight loss, anemia, hypoalbumenemia, hypophosphatemia and transient decreases in serum aspartate transaminase and calcium during the period of nymphal and adult tick engorgement. The animal with intermediate hair loss suffered a mild anemia during the peak time of nymphal engorgement. The animal with the most severe hair loss did not suffer anemia but became very weak and was killed. Post-mortem examination revealed the possibility of immune

complex disease. The animal with the least hair loss did not exhibit increased gamma globulin but the two moose with more severe hair loss had increases in gamma globulin shortly after onset of female tick engorgement. Infested animals did not become anorexic.

Results suggest that the extent of hair loss is related to host resistance to the tick. Moose that groom and develop hair loss likely carry fewer ticks and suffer less severe blood loss:

#### ACKNOWLEDGEMENTS

This research was possible through the assistance, cooperation and inspiration of many people. No matter how small the part, each person played an important role in shaping the outcome of this research. I would like to express my sincere gratitude to the following:

all members of my supervisory committee, John Holmes, Bill Kaufman and Jan Murie, for their invaluable advice and encouragement throughout this project;

personnel of Elk Island National Park, particularly Fred Bamber, Superintendant, and Jack Willman, Chief Park Warden, for their cooperation and help in the collection of moose hides;

all personnel of Alberta Fish and Wildlife Division who provided me with orphan moose calves, Brian Lajeunesse who provided the technical expertise and assistance to capture moose calves by helicopter and, particularly, Bill Wishart for his interest and encouragement;

all technicians who helped raise moose calves and/or worked in the laboratory - Eric Grinnell, Michelle Anholt, David Lawley, Lorne Duncan and Brent Gray;

the Bioscience Animal Service technicians who looked after the moose once they were weaned, particularly Keith Taylor for his considerate care and handling of the animals which kept them tractable and for his assistance during the bleeding procedure;

the parasitologists on the nineth floor, particularly John Holmes, for challenging discussions (and arguments).

I am extremely grateful to my supervisor W.M. Samuel for his guidance, encouragement, advice on manuscripts and financial support to attend scientific conferences. Without Bill's enthusiasm and support this project would never have left the ground.

Last but certainly not least, I thank my husband Dave for his understanding, patience and moral support throughout.

This research was funded by the Alberta Fish and Wildlife Division, the Canadian National Sportsmen's Fund, the Boreal Institute for Northern Studies and the Alberta Recreation, Parks and Wildlife Foundation. I was supported by a Natural Sciences and Engineering Research Council postgraduate scholarship and a University of Alberta teaching assistantship.

viii

	· · ·		Table of (	Contents			
	Chapter	· .	84		•	Page	. :
		DUCTION		· · · · · · · · · · · · · · · · · · ·		1	•
	L	iterature (	Cited	· · · · · · · · · · · · · · · · · · ·		5	•
•	ALBIPI	CTUS, AND	ITS EFFECT	ER TICK DE ON THE HAIR RAL ALBERTA	COAT OF	9	
. •	AŁ	ostract	, , , , , , , , , , , , , , , , , , ,	•••••		9´	1-
. 3	Ir	itroduction	1	••••	•••••	10	•
•	Ма	terials an	d Methods	• • • • • • • • • • • •		,11	
	Re	sults	· · · · · · · · · · · · · · · ·	•••••		13	· ·
•	Di	scussion		· · · · · · · · · · · ·	•••••	15	,
	Li	terature C	ited	•••••	••••	27	4
•	INCUBA	TION PERIO	DS OF DERMA	, PREOVIPOS <i>CENTOR ALBI</i> ERATURES	PICTUS	31	•
- -	. Ab	stract	••••••			31	•
а ,	In	troduction	•••••	••••	• • • • • • • • • • •	32	-
	Ma	terials an	d Methods			33	
	Re	sults	• • • • • • • • • • • • •	• • • • • • • • • • • •	· · · · · · · · · · · · ·	35	7
	Di	scussion	• • • • • • • • • • •	•••••	· · · · · · · · · · · · ·	36	
1. A. 1.		•		• • • • • • • • • • •		48	
•	(ACARI	NA:IXODIDA	RMACENTOR AL E) ON BLOOD AT OF THE MO	L <i>BIPICTUS</i> COMPOSITION DOSE, <i>ALCES</i>	N, WEIGHT ALCES	54	
¢ .	Ab	stract	•••••	• • • • • • • • • • • •		54	
•	In	troduction	••••••				
	Ma	terials and	d Methods	•••••••		57	
	Re	sults	• • • • • • • • • • • •	· • • • • • • • • • • • • •	•••••	62	
•	Di	scussion	•••••••••		• • • • • • • • • • • • •	67	
			ix	•	•		3

	Tables Page
	Chapter II.
•	1. Progression of hair loss on moose in Elk Island National Park during 1980
	2. Location of hair loss on moose in Elk Island National Park during 1 January to 30 May, 1980 and 1981
•	3. Preference of <i>D. albipictus</i> for different parts of the body
•	<ol> <li>Body regions containing the greatest proportion of the most advanced or least advanced instar of <i>D. albipictus</i></li></ol>
	Chapter III.
	<ol> <li>Preovipositional weights of D. albipictus females, their egg production, reproductive efficiency index and survival of eggs at three constant temperatures</li></ol>
	2. Preovipositional period and time from first egg to first larva at three constant temperatures
	3. Reproductive efficiency index of several ixodid ticks
5	4. Development of <i>D. albipictus</i> at constant temperatures
(	Chapter IV >
· · · ·	1. Development of <i>D. albipictus</i> on experimental moosé
	2. Food consumption and weight gains of control and infested moose calves
¥.	3. Food consumption and weight gains of control and infested meose during two periods of the experiment
	4. Reference ranges for hematology and serum chemistry of moose
•	
	xi

		*	
			· .
		14,5	
		•	н. 
	List of Figures		
Figu	re a statistic statistic statistics and statistics and statistics and statistics and statistics and statistics	Page	
-			
Chap	ter II		· · ·
	None bide divided into seven newions	0.4	
1.	Moose hide divided into seven regions	· · · · · · · · · · <sub>*</sub> · · · 24	· · · · ·
2.	Sequence of life stages of D.		
	albipictus on moose of central		•
1. · ·	Alberta		
•			
Chap	ter, IV		
4			• •
	Experimentally tick-infested moose		
· · · · · ·	calf (Mo 35) on 26 February showing very severe hair loss		6
. 2.	Progression of hair loss on moose		• • •
	calves experimentally infested with		2
•	D. albipictus		•
· · · · · · · · · · · · · · · · · · ·	The union to the set of the fore the start		· ·
J.	The weight of tick-infested, pair-fed and control moose calves		•
	before and during the experiment		с с
· · ·		••••••	
<b>4</b> .	Hematology of three moose calves		
· · · · · ·	experimentally infested with D.		•
•	albipictus		
· · E	Total communication allowers and		•
5.	Total serum protein, albumen and albumen/globulin ratio of three		
	moose infested with <i>D. albipictus</i>		
6.	Serum globulin values of three moose		•
	experimentally infested with		
	D. albipictus		
7	Serum chemistry values for three	•	
	moose calves experimentally infested		
•	with D. albipictus		
		-	
		н н. 1 - А	•
		• · · · ·	•
			•
			÷
			••
:			•
• • •			
ىت , <u>د</u> بە			•
•			
	xii		•
			•

## I. INTRODUCTION

The winter tick, *Dermacentor albipictus*(Packard), is a one-host tick that primarily infests wild ungulates, horses and cattle (Bishopp and Wood, 1913; Cooley, 1938). It is found across southern Canada and throughout most of the continental United States (except Alaska) (Cooley, 1938; Bishopp and Trembley, 1945; review of Anderson and Lankester, 1974). As its name implies, this tick infests its host primarily during the winter months. Larvae ascend the vegetation and acquire a host in autumn. Larval and nymphal moults occur on the host and engorged adults drop off in the spring. Engorged females then lay eggs on the ground (Bishopp and Wood, 1913).

Many authors feel this tick is a serious parasite of moose (*Alces alces*), wapiti (*Cervus elaphus*), mule deer (*Odocoileus hemionus*) and free ranging horses and cattle in North America (Bishopp and Wood, 1913; Cameron and Fulton, 1926-27; Bruce, 1927; Fenstermacher and Jellison, 1933; Cowan, 1951; Peterson, 1955; Stelfox, 1962; Berg, 1975). In Alberta, epizootics of winter ticks in Elk Island National Park have been associated with deaths of wapiti during the winter of 1936-37 (Love, 1955) and moose during the winters of 1976-77 (Samuel and Barker, 1979), 1980-81 and 1981-82 (Drew and Samuel unpub.). Elsewhere in the province, deaths of moose, heavily infested with winter ticks, were reported

during the winters of 1957-58 and 1958-59 (Webb, 1959). In northern Alberta, the hunting season for moose was closed during the years 1950, 1951, 1953 and 1954 "in response to unusually heavy tick infestations on moose and alleged overharvest by timber wolves, (*Canis lupus*)" (Lynch, 1973).

2

Interest in this tick reached its zenith in the early 1930's when reports implicated the winter tick in the transmission of neurologic disease of moose (Thomas and Cahn, 1932; Wallace et al., 1933). However, it became apparent that this tick neither caused nor transmitted the disease, and interest in the tick declined. Recent epizootics of winter ticks in Alberta (Samuel and Barker, 1979) and Ontario (Addison and Smith, 1981), associated with deaths of moose, have again given rise to interest in the effects of winter ticks on moose and possible management techniques to reduce tick populations.

There is debate in the literature as to the effects these ticks have on moose. Several authors (Fenstermacher and Jellison, 1933; Wallace, 1934; Cowan, 1951; Peterson, 1955; Webb, 1959; Berg, 1975; Addison et al., 1979; Samuel and Barker, 1979) report that in late winter, moose, heavily infested with winter ticks, often exhibit hair loss. These authors feel that the hair loss is related to the presence of the tick. Others feel hair loss is simply the spring moult and the presence of ticks is coincidental (Ritcey and Edwards, 1958; Peterson, 1977). While many feel this tick is a serious pest of moose, others feel it only causes mortality in conjunction with other factors such as disease or malnutrition (Ritcey and Edwards, 1958; Stelfox, 1962; Berg, 1975).

To help resolve the controversy in the literature and among wildlife managers over the importance of the winter tick in the management of moose populations, an investigation into the physiological effects  $\partial f D$ . albipictus on moose was necessary. However, the controversy was fueled by a general lack of information on the basic life history and population dynamics of this tick in northern climates. Therefore, before experiments on the physiological effects of these ticks were started, I began collecting information on the life history and population dynamics of the winter tick in central Alberta. This information would later allow me to compare the results of the physiological experiment to the situation observed in nature. The life history of D. albipictus on moose was determined by sampling the hides of freshly killed moose and then identifying and counting the ticks on these samples. Once the life history was known, it could then be compared with the timing of the hair loss exhibited by moose in the spring. The hypothesis that the hair loss occurred when the ticks were engorging could be tested.

The life history and population dynamics were further investigated by examining the effects of constant temperatures on the reproductive efficiency, preoviposition and egg incubation periods of *D. albipictus* while off the

host. Populations from different parts of a species' range often show different developmental patterns in response to temperature (Campbell et al., 1974). The information from this experiment allowed a comparison of the developmental patterns of *D*. *albipictus* from central Alberta with the patterns reported in the literature for other populations.

Moose calves experimentally infested with ticks were used to study the physiological effects of winter ticks on moose. Other ectoparasites have been shown to cause anemia, anorexia, weight loss and biochemical changes in the host (see review by Nelson et al., 1977). Therefore, this experiment was designed to test whether *D. albipictus* also caused these changes in moose and if anorexia occurred, to separate the anorectic effects from the specific effects of the tick.

This thesis is arranged in the form of three separate papers. The first deals with the development of *D. albipictus* on wild moose in Elk Island National Park, Alberta, as determined by examination of moose hides. In it I discuss the hair loss observed on moose in relation to development of the tick.

The second paper is concerned with the effect of three constant temperatures on the reproduction and development of female winter ticks and eggs. I discuss how spring and summer weather can affect the population dynamics of the tick.

In the last paper I investigate the effects of winter tick infestation on the weight gains, hair coat and blood composition of experimentally infested moose calves. In the final discussion that follows I examine the factors affecting the population dynamics of the winter tick and the possible management implications of this research.

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### ABSTRACT

The relationship between the life history of the wintertick, Dermacentor albipictus, and late winter alopecia observed in moose, Alces alces, was investigated. Examination of one moose hide collected in the Swan Hills and 27 hides collected in Elk Island National Park (E.I.N.P.), Alberta during September-June, 1979-80 and 1980-81, revealed that larval ticks were present on moose in late September but unengorged nymphs predominated the tick population as early as October. Most nymphs remained unengorged until after January. Engorged nymphs were most prevalent in the tick population of hides collected in March and April. Unengorged adults became numerous during February-March but engorged females were not found until April-May. No ticks were found on hides collected in late May or June.

The highest densities of ticks were found on the neck, hump and shoulders of the hides while advanced instars were found significantly more often than expected on the neck, hump, shoulder; chest and belly. Aerial and ground surveys of moose in E.I.N.P. revealed that moose developed

alopecia during the same period engorged nymphs and female ticks were present on the hides. In addition, hair loss was seen on those portions of the body where high densities of ticks were found.

Observations of hair loss on three moose calves experimentally infested with *D. albipictus* and the normal moult of the winter hair coat on eight uninfested calves confirmed that late winter alopecia in moose was a reaction to the presence of engorging nymphal and female winter ticks.

## INTRODUCTION

The winter tick, *Dermacentor albipictus*, is a one-host tick that is found on its host during the autumn, winter and spring (Bishopp and Wood, 1913). In Canada, moose (*Alces alces*) are the major host for winter ticks. During epizootics of *D. albipictus*, there may be averages of 30,000-40,000 ticks/moose, with some individuals having over 100,000 ticks (Samuel and Barker, 1979, and unpub.).

Many authors have noted that moose, heavily infested with *D. albipictus*, often have hair loss in late winter (Fenstermacher and Jellison, 1933; Wallace, 1934; Peterson, 1955; Webb, 1959; Berg, 1975; Addison et al., 1979; Samuel and Barker, 1979) They suggest the hair loss is related to the presence of this tick; however, others (Ritcey and Edwards, 1958; Peterson, 1977) feel the hair loss is simply the spring moult and the presence of ticks is coincidental.

Although several authors have reported the seasonal occurrence of *D. albipictus* (Bishopp and Wood, 1913; Howell, 1939; Drummond, 1967; Samuel and Barker, 1979), there has never been a detailed study of its life history on moose nor the role this tick may play in the hair loss. The present study was designed to determine the "life history of the winter tick on moose in central Alberta and to discover what relationship exists between *D. albipictus* and the hair loss on moose in late winter.

## MATERIALS AND METHODS

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Twenty-seven moose hides were collected from Elk Island National Park (E.I.N.P.), during the autumn, winter and spring of 1979-80 and 1980-81 and one hide (September, 1979) from the Swan Hills (Appendix I). Hides were placed at -20°C within four hours of collection and remained frozen until examined. Twenty-seven hides were scored with a scalpel into 100 cm<sup>2</sup> squares, then divided into regions. (The hide collected in June, 1980 was examined grossly, had no ticks and was not scored for further examination.) The first five hides were divided into four, approximately equal sections while the rest were divided into eight regions: head (1981 hides only), neck-hump, anterior left, anterior right, back, anus, posterior left, and posterior right (see figure 1)..

Approximately 10% of the squares in each region were chosen, using a random number table, and sampled; the location of each sample within the region was recorded. The samples were digested in 5% KOH at 90°C until the hair and skin had dissolved (1-2 hours): The digest was passed through a #80 (180 um) sieve. The remains in the sieve were washed into a white enamel pan and examined under an illuminated magnifying (2X) lens. Ticks were extracted, identified and counted. The number of ticks sampled in each region of the hide was multiplied by a factor dependent on the proportion of the region sampled. These population estimates within regions were summed to estimate the total tick population on the hide.

The samples from each of 19 hides with little (<10%) or no hair loss were ranked from the sample with the greatest density of ticks to the sample with the lowest density. The locations of the three most heavily and three least heavily infested samples from each hide were recorded. For each region of the body, the null hypothesis that a sample had equal probabilities of being one of the most densely or least densely parasitized samples was tested using a G test with Williams correction for the two cell case ( $n\geq 25$ ) or a two-tailed exact test (n<25) (Sokal and Rohlf, 1981).

The same tests were performed to determine if samples had equal probabilities of having high or low proportions of advanced instars. The three samples from each hide containing the greatest proportion of the most advanced

instar of tick and the three containing the greatest proportion of least advanced instar on the hide were used.

To determine whether the hair loss was related to the presence of this tick, four hand-raised moose calves were each infested with approximately 31,000 *D. albipictus* larvae in October 1980. At regular intervals between January and May 1981, 50-100 ticks along the neck, hump and shoulder of each moose were identified to instar. Eight uninfested moose calves served as controls.

The relationship between hair loss and tick development was investigated further by comparing development data from the hides with surveys of hair loss in the moose population at E.I.N.P. Observations of moose were made from the ground as well as from a helicopter during the winter and spring of 1980: A diagram was made of each moose showing the extent of the hair loss.

## RESULTS

The development of *Dermacentor albipictus* on moose included larval and adult stages of short duration and a nymphal stage of long duration (Figure 2 and Appendix I). Larval *D. albipictus* were found on moose from late September (when moose were first collected) to November. They engorged and moulted into nymphs which predominated the population from October until the end of February. Most of the nymphs remained unengorged and, apparently, in diapause until

January, when a few nymphs began to engorge. Peak engorgement occurred in March or April. A very small proportion of engorged nymphs were found on moose in the autumn. Although adult females (less than 1% of the population) and males were found in very small numbers as early as September, they began to appear in substantial numbers during February. The males accumulated on the host and reached peak proportions in early May. In contrast, females accumulated during February and March but engorged and dropped off from late March to early May.

One of the four moose calves infested with D. albipictus died (as the result of an accident) early in the experiment but the remaining three calves exhibited various degrees of premature winter hair loss. Hair loss began in January and ranged from moderate (on neck and shoulders) to very severe (including neck, hump, sides, shoulders, back and perianal region) by May (see figure 2, Chapter IV). Hair loss was primarily the result of extensive grooming and rubbing (details to be published elsewhere) by the moose which resulted in the hair being broken to a length of approximately 25 mm. In some regions (hump and shoulder) the hair fell out or could be easily pulled out by the roots. Control animals groomed very little or not at all and showed no hair loss of this nature. Shedding of winter hair was clearly distinguishable, in time of occurrence and sequence, from tick-related alopecia. Control animals began the spring moult in late April-early May.

The progression of hair loss on the experimental animals was associated with the development of *D. albipictus.* Hair loss was first seen when nymphs began engorging and became more extensive as a greater number of nymphs and, later, females engorged (see figure 2 and table I, Chapter IV). The progression and locations of alopecia on experimental calves (figure 2, Chapter IV) were similar to patterns observed on wild moose (Tables I and II).

Highest densities of ticks occurred significantly more often than expected on the neck, hump and shoulder (p<0.001) and less often than expected on the back and face (p<0.025) of the hides examined (table III). In addition, greater proportions of advanced instars occurred on the neck, hump, shoulder, chest and belly (p<0.025) and greater proportions of immature instars on the legs and sides of the hides (p<0.001) (table IV).

## DISCUSSION

The timing of development for *D. albipictus* described here differs very little from the predictions of Samuel and Barker (1979). They speculated that moose became infested in October, but it is now apparent that moose can become infested at least as early as September. This fits well with the reports of Wilkinson (1967) that larvae ascend the vegetation sometime before September 13 and as late as October 3-6, and Addison et al. (1979) who found ticks on moose of Ontario as early as September.

An unusual feature of the present study was the presence of a few engorged nymphs and unengorged adults in September-November. *D. albipictus* can take as little as 21 days to complete the parasitic phases of its life cycle (Drummond et al., 1969), but in Alberta the nymphs usually go into diapause from September to January-February and adults appear in February-March (Samuel and Barker, 1979, and this study). Possibly moose acquired some larvae in early September that developed directly into adults without a nymphal diapause. However, some nymphs and adults, which were removed by the host during grooming the previous spring, may have survived the summer to acquire another host in the autumn. No engorged females were ever found before March, indicating that early adults either died or waited until spring to engorge.

The timing of the life cycle in Alberta is different from that described for the winter tick in the southern and western parts of its range where the nymphs do not go into diapause. In California, *D. albipictus* can complete at least two generations each winter (Howell, 1939), whereas in Texas (Drummond, 1967) and Vancouver Island, British Columbia (Cowan, 1946) it completes one generation, with adults prominent in December and the animals free of ticks in March.

A nymphal diapause appears to be a successful strategy for northern one-host winter ticks. In the Soviet Union, the nymphs of Hyalomma scupense also go into diapause from November to February (Pomerantzev, 1959). Perhaps the success of this strategy is due to sub-arctic animals being on their lowest plane of nutrition in late winter and spring when food is less available. Gladney et al. (1973) found that steers on low protein diets consistently allowed more *Boophilus annulatus* females to engorge than steers on high protein diets. In addition to this, steers on low protein diets groomed less vigorously than steers on high protein diets. It may be advantageous then for nymphs of one-host ticks in northern climates to feed in late winter when the host is on a low nutritional plane. This may ensure greater success for the nymphs and, later, the adults.

There may be another reason for *D. albipictus* nymphs to go into diapause. Alopecia on experimental animals was first noted when nymphs were beginning to feed and became more extensive as more nymphs and, later, females engorged. This was also seen in the wild. By comparing the 1980 hair loss surveys (table I) with the tick development data from the 1980 hides (figure 2) one sees that a few moose had hair loss in January-February when the nymphs were beginning to engorge. As greater proportions of engorged nymphs were found in the tick population, more moose showed hair loss. In April-May, the adult ticks were engorging and 95% of the moose observed had hair loss. It is evident that alopecia is associated with nymphal and adult engorgement. Thus, a nymphal diapause ensures that the moose suffer hair loss in

late rather than early winter. This could be significant because an animal with alopecia in early winter might not survive to spring, when the adult ticks must feed.

The association between alopecia and nymphal and adult engorgement was also evident in the pattern of hair loss on moose. I found the highest tick densities on the neck, hump and shoulders of the hides examined. Also, I found the most advanced instars on the neck, hump, shoulder, chest and belly. This distribution of ticks coincided with the locations of the hair loss on the moose observed in the park (table II).

Tick-related alopecia is not common but has been reported for humans infested with *Dermacentor variabilis* (Ross and Friede, 1955), cattle infested with *Boophilus microplus* (Riek, 1956; Qorrier et al., 1979), and *B. calcaratus* (Antipin et al., 1960) and sheep infested with *Hyalomma transjens* (Nietz, 1959). Premature loss of winter hair must affect the thermoregulatory capacity of moose. This problem could become severe in late winter when food is less available. However, the extensive grooming that causes the hair loss also gets rid of many ticks (Snowball, 1956). In years of high tick populations, the stress associated with the hair loss may be less than the stress associated with blood loss to the ticks.

TABLE I. Progression of hair loss on moose in Elk Island National Park during 1980.

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hair lost	Jan.	Feb.	Mar.	early April	late April- early May	
	(n=23)	(n=99)	(n=99)	(n=22)	(n=21)	
little (<10%) or none	78	84	61	9	5	
some (10-20%)	17	15	38	91	52	
moderate (30 40%)	-4	1	1	0	33	
severe (40-80%)	0	0	0	0	10	
total percent of moose observed.					<u> </u>	
with >10% hair loss	22	16	39	91	95	

TABLE II. Location of hair loss on moose in Elk Island National Park during 1 January to 30 May 1980 and 81 as determined by aerial survey and examination of hides. Shown is the percent of the alopecic moose observed with hair loss in various regions of the body.

region of body	percent						
	Jan.	Feb.	Mar.	Apr.	May	ar L	
	(n=9)	(n=19)	(n=41)	(n=41)	(n=10)		
shoulder	89	74	68	85	100		
neck	11	32	56	56	80		
hump	44	. 10 •	20	29	80		
anus and genitals	11	Q	7	37	70		
chest and belly	0	10	15	5	30 <sup>-</sup>	•	
ear	0	0	0	7	30	•	
side	0	0	12	20	20	•	
back	11	0	10	17	10 -		
rump	0	0	2	7	0	• •	
nead	0	0	0	10	Q		
lank	0	0	2	0	0		
leg	0	° 0	Ó	0	0		
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TABLE III. Preference of <u>D</u>. <u>albipictus</u> for different parts of the body (based on 19 moose hides with little (<10%) or no hair loss).

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region of the body		of samples l least infested	G <sub>adj.</sub> 1 df.	exact test (p)
neck, hump, shoulder	21 .	. 4	12.425***	
chest and belly	7	9		0.8036
back	2 '	11		0.0224**
face	0	8 5	• .	0.0080**
leg	8	15	-	0.2100
anus and genitals	10	3		0.0922
side	7	16		0.0932
rump and flank	3	9	•	0.1460
(hump and shoulder)	. 8	1		0.0390*
		$\delta = \frac{1}{2} \lambda_{\rm eff} + $	4 .	

\*significant at 0.05 level

\*\* significant at 0.025 level

\*\*\* significant at 0.001 level

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TABLE IV. Body regions containing the greatest proportion of the most advanced or least advanced instar of <u>D</u>. <u>albipictus</u> (based on 19 moose hides with little (<10%) or no hair loss).

region of body	number of most advanced le instar		G adj. 1 df.	exact test (p)	
hump and shoulder	13	3		0.0212**	•••
head	10	8		0.8144	
anus, genitals rump and flank	6	12		0.2378	
chest and belly	20	6	7.803**		
neck	14	3	$\odot$ ,	<b>`0.0127**</b>	-
back	4	9		0.2668	
leg	5	36	26.114***	· · · · · · · · · · · · · · · · · · ·	
side	4	25	21.053***		•

\* significant at 0.05 level
\*\* significant at 0.025 level
\*\*\* significant at 0.005 level
Figure 1. Moose hide divided into seven regions: (ar) anterior right,

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(nh) neck-hump, (al) anterior left, (pr) posterior right, (bk) back,

(pl) posterior left and (a) anal region.

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# III. THE REPRODUCTIVE EFFICIENCY, PREOVIPOSITION AND INCUBATION PERIODS OF DERMACENTOR ALBIPICTUS HELD AT THREE CONSTANT TEMPERATURES

### ABSTRACT

Reproductive efficiency, survival of eggs, preoviposition and egg incubation periods of the winter tick, Dermacentor albipictus (Packard) of central Alberta, Canada were studied at three constant temperatures: 19°, 25° and 30°C at > 85% RH. The reproductive efficiency index (no. eggs/g female) increased with decreasing temperature. The total number of eggs produced per female was positively correlated to the engorged weight of the female at 19° and 25° but not at 30°C. Both preoviposition and egg incubation periods were negatively correlated to temperature. The linear regression of development rate on temperature was used to estimate a lower threshold of oviposition of 15.1°C. The average percent survival of eggs (no. larvae/total no. of eggs/female) was 93.6% at 19° and 97.0% at 30° but was not measured at 25°C. The high reproductive efficiency of these ticks at low temperatures probably reflects the northern latitude from which this tick population was taken.

## INTRODUCTION

The winter tick, Dermacentor albipictus, can be a serious parasite of moose (Alces alces), wapiti (Cervus elaphus) and free-ranging horses and cattle in North America (Cameron and Fulton, 1926-27; Fenstermacher and Jellison, 1933; Love, 1955; Peterson, 1955; Berg, 1975; Samuel and Barker, 1979). Recent epizootics of the winter tick in Alberta (Samuel and Barker, 1979) and Ontario (Addison and Smith, 1981) have been associated with the deaths of moose. These epizootics have drawn the attention of wildlife manager's to the importance of knowing the population dynamics of *D. albipictus* and developing management techniques to control these ticks.

D. albipictus is a one-host species in which oviposition and egg incubation occur on the ground during the spring and summer. A knowledge of how temperature affects the development and reproductive efficiency of D. albipictus, while off the host, is essential to understanding the population dynamics of this tick. This is particularly true in the higher latitudes where the daily temperatures during the relatively short summer may be the critical factor determining the success of oviposition and the proportion of eggs that hatch. Few studies have examined the effects of temperature on the development of D. albipictus. Howell (1939) and Drummond et al. (1969a) examined the effects of constant temperature on the development of winter ticks from southern parts of the tick's range (California and Texas, respectively), while Wilkinson' (1967) and Addison and Smith (1981) looked at more northern tick populations (British Columbia and Ontario, respectively). However, populations in different parts of a species range can show different developmental patterns in response to temperature (Campbell et al., 1974). Therefore, developmental patterns reported in the literature may not hold true for local populations.

I was able to examine the effects of temperature on the development and reproductive efficiency of winter ticks from a northern population and to compare the developmental pattern with that of winter ticks in other geographic locations. These ticks, collected in central Alberta, were from a more northern population than any studied previously.

MATERIALS AND METHODS

In the spring of 1980, unengorged and engorged female D. albipictus were removed from hides of freshly-killed moose, collected in Elk Island National Park, Alberta. Unengorged females were allowed to engorge on rabbits (Oryctolagus cuniculus). These, and the almost fully-engorged females taken directly from the moose, were maintained in the laboratory, allowed to oviposit and the larvae were used to infest tame moose calves in autumn, 1980. The calves were housed outdoors and the ticks completed their life cycle as they<sup>32</sup> would in the wild. From 26 March- 2 April, which appeared to be during the peak of female tick drop-off, the pens were inspected daily and all engorged females that were on top of the straw bedding were removed. The daily temperature during this time did not exceed 10°C and the females were inactive when collected. I assumed that because the females were inactive, those found on top of the straw bedding had dropped from the moose during the previous 24 hours.

34

The engorged females were brought to the laboratory, carefully washed, dried and weighed on a Mettler analytical balance to the nearest 0.1 mg. They were then housed individually in cloth-covered glass jars and incubated over water (>85% RH) at 19° (n=25), 25° (n=27) and 30° C ( $\pm$ 1° C)(n=26). The ticks at 19° and 25° C were kept under a 12L:12D diel cycle while those at 30°C were kept in constant darkness. The desiccators containing the glass jars housing the ticks were covered with cotton cloth; this minimized the intensity of the light reaching the ticks.

The ticks were examined daily. Oviposetion and larval eclosion were recorded. Females were allowed to oviposit and the larvae to hatch undisturbed.

Live larvae and unhatched eggs were counted in September. This was done by brushing clumps of larvae onto white filter paper in a petri dish, then sucking them gently into a small glass jar attached to a vacuum. Larvae were counted in group's of five as they were sucked into the bottle. This allowed an accurate count of the larvae, without damaging them. These larvae were then used for experimental infestations.

The relationship between temperature and development during preoviposition of *D. albipictus* was estimated using a linear function (Campbell et al., 1974). Development rates were obtained by taking the reciprocals of the developmental period (in days). The equation Rt=a+bT, where a and b are empirical constants calculated by the least sum of squares method, Rt is the development rate and T is the temperature in °C, was used to estimate the lower threshold temperature t=-a/b. The developmental period k, expressed as the number of degree days above t required to complete development, was calculated as the reciprocal of the slope b. Campbell et al. (1974) discuss the precision of these estimates: A t-test was used to test whether the skewness and kurtosis of data deviated significantly from 0 (normality) (Sokal and Rohlf, 1969).

### RESULTS

The average weight of 111 engorged female D. albipictus, dropped from moose, was 0.609 g (SD=0.139). The weights of females and the number of eggs laid by each, were distributed normally at all three temperatures. The number ς

of eggs laid was positively correlated with the weight of the female at 19° and 25°C but not at 30°C. The survival of eggs and larvae was high at both 19° and 30° but was not measured at 25°C. The reproductive efficiency index (R.E.I.=no. eggs/g female) was highest at 19°C and lowest at 30°C (table I).

Preoviposition period, in days, was distributed normally for each of the three temperatures. Incubation periods were distributed normally at 19° and 25° but, not 30°C. Both preoviposition and incubation periods were sign ficantly correlated with temperature (table 11). Because of the non-normal nature of the hatch data at 30°, linear regression was not performed for the relationship of temperature to development rate during incubation.

The linear regression of development rate of egg maturation, within the female, to temperature is given by the equation 1/P=0.0248T-0.1884, where P is the preoviposition period and T is temperature in °C. The threshold temperature for development was calculated as 15.1°C and the degree days (k) as 80.1 degree days.

### DISCUSSION

# **Productivity**

The average weight of *D.\_albipictus* females in this study (0.61g) was higher than that of engorged females from moose in Ontario (0.47 and 0.40g) (Addison and Smith, 1981) and cattle in Oklahoma (0.28g) (Barker, pers. comm.) or Texas (0.42g) (Drummond et al., 1969a), but slightly lower than that of engorged females taken from mule deer in New Mexico that were fed on cattle (0.69g) (Ernst and Gladney, 1975). The lower weight of female ticks from moose in Ontario is likely because in that study both partially and fully engorged ticks were used. The lower weight of the ticks from cattle in Texas and Oklahoma may reflect either the resistance of the hosts or local population differences in the size of the ticks.

The number of eggs laid by a female tick is strongly correlated to her engorged weight (Snow and Arthur, 1966; Sweatman, 1967; Sweatman, 1968; Drummond et al., 1969a, 1969b; Drummond and Whetstone, 1970; Drummond et al., 1971; Campbell and Glines, 1979; Campbell and Harris, 1979; Davey et al., 1980; Addison and Smith, 1981). However, this correlation breaks down at extreme temperatures (Campbell and Glines, 1979; Campbell and Harris, 1979). In this study, female weight was correlated to egg number at 19° and 25° but not at 30°C. At 19° and 25° C, the average number of 'eggs per female (table I) was higher than any previously reported for this tick, while at 30° C, the average was comparable to those reported by Drummond et al. (1969a) and Howell (1939) at 27° C and 26.6° C respectively.

It is difficult to assess the effect of temperature on female productivity without eliminating the influence of

engorged weight. For this reason, it is convenient to compare the reproductive efficiency index (R.E.I.), equal to the number of eggs per gram of female engorged weight (Drummond and Whetstone, 1970) rather than the number of eggs laid/female. This value can be calculated directly from the data or can be predicted from the regression of number of eggs on engorged weight.

Although the average number of eggs/female winter tick in this study was higher than previously reported, the average R.E.I. was lower than those in the literature (table III). In addition, the literature suggests that the maximum reproductive efficiency is found at 26° C rather than 19° C, as was found in the present study. These differences may reflect the fact that the ticks in this study are from the most northern winter tick population examined and may be better adapted for cold climates, but it may also reflect differences in methodology. Addison and Smith (1981) estimated the total egg number by multiplying the weight of the egg mass by the average egg weight. They didn't mention if the egg weights were distributed normally. Considering the range in the weights of the eggs they measured, there is a very large margin for error.

The ability of the winter tick from Alberta, to reproduce more efficiently at low temperatures, is shared with Haemaphysalis leporispalustris, but not Dermacentor variabilis, from Nova Scotia (table III). It is likely this ability that allows D. albipictus and H. leporispalustris to

38

extend further north than *D. variabilis* (Bishopp-and Tremblay, 1945; Wilkinson, 1967; Sonenshine, 1979).

The average R.E.I.'s reported here for the winter tick are lower than R.E.I.'s calculated from the literature for most other species, but are most comparable to those of *D. variabilis*. Perhaps, *Dermacentor* reproduces less efficiently than other genera, or populations of *D. variabilis* in Nova Scotia (Campbell and Harris, 1979) and *D. albipictus* in central Alberta may have sacrificed reproductive efficiency for hardiness, in order to exist so near the northern limits of their distributions.

D. albipictus females drop from moose in Alberta from late March to early May (Samuel and Barker, 1979 and Chapter I). The average mean daily temperatures for March, April and May (at the Edmonton International Airport) are  $-7.2^{\circ}$ , 2.8° and 10.0° C, respectively (Alberta Environment, 1974) Females are, therefore, under selection to develop eggs at low temperatures. The high reproductive efficiency at 19° C testifies to this. As well, the lack of correlation between female engorged weight and number of eggs at 30° C suggests that the upper limit of temperature tolerance is lower than that of other ticks. The correlation of eggs to weight does not break down until a temperature of 35° C is reached for both D. variabilis (Campbell and Harris, 1979) and H. leporispalustris (Campbell and Glines, 1979).

The eggs, on the other hand, incubate during the summer and would be selected for survival at higher temperatures.

39

The very high survival at 30° C indicates that this is well within the range of temperature tolerance for egg development.

No work has been done to determine the effects of relative humidity on egg survival of *D. albipictus*. However, the high survival of eggs and larvae in this experiment indicates that >85% RH is a favorable range. Several ixodid ticks require high humidity for egg survival; *Ixodes ricinus* requires >80% RH (MacLeod, 1935; Czapska, 1967), *D. variabilis*,  $\geq$ 65% RH and *Amblyomma americanum*, >65% RH (Sonenshine and Tigner, 1969). Since female *D. albipictus* prefer relative humidities higher than 85% (Howell, 1939), it is possible that egg survival or development is impaired at humidities less than this.

### Development:

Although some authors have recorded preoviposition and incubation periods for *D. albipictus* at unspecified and fluctuating temperatures (Bishopp and Wood, 1913; Patrick and Hair, 1975), most have recorded these periods at constant temperatures (table IV).

The preoviposition and incubation periods reported in the present study are different from those in the literature and there are several possible reasons for this. The preoviposition periods reported by Wilkinson (1967) are much shorter than those of this study or of Addison and Smith (1981), because Wilkinson kept the females at 10° C for 12 weeks before starting the experiment. He found that females would oviposit at 10° C. Therefore, the ticks must have undergone development prior to the experiment.

At 25° C, preoviposition of ticks in this study was shorter than that found by Addison and Smith (1981) at 26° C. Those authors however, kept the ticks at 30-50 %RH and such low humidity might have affected the development.

Differences in methodology can explain some of the differences but not all. Incubation of the eggs took longer at 25° C (but shorter at 30° C)<sup>3</sup> than reported by other authors. The relative humidity was similar in all experiments and albeit different photoperiods were used, these differences cannot adequately explain this variation. Wright (1969a) found no effect of photoperiod on the incubation time of D. albipictus. Furthermore, photoperiod has been shown to affect only the length and pattern of oviposition (Snow and Arthur, 1966; Wright, 1969b) not the size of the oviposit (Wright, 1969b; Bennett, 1974) or the preoviposition period of ixodid ticks (Wright, 1969b). The ticks used in these various experiments came from vastly different localities and climates and have been subjected to different selection pressures. Many of the developmental differences previously discussed likely evolved in response to the various climates.

The threshold temperature of oviposition was calculated as 15.1° C. Although this is not a biological threshold at which no oviposition will occur, it is a point at which the linear function of development rate to temperature has lost

any meaning (Campbell et al., 1974). Near the threshold, preoviposition period does not change as greatly in response to temperature as it does in the linear portion of the curve. In addition, one might expect differential mortality at or below the threshold as well as a loss of correlation between egg number and female engorged weight.

On average, near Edmonton, only July and August have mean daily temperatures above this threshold (Alberta Environment, 1974). The fact that these ticks can persist in such an environment implies that fierce selection has occurred for individuals that can develop and oviposit at these temperatures. Indeed, Wilkinson (1967) found that winter ticks from British Columbia could oviposit at 10° and 15° C but that eggs would only develop at 15° C.

The fact that the maximum reproductive efficiency in this experiment was found at a temperature so close to the theoretical threshold and that in nature these ticks must develop at temperatures near or below this threshold suggests that central Alberta is very close to the northern limit of distribution. This corroborates Wilkinson's (1967) claim that 60° N (the Alberta-N.W.T. border) is the northern limit of D. albipictus.

Species at or near the edge of their distribution develop under precarious conditions. It is possible that during some years, in central and northern Alberta, average daily temperatures are low enough that few or no larvae are able to hatch. Conversely, since maximum reproductive efficiency occurred at 4° C above the theoretical threshold, an increase of only a few degrees in the average daily temperature during the spring and early summer could allow the tick population to increase dramatically.

43

Certainly more information is needed in order to understand the development and reproductive efficiency of these ticks under natural conditions. Information on development at temperatures below 19° C and fluctuating temperatures near the theoretical threshold is needed before any models can be generated to predict development and reproduction of this tick in the field.

constants refer to the linear regression E= a+bW, where E equals total number of eggs, W equals the weight of the engorged females (in grams), r equals Pearson's correlation coefficient and SE is the standard index (R.E.I.= no. eggs/g female) and the survival of eggs at three constant temperatures. The regression TABLE I. Preovipositional weights of  $\underline{D}$ . <u>albipictus</u> females, their egg production, reproductive efficiency ; •

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C tr	temperature C	weight of female (g)	TICK PRODUCTIVITY total no. R.E. of eggs	JCTIVITY R.E.I.*	%surv1val of eggs**	ro -	REGRESSION CONSTANTS b r	N CONSTAL	VTS SE
0 <sup>0</sup>	mean(median) S.D. range n=20	0.589 0.149 0.286-0.809	4754 1015.5 3200-7041	(7927) 6391-14538	93.6 6.66 79.7-98.9	1439.8	5426.3 0.826	0.826	558.02
25 <sup>0</sup>	mean(median) S.D. range n=19	0.610 0,136 0.304-0.844	4149 1143.7 1489-6281	(6933) 4399-8842	NA NA NA	124.4	6595.4	0.782	732.85
300	mean(median) S.D. range n=22	0.626 0.112 0.404-0.790	3626 1133.4 1225-6144	(5977) 1550-8977	97.0 3.54 82.4-100.0	•	•	0.307	•
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\*Using a Mann-Whitney rank sum test, the R.E.I.'s at these temperatures are significantly different (p<0.05) \*\*The survival (number of live larvae/total number of eggs X 100) was determined for each egg mass. Survival

TABLE II. The preoviposition period (days) and the time from first egg to first larva (days) at three constant temperatures. Pearson's correlation coefficient r is given as a measure of correlation between developmental period and temperature. . **1** 

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developmental .		temperature	·	
period	19 <sup>0</sup>	25 <sup>0</sup>	, 30 <sup>0</sup>	r
preoviposition 🧳			•	· · ·
mean	21.3	8.7	5.6	-0.871
S.D.	4.88	2.09	1.32	
range	14-32	5-12	3-9	<b>5</b>
ncubation of ggs		· ·		
mean	68.5	41.5	24.3	-0.991
S.D.	2.45	1.58	1.85	
range	64-73	39-46	21-30	
sample size	25	27 ·	26	
· · · · · · · · · · · · · · · · · · ·				• • • • • • • • • • • • • • • • • • •
· · ·		,	•	c.,
		∎ statistics Statistics		•

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species	R.E.I.	°c	authors
Dermacentor			
albipictus .	7621	22	Addison and Smith, 1981
	9240	26	H
· · · · · · · · · · · · · · · · · · ·	8778	27	Drummond et al., 1969a
). variabilis	3266	15.0	Campbell and Harris, 1979
¢	6550	20.0	
	7037	25.0	
	8057*	27.0	Drummond et al., 1971
	6938	29.7	Campbell and Harris, 1979
	2334	. 35.6	10 <b>1</b> 1 <b>1</b>
laemaphysalis	•	•	
leporispalustris	3713	14.7	Campbell and Glines, 1979
· · · ·	9470	19.9	H T
	9386	25.2	H
	8439	29.7	
· · · ·	4545	35.6	"
hipicephalis			
sanguineus	11214	20-30	Sweatman, 1967
oophilus microplus	12543	27	Davey et al., 1980
yalomma aegyptium	7075	20-35	Sweatman, 1968
nocentor nitens	9919	30	Drummond et al., 1969b
mblyomma maculatum	9721*	27	Drummond and Whetstone, 1970

TABLE III. Reproductive efficiency index of several ixodid ticks.

\* These R.E.I.'s were reported in the literature. All other values were calculated from the regression of number of eggs on engorged weight of undisturbed females.

temperature <sup>O</sup> C	preoviposition (days)	incubation (days)	authors
15	6-34 v.	151 little dev.	Wilkinson, 1967 Howell, 1939
20	6-8	57-62 56-60	Wilkinson, 1967 Howell, 1939
22	9-18	22-52	Addison and Smith, 1981
25	2-3	34-39 36	Wilkinson, 1967 Howell, 1939
26	10-11	30-34	addison and Smith, 1981
27	8-26	24-27 28-30 30-35	Drummond et al., 1969a Howell, 1939 Wright, 1969a
30	2-3	27-29 29	Wilkinson, 1967 Howell, 1939 4
35	2-3	27-39 20	Wilkinson, 1967 Howell, 1939

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TABLE IV. Development of <u>D</u>. <u>albipictus</u> at constant temperatures.

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IV. THE EFFECT OF DERMACENTOR ALBIPICTUS (ACARINA: IXODIDAE) ON BLOOD COMPOSITION, WEIGHT GAIN AND HAIR COAT OF THE MOOSE, ALCES ALCES.

# ABSTRACT

The physiological effects of the winter tick, Dermacentor albipictus (Packard), on moose, Alces alces; were investigated. The blood composition, weight gain, food intake and change in the hair coat of four moose calves, each experimentally infested with 31,000 D. albipictus larvae, and eight uninfested moose calves were monitored. One infested animal accidently died early in the experiment and showed no clinical signs associated with the infestation. The three remaining infested moose developed moderate to very severe alopecia because of their extensive grooming, apparently in response to feeding nymphal and adult ticks. No uninfested animals developed alopecia.

The infested animal with the least amount of hair loss (30-40%) suffered the most severe clinical signs: chronic weight loss, anemia, hypoalbumenemia, hypophosphatemia and transient decreases in serum aspartate transaminase and calcium during the period of nymphal and female tick engorgement. The animal with intermediate hair loss (60%)

only suffered mild transient anemia during the period of nymphal engorgement. The animal with very severe hair loss (>80%) did not suffer anemia but became very weak and was killed. Post-mortem examination revealed mild glomerulitis and mild subacute vasculitis of the heart and lungs, possibly as a result of immune complex disease. Infested animals did not become anorexic. The two moose calves with more than 50% hair loss had increases in gamma globulin shortly after the onset of female engorgement, whereas the animal with moderate hair loss did not.

These results suggest hair loss may be associated with tick resistance. Animals that groom and develop hair loss likely carry fewer ticks and therefore suffer less severely from blood loss.

### INTRODUCTION

The effect of ticks on their host is complex. Several authors have shown that ticks, in heavy infestations, can cause anemia and reduced weight gain in the host (Jellison and Kohls, 1938; Riek, 1957a; O'Kelly and Seifert, 1969; 1970; Gee et al., 1971; O'Kelly et al., 1971; Seebeck et al., 1971; Williams et al., 1977; 1978; Corrier et al., 1979; Rechav et al., 1980). One species, *Boophilus microplus*, has also been shown to cause anorexia (Seebeck et al., 1971) and biochemical changes in the serum composition of cattle (O'Kelly et al., 1971). Seebeck et al. (1971) estimate that tick-induced anorexia is responsible for 65% of the reduction in body weight of cattle infested with B. *microplus* while 35% is due to the specific effects of the tick.

In addition to the systemic effects resulting from heavy tick infestations and the loss of blood, the effects of tick bite can be: dermatosis, pain, itch, urticarial swelling, subcutaneous hemorrhage, necrosis, ulceration, alopecia, secondary infection and anaphylaxis (Marshall, 1966; Rearn, 1977), Tissue destruction associated with Amblyomma americanum infestations have caused blindness and ubsequentideath of white-tailed deer (*Odocoileus virginianus*) fawne (Boite et al., 1970). As well, the salivary secretions of some ticks are toxic and can result in paralysis or other forms of toxicosis of the host (Nelson et al., 1975).

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Like other ticks, the winter tick (*Dermacentor* albipictus) is thought to cause anemia (Fenstermacher and Jellison, 1933). Alopecia has also been reported in moose (*Alces alces*) heavily infested with winters ticks (Fenstermacher and Jellison, 1933; Wallace, 1934; Peterson, 1955; Webb, 1959; Berg, 1975; Addison et al., 1979; Samuel and Barker, 1979) but some observers (Ritcey and Edwards, 1958; Peterson, 1977) feel that alopecia is actually the spring moult and the presence of ticks is coincidental. Horses, heavily infested with *D. albipictus*, sometimes

56

suffer an edematous condition known as "water belly" (Diamant and Strickland, 1965) but this condition has not been reported in wild hosts. Very little is known about the effects winter ticks have on their host, although outbreaks of this tick have been associated with the deaths of moose (Bruce, 1927; Webb, 1959; Berg, 1975; Samuel and Barker, 1979; Addison and Smith, 1981), wapiti (*Cervus elaphus*) (Love, 1955) and free-ranging horses and cattle (Cameron and Fulton, 1926-27).

The purpose of this study was to determine the physiological effects winter ticks have on moose. Hand-reared moore calves were experimentally infested with *D. albipictus* larvae and held on a high plane of nutrition. The food intake, weight gain, hematology, serum chemistry and change in hair coat of infested and uninfested moose were carefully monitored. A specific goal of the experiment was to separate possible anorectic effects induced by the tick from the specific effects of the tick.

#### MATERIALS AND METHODS

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Neonatal moose were obtained as orphans during late May and early June of 1980 from personnel of the Alberta Fish and Wildlife Division and held at the University of Alberta Biomedical Animal Centre in Ellerslie Alberta. They were hand-raised and halter-trained so the later, during the experiment, they would be tractable (see Appendix II for rearing techniques). In September 1980, calves were placed in individual outdoor pens (36 or 50 m<sup>2</sup>). Uninfested animals were separated from tick-infested animals by a 3m wide concrete walkway and two 1m high concrete walls as well as chainlink fencing. All animals were fed a modified Moose Research Center 'MRC special'pelleted ration (Schwartz et al., 1980) and mixed timothy-alfalfa hay *ad libitum*.

In order to separate any anorectic effects from the specific effects of the tick, three groups of animals were used:

I. Experimentally infested with ticks.

Four moose calves were each infested, over a three week period, with 31,000 *D. albipictus* larvae as follows:

10,000...17 Öctober 1980 🕔

10,000...22 October

5,000...24 October

5,000...30 October

1,000...6 November

, These animals were allowed to feed *ad libitum* and their food consumption was monitored daily.

II. Pair-fed

Each infested moose was paired to one uninfested moose of the same sex and approximate weight.<sup>35</sup> The pair-fed moose were each fed the amount of food eaten by its infested partner the previous day. Food consumption was monitored daily.
## III. Controls

Four uninfested moose were allowed to feed ad libitum and served as controls. Food consumption was measured from Monday to Friday.

Moose were weighed regularly at one or two week intervals. Beginning 16 October at regular one or two week intervals, 15ml of blood were taken by syringe from the left jugular vein. Each moose was brought into a barn, which bordered the outdoor pens, haltered, and tied to a support. An assistant fed bananas to the moose (young moose love bananas) while blood was extracted. This kept the moose distracted and served to minimize stress connected with the bleeding procedure.

Five ml of blood were transferred from the syringe to a sterife test tube containing disodium edetate. The remaining 10ml of blood were transferred to two sterile test tubes and allowed to clot. They were then taken immediately to the University (14km from the Animal Centre), where erythrocyte and leucocyte counts were determined on a Coulter Counter TA II. The hematocrit was determined by the microcentrifuge technique and differentials were determined from blood smears stained in either Giemsa or Wright's stain. Serum was collected and stored at -70°C. The blood was refrigerated until the following day when hemoglobin was determined by the cyanometer hemoglobin method (Sigma Technical Bulletin #525) of the blood by the technical Bulletin Frozen sera were shipped in dry ice to the Western Veterinary Diagnostic Laboratory in Vancouver, British Columbia, where total protein, albumen, calcium, phosphate, cholesterol, urea nitrogen (BUN), creatinine, total bilirubin, alkaline phosphatase and aspartate transaminase (ASAT=SGOT) were determined on a Technicon SMA II. Serum iron and iron binding capacity were determined by the method described in Giovaniello et al.(1968). Protein electrophoresis was performed on cellulose acetate using the Helena Electrophoresis system. Thyroxine (T4) was determined by radioimmunoassay using a Clinical Assays Kit and an LKB 'Rack Gamma' counter.

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Serum magnesium and copper concentrations were determined by the Manitoba Department of Agriculture using a Perkin-Elmer 403 Atomic Absorption Spectrophotometer.

As a back-up to the hematology run at the University, beginning 29 January 1981, whole blood was shipped to the Western Veterinary Diagnostic Laboratory where RBC, WBC, PCV and hemoglobin were determined on a Coulter-S-Plus and differentials were run.

From 20 January 1980-14 May 1981, at irregular intervals, I inspected the neck, hump and shoulder of each experimental moose. On each inspection I counted and identified between 50-100 ticks. Periodically I checked the control moose for ticks by running my fingers through their hair, feeling for ticks. At irregular but numerous intervals, photographs were taken of the experimental and

control moose. The photographs were used to diagram hair loss on these animals.

Analysis

To determine whether experimental values were distributed normally; a t-test was performed to test the significance of the skewness and kurtosis deviating from 0. (Sokal and Rohlf, 1969). Log transformation was required to normalize the alkaline phosphatase values.

T-tests or Mann-Whitney U-tests were performed to test whether the blood values of the pair-fed moose differed significantly from the controls. Values that did not differ significantly (p>0.10) were combined in order to determine the reference or 'normal' range of values. All significant differences (p<0.05) between the values of pair-feds and controls were attributable to differences between males and females. (All control animals were female; two pair-fed animals were male.) In these instances, reference ranges were determined for males (based on the values of the two pair-fed males) and for females (based on the values of the two control females). A pair-fed animal, whose infested partner died during the experiment, was considered to be a control animal after two weeks on an *ad libitum* diet.

Reference ranges were determined by three methods. Ninety-five percent tolerance intervals were calculated for values that were distributed normally (Bermes et al., 1976). Non-parametric 95% confidence intervals were calculated for values without Gaussian distributions having sample sizes of

120 or more. The two extremes of the values served as the reference limits for values with non-Gaussian distributions, having sample sizes less than 120 (Henry and Reed, 1974).

RESULTS

## Development and number of ticks

,Two of the infested moose calves did not survive to the end of the experiment. On 21 November 1980, Moose #17 (Mo 17) died, after suffering an accident. Her hide was removed and the number of ticks on the hide was estimated by the technique described in Chapter II. Of the 31,000 ticks placed on her, an estimated 22,890 had attached. Of these ticks, 2.5% were unengorged larvae, 11.7% were engorged larvae, 85.7% were unengorged nymphs and less than 1% were engorged nymphs.

Later in the experiment (27 February), Mo 35 was killed because she was weak and could barely stand. She was suffering very severe hair loss (figure 1) and her hide had an estimated 9,870 ticks. Of these, less than 1% were engorged larvae, 82.3% were unengorged nymphs, 13.2% were engorged nymphs, 2.2% were males and 1.5% were females. No engorged females were found on the hide even though they were prevalent 8 days earlier (table I).

The timing of tick development on Mo 23 and Mo 41 was similar (table I). Nymphs began to engorge sometime before 20 January and engorged nymphs predominated the tick population by 12 March. The proportion of engorged females in the population increased during March to reach a peak on 2 April (Mo 23) and 8 April (Mo 41). The moose were free of ticks by 7 May (Mo 41) and 14 May (Mo 23).

Tick development on Mo 35 was markedly different from development on Mo 23 or Mo 41. Engorged females represented almost half of the tick population in the neck, hump, shoulder region of Mo 35 by 19 February. This is more than a month before similar proportions were reached on Mo 23 or Mo 41.

Hair loss

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All of the infested moose (except Mo 17) showed tick-related hair loss. Hair loss was primarily caused by the extensive grooming and rubbing by these animals in response to 'the irritation caused by the feeding ticks. This caused much of the hair in the affected areas to be broken to a length of approximately 25mm, but in some places, the hair fell out or could be pulled out by the roots. Tick-related hair loss ranged from moderate (on head, neck, shoulder and hump) to very severe (head, neck, shoulder, hump, back, sides, belly, legs and perianal region)(figure 2), None of the tick-free moose showed these types of hair loss. The spring moult began late April-May, starting on the hind legs and extending anteriorly and dorsally (details to be published elsewhere).

## Weight of ticks dropped from experimental moose

On 26,28,30,31 March and 2 April 1981, any engorged female ticks, lying on top of the straw bedding in the pens, were taken to the laboratory and weighed on a Mettler analytical balance. Thirty-four females, dropped from Mo 41, had a mean weight of 0.520g (SD=0.130; range 0.232-0.716g). Seventy-seven females, from Mo 23, had a mean weight of 0.648g (SD=0.126, range 0.304-0.946) (t=4.87, df=110, p=0.001).

## Food consumption and weight gains

There was no difference in the mean daily pellet consumption/wk between infested and control animals (Wilcoxon two sample test, p>0.05). Although food consumption was not depressed, Mo 23 gained less weight than other animals (table II).

All the animals grew rapidly from 13 Nov. to 12 Mar., but put on every little weight after 12 Mar. despite the increased food intake (table III). Mo 23 had no net weight gain between 12 Mar.-21 May, the period during which nymphs and adults engorged, and figure 3 shows that he actually lost weight during this period.

Hematology; serum chemistry and post mortem results

The reference or 'normal' hematology and serum chemistry values are presented in table IV. (Appendices III and IV show when blood values of the infested moose were outside the reference ranges).

Mo 23

Mo 23 was the only animal to show extensive clinical changes during the infestation. He suffered a normocytic, normochromic anemia from 19 March to 28 April (figure 4), as well as hypoalbumenemia from 26 March to 7 May (figure 5). Although beta and gamma globulin levels were chronically below those of the pair-fed males early in the experiment, these levels, as well as levels of alpha 1 and alpha 2 globulins, increased during the period of hypoalbumenemia (figure 6). Alpha 1 levels rose above the reference ranges from 8-23 April. Alpha 2 levels rose above the reference range on 28 April and gamma globulin rose above the 95% tolerance interval on 7 May (figure 6 and Appendix III). Transient increases in neutrophils, lymphocytes and basophils also occurred (Appendix III). ASAT levels increased somewhat during the initial period of nymphal engorgement (29 Jan. - 5 Mar.) but decreased during the period of peak nymphal and adult engorgement until levels dipped below the reference range 28 April-5 May (figure 7.). Calcium levels also dropped below the reference range 23-28 April. Phosphate levels were chronically depressed 19 March-7 May (figure 7).

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While five of the six total iron binding capacity values were below the reference range (Appendix III), the serum iron and transferring saturation values were normal. This, plus the small sample size of reference values, makes it imprudent to consider these values different from the controls.

Mo 23 died, one month after the end of the experiment, from a chronic abscessing bronchopneumonia that could have been contracted during the experiment. Mo 41

Mo 41 had few clinical changes during the infestation. He developed a mild normocytic, normochromic anemia on 19 March (figure 4). Although he also had several low values of gamma globulin early in the experiment, gamma globulin levels rose after 15 January to reach a peak, above the 95% tolerance interval on 8 April (figure 6). Moderate increases in alpha 1 and beta globulin in addition to the high gamma globulin on 8 April pushed globulin levels above the reference range. From 16-28 April, he exhibited eosinophilia. He also had transient increases in neutrophils and basophils (Appendix III).

Mo 35

Shortly before Mo 35 died, she showed several clinical changes, including increased RBC, hemoglobin, PCV, neutrophils, monocytes, cholesterol, globulins, gamma globulins and serum copper, as well as, decreased albumen (figures 4-6, Appendices III,IV). On 21 February 1981, Mo 35 was acting weak and dizzy. Her condition became worse and by 27 February when she stopped eating and drinking, she was so weak she could barely stand. We killed her and sent her body to the Provincial Veterinary Laboratory Services for post mortem examination. The post mortem revealed a mild glomerulitis and mild subacute vasculitis of the heart and

lungs. Examination of tissues for bacterial infection and feces for parasites was negative. Skin sections showed a chronic parasitic dermatitis.

Mo 17 died after suffering a severe blow to her head. She slipped, hitting her head on a concrete wall. All of the clinical changes observed were associated with the hemoconcentration that occurred as edema developed in the brain.

DISCUSSION

Late winter and spring hair loss on moose is clearly related to the presence of *D. albipictus* and appears to be associated with the engorgement of nymphal and female ticks. The association between tick engorgement and hair loss was first observed in wild moose of Elk Island National Park (E.F.N.P.) (Chapter II) and the results of this experiment further strenghthen this association.

Hair loss was first seen on Mo 41 when engorging nymphs represented 3.4% of the ticks examined (table I, figure 2). Although 3% may seem insignificant, it could easily represent a few hundred ticks. The irritation caused by the engorging nymphs likely prompted Mo 41 to groom and rub the affected areas causing hair loss. Hair loss became more extensive when female ticks began to feed.

A similar pattern was observed on Mo 35. The high proportion of adult ticks present on 20 January suggests that nymphal engorgement began sometime in December. Hair loss was first observed 17 December and became very severe after engorging females were present. Mo 23 first showed hair loss when engorging nymphs represented half of the ticks examined and hair loss became more severe as females engorged.

The timing of tick development on Mo 41 and Mo 23 was slightly advanced of that seen on wild moose in E.I.N.P. (Chapter II), However, the tick development on experimental moose was determined by ageing ticks along the neck, hump, and shoulder of each animal. High proportions of advanced instars were found on the neck, hump and shoulder of wild moose (Chapter II) therefore percentages of life stages in table I may be biased towards engorged nymphs and adults. The timing of hair loss on Mo 23 and Mo 41 was similar to that seen on moose in the park (Chapter II) suggesting that tick development was also similar.

Tick engorgement and corresponding hair loss occurred earlier on Mo 35 than on Mo 41, Mo 23 or moose observed in E.I.N.P. Although this advanced development and early hair loss seems anomalous, it appears to happen occasionally in nature. On 18 January 1980, a moose calf was spotted in E.I.N.P. with hair loss similar to that oftomo 35 on 20 January. This calf had more extensive hair loss when seen again in mid-February (Samuel, pers. comm.) suggesting that

the timing of tick development was similar to that of Mo 35.

Although all experimental moose received the same, number of ticks, the extent of the hair loss differed between individuals. The extent of hair loss may not be dependent on the number of ticks, but instead, on the animal's ability to mount a resistance to the ticks. Individuals within the same species (and breed) exhibit different levels of resistance to ticks (Roberts, 1968; Hewetson, 1971; Wagland 1975; 1978; 1979). Resistance can be measured by several criteria but in general, resistant animals allow fewer female ticks to feed to repletion and the females that drop off have lower encomped weights (Riek, 1962; Musatov, 1967; Hewetson, 1972; Brown, 1977). As well, some authors have found that resistant animals groom more than susceptible ones (Bennett, 1969; Koudstaal et al., 1978).

Mo 35 lost the most hair. Although many females were found engorging on her 11 days before her death, no engorged females were found on her hide at the time of her death. In addition, at the time of her death, she carried less than half the number of ticks carried by Mo 17 in October (9,870 vs. 22,890). This suggests she was very successful at removing her ticks and was therefore quite resistant to them.

Mo 41 also had extensive hair loss and he appeared to be more resistant to ticks than Mo 23. Consistently low proportions of the tick population on Mo 41 were represented

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by engorged females and the females that dropped off weighed. less than those from Mo 23. This, coupled with the more extensive hair loss and grooming, suggests that Mo 41 allowed fewer females to engorge and those that did took smaller blood meals. If this association between hair loss and resistance is real; it implies that in the wild, although animals with hair loss might face sizes from hypothermia, they would not suffer the debilitating effects of the tick.

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Mo 23 appeared to be most susceptible to the ticks. He groomed the least of the three infested animals (unpub.)) lost the least and the first and therefore likely carried the highest tick burden (Snowbal) 1956; Nikitina and Aristova, 1963; Rich, 1973). This, along with the greater engorged weight of the females, implies that he lost the greatest amount of blood to the ticks. This is, of course, corroborated by the fact that Me 23 suffered chronic anemia, hypoalbumenemia and weight loss during the period of nymphal and female engorgement, while Mo 41 registered borderline anemia on only one occasion.

Other authors have also found that heavy tick infestations can cause anemia (Jellison and Kohls, 1938; Riek, 1957a; O'Kelly and Siefert, 1970; O'Kelly et al., 1971; Williams et al., 1978; Corrier et al., 1979; Rechav et af., 1980) becreased albumen (O'Kelly and Seifert, 1970; O'Kelly et al., 1971) and weight loss or reduced weight gains (CKelly and Seifert, 1969; 1970; Gue et al., 1971; Seebeck et al., 1971; Williams et al., 1977, 1978; Corrier et al., 1979; Rechav et al., 1980). Although some ticks may cause anorexia, it is apparent from this study and others (0'Kelly et al., 1971; Seebeck et al., 1971; Springell et al., 1971) that anorexia is not a precondition for these changes to occur.

71

In both long term experimental animals, the anemia was brought on by the engorging nymphs. Mo 23, however, registered the lowest erythrocyte count on 28 April after most of the ticks had dropped off, Tick-caused anemia may not be solely the result of blood loss, but may also involve metabolic changes that prevent the animal from exhibiting the appropriate erythropoietic response to blood loss (dellison and Kohls, 1938; Rechav et al., 1980). The salivary secretions of *D. albipictus* may contain substances that depress erythrocyte production; therefore, even though fewer ticks were engorging on 28 April, Mo 23 may not have replenished the blood lost previously, and thus suffered further anemia.

The hypoalbumenemia, suffered by Mo 2? was associated with a rise in globulin that only partially compensated for the loss of protein, thus hypoproteinemia resulted.

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Decreased albumen is a nonspecific clinical change common in a variety of conditions (Wallach, 1978), and may simply reflect the stress associated with the heavy tick burden. However, O'Kelly et al. (1971) suggests that the hypoalbumenemia suffered by cattle infested with *Boopbill*  microplus could also be caused by losses to the tick or by altered catabolism and/or synthesis of albumen, resulting from either reduced protein intake or liver damage.

Reduced protein intake could not be a factor in this experiment. BUN levels are directly related to the dietary intake and absorption of protein (Preston et al., 1965). The values reported here are comparable to the levels in moose on summer range (Houston, 1964). Liver damage, however, has been observed in animals after injection with extracts of ixodid ticks, including *B. microplus* and *D. albipictus* (Riek, 1957b). Therefore, a possibility exists that these ticks may secrete a toxin that can affect liver function.

Mo 23 suffered other clinical changes that further suggest possible metabolic interference by *D. albipictus*. During the period of nymphal and female engorgement, Mo 23 shown decreased levels of ASAT, phosphate and total serum calcium. ASAT is important in the catabolism of several amino acids and in oxidative phosphorylation carried out in liver and heart mitochondria. A decrease in the activity of this enzyme may reflect a reduced body metabolism. The decreased serum calcium coincided with the greatest loss of the albumen-bound calcium fraction. The low phosphate levels, however, suggest there may have been a decrease in ionized calcium as well, since this would stimulate phosphate excretion into the urine.

The similarities between the clinical changes observed in Mo 23 and those observed in cattle infested with B.

*microplus* are striking. The effects of *B. microplus* include: hair, loss (Riek, 1956; Corrier et al., 1979), anemia (Riek, 1957a; O'Kelly and Siefert, 1970; O'Kelly et al., 1971; Corrier et al., 1979), hypoalbumenemia (O'Kelly and Siefert, 1969; O'Kelly et al., 1971), weight loss (Corrier et al., 1979) and marginal decrease in the activity of glutamate oxaloacetate transaminase (=ASAT) (O'Kelly et al., 1971).

O'Kelly et al. (1971) found that activity of other enzymes, not studied in this experiment, was depressed as well. Ticks may secrete substances that affect the production of some enzymes or react with certain enzymes to denature them and thereby impair their function. In the case of aspartate transaminase (ASAT), which is found in large quantities in the liver, depressed activity might impair liver function and thereby affect albumen production. This enzyme participates in a vaniety of metabolic pathways, but its role in protein metabolism and exidative phosphorylation suggest that depression of its activity could result in less efficient cell metabolism. This might weaken the body's response to the tick.

Although there appeared to be considerable metabolic disturbance associated with *D. albipictus* infestation, the clinical picture of Mo 23 is complicated by the fact that he may have contracted pneumonia during the experiment. The increase invalpha 2 globulin levels on 23 and 28 April could be further evidence that liver damage had occurred but could also represent an acute phase reaction, marking the,

initial contact with pneumonia (Grant and Kachmar, 1976). The high neutrophil count on 23 April further suggests bacterial invasion. Since Mo 41 also registered neutrophilia on 23 April and these animals were penned beside one another, it is likely that both animals came in contact with the organism.

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Pneumonia is often associated with decreased albumen and albumen/globulin ratio (Woolf et al., 1973). If Mo 23 contracted pneumonia during the week of 23 April, it might explain why the albumen levels were more depressed at that time and why the albumen/globulin ratio was still depressed at the end of the experiment.

In contrast to both Mo 35 and Mo 41, Mo 23 did not show any signs of having mounted an immune response to the ticks. He did have a rise in gamma globulin at the end of the experiment but this was likely in response to the pneumonia. However, both Mo 35 and Mo 41 had elevated gamma globulin levels shortly after female ticks began to engorge and Mo 41 followed this with eosinophilia. There is also the possibility that the mild vasculitis and glomerulitis suffered by Mø 35 resulted from the deposition of immune complex on or near blood vessel walls.

Ticks are known to contain substances antigenic to their hosts (Willadsen, 1980). If large amounts of tick antigen were circulating in the blood, the resulting antigen-antibody complexes could passively deposit beneath the endothelium of arteries and glomerular capillaries, setting up an inflammatory response known as immune complex disease (Benveniste et al., 1972; Knicker, 1972). Although vasculitis is a rather non-specific lesion, the intense grooming by Mo 35, in response to the feeding ticks, suggests that she suffered extreme irritation. The possibility that large amounts of antigen were being deposited in the skin and blood, cannot be overlooked. Other clinical signs suffered by Mo 35 immediately prior to her death can either be attributed to the vasculitis and glomerulitis, or to dehydration since she drank very little, during the final days.

In nature, moose are on a low plane of nutrition in the winter and early spring, before the leaves come out (Gasaway, and Coady, 1974). It is during the late winter and early spring, when browse is less available, that the nymphs and female ticks engorge. Poor nutrition not only affects the immune system (Dreizen, 1979), but also affects an animal's ability to handle blood loss (Alexander and Kieser, 1965). Animals on a low plane of nutrition are less resistant to ticks (Gladney et al., 1973) and suffer more severely from tick infestation than counterparts on a high plane of nutrition (O'Kelly and Seifert, 1970). The moose in this experiment were kept on a plane of nutrition similar to that of summer range and therefore would have suffered less severe consequences to the tick infestation than would be expected from animals in the wild. During winters when food is scarce even animals normally resistant to D. albipictus

would be likely to suffer the debilitating effects of this tick.

76

The full consequences of winter tick infestation are complicated by the fact that those animals that groom and remove the ticks may suffer severe hair loss. How this hair loss affects the sufficient of moose is not known. Further experimentation is needed to fully understand the complex physiological relationships that exist between the winter tick and its host.

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	-	•									i		•

<sup>1</sup>UN=unengorged nymph<sub>≪</sub>EN=engorged nymph, <del>M=m</del>ale, UF=unengorged female, EF=engorged female

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<sup>2</sup>The percentages overestimate the proportion of adults and engorged nymphs because only the neck, hump and shoulder regions (where high proportions of advanced instars are found (see Chapter II)) were

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TABLE II. Food consumption and weight gains of control and infested moose calves. ı, D 1. .

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Courtrol. <sup>15</sup> 13 131.82 220.91 2.91 4.954 13.44 <sup>15</sup> 152.27 244.54 3.00 <sup>1</sup> 3.32 3.214 8.70 <sup>16</sup> 152.27 244.54 3.00 4.990 13.66 <sup>39</sup> 154.54 240.45 2.82 4.758 14.25 <sup>112.28</sup> <sup>111.28</sup> <sup>111.28</sup> <sup>111.28</sup> <sup>111.28</sup> <sup>111.28</sup> <sup>111.28</sup> <sup>111.28</sup> <sup>111.28</sup> <sup>111.28</sup> <sup>111.28</sup> <sup>111.28</sup> <sup>111.28</sup> <sup>111.28</sup> <sup>111.28</sup> <sup>111.28</sup> <sup>111.28</sup> <sup>111.28</sup> <sup>111.28</sup> <sup>111.28</sup> <sup>111.28</sup> <sup>111.28</sup> <sup>111.28</sup> <sup>111.28</sup> <sup>111.28</sup> <sup>111.28</sup> <sup>111.28</sup> <sup>111.28</sup> <sup>111.28</sup> <sup>111.28</sup> <sup>111.28</sup> <sup>111.28</sup> <sup>111.28</sup> <sup>111.28</sup> <sup>111.28</sup> <sup>111.28</sup> <sup>111.28</sup> <sup>111.28</sup> <sup>111.28</sup> <sup>111.28</sup> <sup>111.28</sup> <sup>111.28</sup> <sup>111.29</sup> <sup>111.99</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup> <sup>111.92</sup>	MOOSE .	WEIGHT ON OCT. 22	WEIGHT ON MAY 22	X WEIGHT GAIN KG/WK	PELLETS CONSUMED (X) KG/DAY	KG PELLETS/KG OF Weight cain	د.
.82220.91 $2.91$ $4.954$ .18185.00 <sup>1</sup> 3.323.214.27 $244.54$ 3.00 $4.940$ .54 $240.45$ $2.82$ $4.758$ .60 $258.82$ $3.36$ $4.957$ .18 $254.09$ $3.00$ $4.897$ .18 $254.09$ $3.00$ $4.897$ .18 $254.09$ $3.14$ $4.147$ .00 $229.09$ $3.14$ $4.147$ .01 $222.73^2$ $3.23$ $4.664$ .02 $232.73^2$ $3.23$ $4.252$ .03 $210.91$ $1.82^3$ $4.252$ .03 $210.91$ $1.82^3$ $4.252$	CONTROL						
$.18$ $185.00^1$ $3.32$ $3.214$ $.27$ $244.54$ $3.00$ $4.940$ $.54$ $240.45$ $2.82$ $4.758$ $.00$ $258.82$ $3.36$ $5.365$ $.00$ $258.82$ $3.00$ $4.897$ $.18$ $254.09$ $3.00$ $4.897$ $.18$ $254.09$ $3.00$ $4.897$ $.18$ $242.73$ $2.68$ $5.013$ $.09$ $229.09$ $3.14$ $4.147$ $.00$ $232.73^2$ $3.23$ $4.664$ $18$ $240.91$ $3.23$ $4.252$ $73$ $210.91$ $1.82^3$ $4.252$ $73$ $210.91$ $1.82^3$ $4.252$	Mo 13	131.82	220.91	2.91	4.954	13.44	
$27$ $244.54$ $3.00$ $4.940$ $54$ $240.45$ $2.82$ $4.758$ $240.45$ $2.82$ $3.36$ $5.365$ $500$ $258.82$ $3.36$ $5.365$ $18$ $254.09$ $3.00$ $4.897$ $36$ $242.73$ $2.68$ $5.013$ $36$ $242.73$ $2.68$ $5.013$ $36$ $242.73$ $2.68$ $5.013$ $36$ $242.73$ $2.68$ $5.013$ $09$ $229.09$ $3.14$ $4.147$ $00$ $232.73^2$ $3.23$ $4.664$ $18$ $240.91$ $3.23$ $4.252$ $73$ $210.91$ $1.82^3$ $4.252$ $73$ $210.91$ $1.82^3$ $4.252$	15	98,18	185, 00 <sup>1</sup>	3.32	3.214	8.70	
$54$ $240.45$ $2.82$ $4.758$ $.00$ $258.82$ $3.36$ $5.365$ $.18$ $254.09$ $3.00$ $4.897$ $36$ $242.73$ $2.68$ $5.013$ $36$ $242.73$ $2.68$ $5.013$ $36$ $242.73$ $2.68$ $5.013$ $36$ $242.73$ $2.68$ $5.013$ $09$ $229.09$ $3.14$ $4.147$ $00$ $232.73^2$ $3.23$ $4.664$ $18$ $240.91$ $3.23$ $4.252$ $73$ $210.91$ $1.82^3$ $4.252$ $73$ $210.91$ $1.82^3$ $4.252$	ر <mark>.16</mark>	152.27	244.54	3,00	4.940	13 66	
.00 $258.82$ $3.36$ $5.365$ .18 $254.09$ $3.00$ $4.897$ .36 $242.73$ $2.68$ $5.013$ .36 $242.73$ $2.68$ $5.013$ .09 $229.09$ $3.14$ $4.147$ .00 $232.73^2$ $3.23$ $4.664$ .18 $240.91$ $3.23$ $4.664$ .18 $240.91$ $3.23$ $4.252$ .03.03(0.24) $1.82^3$ $4.252$	39	154.54	240.45	2.82	4.758	11, 25	
18 $254.09$ $3.00$ $4.897$ $36$ $242.73$ $2.68$ $5.013$ $36$ $242.73$ $2.68$ $5.013$ $09$ $229.09$ $3.14$ $4.147$ $00$ $232.73^2$ $3.23$ $4.664$ $18$ $240.91$ $3.23$ $4.664$ $73$ $210.91$ $1.82^3$ $4.252$ $73$ $210.91$ $1.82^3$ $4.252$		155.00	258,82	3.36	5.365	11.28	
18 $254.09$ $3.00$ $4.897$ 36 $242.73$ $2.68$ $5.013$ $09$ $229.09$ $3.14$ $4.147$ $00$ $232.73^2$ $3.23$ $4.664$ $18$ $240.91$ $3.23$ $4.664$ $73$ $210.91$ $1.82^3$ $4.252$ $73$ $210.91$ $1.82^3$ $4.252$			•				
36       242.73       2.68       5.013         09       229.09       3.14       4.147         00       232.73 <sup>2</sup> 3.23       4.664         18       240.91       3.23       5.369         73       210.91       1.82 <sup>3</sup> 4.252         73       210.91       1.82 <sup>3</sup> 4.252         3.03(0.24)       3.03(0.24)       3.03	Mo 187 to Mo 35).	163, 18	254.09	, 3 <b>.</b> 00	4.897	07 11	
$09$ $229,09$ $3.14$ $4.147$ $00$ $232.73^2$ $3.23$ $4.664$ $18$ $240.91$ $3.23$ $5.369$ $73$ $210.91$ $1.82^3$ $4.252$ $73$ $210.91$ $1.82^3$ $4.252$ $73$ $210.91$ $1.82^3$ $4.252$	34 (to Mo 47)	161.36	242.73	2,68	5,013	11.07 15 55	
00 232.73 <sup>2</sup> 3.23 4.664 18 240.91 3.23 5.369 73 210.91 1.82 <sup>3</sup> 4.252 3.03(0.24)	42 (to Mo 23)	132,09	229,09	3.14	C1010		ι.
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	INFESTED					7.47	
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	Mo 35	175 00				A.	
18 $240.91$ $3.23$ $5.369$ 73 $210.91$ $1.82^3$ $4.252$ $*$ $3.03(0.24)$ $4.252$	7 1			<b>3.</b> 2 <b>3</b>	4.664	10.74	
73 210.91 1.82 <sup>3</sup> 4.252 3.03(0.24)		143.18	240.91	3.23	5.369	11.92	
3.03(0.24)	23	147.73	210.91	1,82 <sup>3</sup>	4,252	14.60	•
3.03(0.24)	X (SD) OF, COMBINES, A.	<b>A</b>		•			
	PAIR-FED AND CONTRACT	***	÷.	3.03(0.24)		12.26(2.39)	
<sup>2</sup> Weight taken on February 24.	<sup>1</sup> Weight taken on April	23.					1
	<sup>2</sup> Weight taken on Februa	Iry 24.				- - - -	

ABLE III, Food consumption and weight gain of the control
nd infested moose during two periods of the experiment. The first
eriod was characterized by larval engorgement and nymphal diapause
f winter ticks. Peak nymphal and female engorgement occurred during
he second period.

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MOOSE	(NOV. 13- PELLETS CONSUMED (X) KG/DAY	MAR. 11) OWEIGHT GAIN (X). KG/WK	(MAR.12- MAY PELLETS CONSUMED (X.) KG/DAY	22) WEIGHT GAIN (X) KG/WK	<b>.</b>
CONTROL	· · · · · · · · · · · · · · · · · · ·	,			J.
Mo 39	4.488	2.84	5.174	1.66	• L.
16	4.612	3.60	5.448	0.97	
13	4.394	4.04	5.820	0.32	• •
40	5.223	3.95	5.675	1.62	
15 <sup>1</sup>	3.611	3.83	8	, ·	• د
•	(OCT.23 1	MAR, 11)	(MAR. 12- MA	Y 22)	
INFESTED	•				
10 35 <sup>2</sup>	4.660	NI CONTRACTOR			<b>9</b>
23	3.842	3.14	4.990	• 0.00	
41	4.893	-4.40	.234	0.90	
Mo 15 be	came lame and wa	s killed on Apr	-1-28.	درب در	
Mo 35 wa	s killed on Febr	uary 27.	<b>b</b>	, Ar	•

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TABLE IV. The reference 1 95% confidence interval, and control moose. When v on values of two pair-fed	ales and six contre	les.	anges for hematology and serum chemistry of mogse, based on the nonparametric 95% tolerance interval and the range of the combined values for the pair-fed alues for males and females differed significantly, reference ranges were based males and six control females.	onparametric he pair-fed ges were based	
Assay	sample size	nonparametric 95% confidence interval	95% tolerance intęrval	range	• • • • • •
HEMATOLOGY red blood cells(10 <sup>6</sup> /mm <sup>3</sup> ) white blood cells(10 <sup>3</sup> /mm <sup>3</sup> ) packed cell volume(volume cells/ 100 ml) hemoglobin(g/100 ml) MCH(10 <sup>-12</sup> g/100 ml) MCH((0 <sup>-12</sup> g/100 ml) MCH((0 <sup>-12</sup> g/100 ml) MCH((0 <sup>-12</sup> g/100 ml) MCH((10 <sup>-12</sup> g/100 ml)) mCH((10 <sup>-12</sup> g/10	132 132 132 132 132 69 69 69	4.52-6.51 3.43-8.37 29.9-42.3 9.9-17.9 59-81 16.8-33.8 16.8-33.8 28-44	4.54-6.74 4.54-6.74 30.1-42.6 4.8-7.2 32-47 11.7-16.9	4.38-7.50 2.82-8.95 2.82-8.95 29.5-45.2 8.0-20.0 46-92 14.8-35.1 25-51 25-51 25-51 3.9-8.3 32-47 11.9-16.9	81

	•			° 82
range	61.3-73.3 21.6-26.0 33.5-38.9	38-85 14-62 0-14 0-2 0-4	1.60-5.98 0.74-3.02 0.00-0.67 0.00-0.11 0.00-0.22	
95% tolerance interval	60.3-71.2 21.4-26.4 33.6-39.0			
nonparametric 95% confidence	interval			
size	60 69	69 66 69 69 69 69	€9 69 69	
TABLE IV (cont.) Assay	MCV MGH MCHC DIF	necestratis (Z) lymphocytes (Z) eosingehitis (Z) basophilis (Z) monecytes (Z)	<pre>neutrophils(<math>10^3/m^3</math>) lymphocytes(<math>10^3/m^3</math>) eoginophils(<math>10^3/m^3</math>) basophils(<math>10^3/m^3</math>) monocytes(<math>10^3/m^3</math>)</pre>	ð

	95% tolerance range interval	4.04-5.24 3.97-6.33 1.72-2.78 1.79-2.74 2.09-2.99 2.14-2.98	1.57-2.59 1.69-2.78 1.43-2.20 1.54-2.14 0.08-0.26 0.05-0.33	$\begin{array}{cccccccccccccccccccccccccccccccccccc$		61-72 60-74 60-74 6-10 6-11 6-10 7-10 7-10	83
	sample nonparametric size 95% confidence interval	127 4.05-5.20 73 37	2 73 37 127 0.10-0.27	73 37 37 37	73 37 37 37	127 61-71 1 127 1-4 73 37	æ
TABLE IV (cont.)	0.3		grobulin female male globulin(g/100 ml)	alpha 2 globulfn(g/100 ml) female male , beta globultn(g/100 ml) female , male	gamma globulin (g/100 ml) female male total protein(g/100 ml) female male	albumen (percent of total protein) alpha 1 globulin (%) alpha 2 globulin (%) female male beta globulin (%) female	mare

1 dence	sample nonparametric 95% toler size 95% confidence interval interval interval 37 10-17 33 37 10-12.0 93 38 93 4 147 10.0-12.0 149 0.2-0.5 149 0.8-1.9 149 0.8-1.9 149 2.3-6.9 76-614 59-646 149 50-73 149 71-4 149 71-4 149 71-2.0 149 76-614 50-73 149 71-2.1 74-243 74-243 74-243 74-243 74-243 74-243 74-243 74-243 74-243 74-243 75 0.08-0.90		13-19 5.0-11.8 6.6-11.4 46-195 44-116 9.4-12.6	0. / - 2. 1 1. 7 - 8. 0 38 - 82 7 - 36 92 - 300	240-366 33-93 1.30-7.39 0.10-0.96
		etric idence			0.08-0.90

85 <sup>1</sup>Cell counts run on a Coulter Counter TA II. Packed cell volume determined by the microcentrifuge technique. . • Hemoglobin determined by the cyanomethohemoglobin method. <sup>2</sup>Values were determined on a Coulter-S-Plus.

Figure, 1. Experimentally tick-infested moose calf (Mo 35) on February

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26 showing very severe hair loss.

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Figure 2. The progression of hair loss on moose calves experimentally infested with 31,000 <u>D</u>. <u>albipictus</u> larvae. **M** areas with hair broken to a length of  $\leq 25$ mm. **E** areas with patches of hair loss. In no hair loss. The summer hair coat had grown in by May 18.



Figure 3. The weight of tick-infested, pair-fed and control moose calves

before and during the experiment,



Figure 4. Hematology of three moose calves experimentally infested with <u>D</u>. <u>albipictus</u>. The stippled area between short dashed lines represents the 95% tolerance interval of reference ('normal') values. Long dashed lines delimit the range of reference values.

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Figure 5. Total serum protein, albumen and albumen/globulin ratio of three moose calves experimentally infested with <u>D</u>. <u>albipictus</u>. The stippled area between short dashed lines represents the 95% tolerance interval of reference values. Long dashed lines delimit the range of deference values. Where values differ between males and females, the reference ranges for males are shown in the body of the figure and reference ranges for females are shown on the right of the figure. Mo 23 and Mo 41 were male. Mo 35 was female.


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Figure 6. Serum globulin values of three moose calves experimentally infested with <u>D</u>. <u>albipictus</u>. The stippled area between short dashed lines represents the 95% tolerance interval of reference values. Long dashed lines delimit the range of reference values. Where values differ between males and females, the reference ranges for males are shown in ~ the body of the figure and the reference ranges for females are shown on the right of the figure. Mo 23 and Mo 41 were male. Mo 35 was female.



Figure 7. Serum chemistry values for three moose calves experimentally infested with <u>D</u>. <u>albipictus</u>. Aspartate transaminase and phosphate- short dashed lines delimit the range of reference values for males; long dashed lines delimit reference range for females. Mo.23 and Mo 41 were male; Mo 35 was female. Total calcium- the stippled area between short dashed lines represents the 95% confidence interval of reference values; long dashed lines delimit the range of reference values.

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### V. THESIS DISCUSSION

The winter tick is indeed a serious parasite of moose. The experiments in this study show that even moose on a high plane of nutrition can suffer anemia, hypoalbumenemia, weight loss, alopecia and very likely, metabolic disturbance. The calves in this experiment had blood values similar to that of moose in summer (Houston, 1969) indicating that the diet was similar in quality to summer browse. Winter browse is of poorer quality, and in nature, moose are in negative energy balance during the winter (Gasaway and Coady, 1974). Thus moose calves in the wild would be expected to suffer more severely from similar tick infestations than did the experimental calves.

There will always be a certain portion of the moose population, like Mo 23, that is susceptible to winter ticks. The combined effects of anemia, increased weight loss and general debilitation caused by heavy tick infestations could make these animals more susceptible to disease and predation. Although *D. albipictus* did not cause anorexia under experimental conditions, the listlessness associated with anemia may prevent an animal in the wild from travelling the distances needed in order to consume an adequate amount of food. Reduced food intake over a long period could affect the microbial fauna of the rumen, thereby affecting the animal's ability to digest the food

(Gasaway and Coady, 1974). The sum of the natural weight loss during winter, weight loss due to ticks and weight loss due to reduced food intake could cause these moose to become emaciated and die.

Therefore, in years of high winter tick infestations, even the moose populations on good range would be expected to suffer some losses. These losses would likely be restricted to susceptible animals and those few resistant animals, like Mo 35, that suffered extreme hair loss early in the season. However, if the habitat became overbrowsed, or heavy snow restricted browsing, then many animals could become nutritionally stressed. Under these conditions, animals normally resistant to the winter tick might not be able to mount a resistance and rid themselves of the ticks. These animals would also suffer the debilitating effects of the infestation. In these circumstances, high mortality of moose could be expected.

The full extent of the losses to winter ticks are difficult to predict without further knowledge of the thermoregulatory capacity of moose. Scholander et al. (1950) suggest that most large northern animals do not increase their metabolic rate until temperatures are at least -40°C. In agreement with this, Hart et al. (1961) found no increase in the metabolic rate of a nine month old caribou calf exposed to -55°C. The ambient temperature below which an animal must increase the rate of heat production to maintain constant body temperature, is affected by plane of

nutrition, insulation, exercise and access to solar energy (Ames, 1980). Moen (1968) found that fully-fed white-tailed deer in Minnesota could withstand exposure to -40°C and wind velocities of 8mph, without suffering thermal stress, but that deer on starvation diets went into negative energy. balance at temperatures approaching 0°C and wind velocities of 3mph.

Tick-induced hair loss on moose must affect their ability to withstand the cold. However, an animal with alopecia can minimize the heat loss by adopting certain postures, seeking shelter from the wind, increasing exposure to sunlight and increasing exercise (assuming the diet provides sufficient calories). Therefore, without further research, it is difficult to predict under what conditions these animals would become hypothermic.

It is clear that heavy infestations of winter ticks are injurious to moose. This, then, poses the question: how can  $\overline{D}$ . albipictus populations be controlled?

Ticks on livestock can be controlled by a variety of methods including: acaricide treatment, pasture spelling, the use of resistant hosts or altering vegetation to minimize larval transmission (Wilkinson, 1979). Dipping, spraying, or dusting wild animals with acaricides, or the use of chemically impregnated collars or eartags, are not feasible approaches to the control of winter ticks. The manpower and expense required to treat enough animals often enough to reduce the tick population would be prohibitive.

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The use of systemic acaricides is also not suggested. A minimum dose of acaricide is needed to kill the tick. Presuming animals could be attracted to bait (ie. salt licks or apple mash) containing a systemic acaricide, there is no control over the dose each animal receives. This approach may help some animals temporarily, but would ultimately promote the dévelopment of an acaricide resistant strain of ticks (U.S. Dept. Agric., 1981) and defeat the purpose of the program.

Pasture spelling allows animals to graze alternately in two adjacent paddocks; the period between each transfer must be sufficient to ensure that most of the ticks in the unstocked paddock have died. Again, the expense involved in applying this technique to wild ungulate populations would be prohibitive.

At present we are not in a position to genetically select moose or any other wild animal for tick resistance beyond that which occurs naturally. Therefore, the use of immune hosts to control winter ticks is not possible. We are, however, in a position to manage the wild populations so that the animals exhibit their own level of resistance to a maximum degree. This will be discussed later.

Lastly, altering vegetation to minimize larval transmission requires changing the height of the vegetation to eliminate tick-host contact. This is usually accomplished through the use of herbicides, plowing, fire or by overgrazing pastures (Wilkinson, 1979). It is unlikely this

technique would be used to control winter ticks, because it would also destroy moose habitat.

Conventional methods of tick control cannot be successfully applied to populations of wild animals; therefore, a different approach must be used. Insights into winter tick control can be found by examining the factors that affect *D. albipictus* population dynamics. Several factors can affect the size of the winter tick population: 1. Spring and summer weather

As was discussed in Chapter II, the temperature during the preoviposition period can affect the reproductive efficiency of the females. A cold spring would reduce the number of eggs laid by the females, but warmer weather than usual could allow the females to lay maximum numbers of eggs. Summer temperatures would determine whether the eggs developed fast enough for the larvae to hatch before the cold weather of autumn stopped their development. A cold summer may prevent many eggs from hatching.

2. Autumn weather

Temperatures during the autumn affect the activity of the larvae in the vegetation (Drew, pers. comm.). A long warm autumn would promote larval activity and lengthen the transmission period. A cold autumn would reduce larval activity and minimize successful tick-host encounters. 3. Host stocking density

The host stocking density affects the probability of the larvae encountering a host. A low host stocking density

would lower the chances of a tick-host encounter; therefore, few larvae would obtain a host and the tick population would remain low.

4. Nutrition and host resistance

Host resistance affects the number of ticks that engorge successfully on the host (Kemp, 1980). This resistance can be affected by nutrition (Gladney et al., 1973). Moose overwintering on poor range, or animals restricted from browsing by deep snow, could become. nutritionally stressed in late winter when the nymphs and female ticks engorge. These animals might not be able to mount an adequate resistance to the ticks and would therefore allow greater numbers of female ticks to engorge successfully. Animals on poor or overbrowsed range would maintain higher tick populations than animals on good range.

Further information on the development and reproductive efficiency of winter ticks at low temperatures (5-19°C) would enable us to predict years of high larval populations. Information on the effect of temperature on larval activity would enable us to estimate the length of the transmission period. With this information we could predict whether animals, in a given winter, would be heavily infested with ticks.

Although we have no control over spring, summer or autumn weather and its effect on the tick population, we can control host nutrition and stocking density. Evidence is mounting that animals on good nutrition are not only more resistant to ticks but also groom more than counterparts on poor nutrition (Gladney et al., 1973; Strickland et al., 1981). Grooming is important for the removal of ticks (Snowball, 1956; Bennett, 1969) and may also reduce the engorged weight of the females that drop off (Kemp, 1980). Ticks feeding on resistant hosts do not imbibe as much blood as ticks on susceptible hosts; therefore, the engorged females weigh less and produce fewer eggs (Riek, 1962; Musatov, 1967; Hewetson, 1972; Brown, 1977). Animals kept in good condition allow fewer female ticks to engorge to completion and those females that engorge successfully, lay fewer eggs. Therefore, these animals support smaller tick populations than animals in poor condition.

In regions where winter ticks cause significant mortality, wildlife managers should examine the host stocking density in relation to the condition of the winter range. They should try to maintain host populations at levels that let the maximum number of animals pass through the winter in the best possible condition.

remove the ticks, but the animals that groomed and removed the ticks would stand a better chance of survival than those that didn't. Although this approach wouldn't eliminate the problem, it would minimize tick-related mortality.

At present, this is the only feasible method of controlling the winter tick on wild cervids, but in the future, as wilderness areas become smaller and wildlife management becomes more intensive, it may be desireable to combine proper host management with other tick control measures. Agricultural methods of tick control could only be applied to cervids in game ranching situations. The very nature of wild populations makes conventional methods of tick control impractical; therefore, other approaches must be sought. Further information about the ecology of D. albipictus and the nature of the tick-host interactions should be obtained. Information such as how vegetation affects the transmission of larvae, the importance of different host species in the maintenance of the tick population, further information on the role of nutrition in host resistance and the effect of hair loss on the metabolism of moose could be integrated to develop a sound ecological approach to the management of ticks, hosts and habitats.

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APPENDIX I. Life stages and estimated winter tick population on moose hides collected in Elk Island National Park. UL= unengorged larvae, EL=engorged larvae, UN=unengorged nymphs, EN=engorged nymphs, M=males. UF=unencorved females FF=encorred females

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MOOSE NO.	AGE	SEX	DATE mo/day	IJ	EL	UN % of est	UN EN of estimated t	M total	UF	EF	ESTIMATED NUMBER OF	TOTAL TICKS	
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M-80-1	A	<b>.</b>	1/30	0.0	0.0	82.6.	15.5	2.9	1.0	0.0	18	18,902	
M-80-2	, D	W	1/29	0.0	0.0	76.2	8,5	9.6	5.7	0.0	9	6,026	
M-80-3	ວ	بىز	2/29	0.0	0:0	45.4	13.0	27.2	14.4	0.0	<b>2</b>	5, 125	
M-80-4	A	ţr.	2/29	0.0	0.0	53.8	14.6	23,5	8.1	0.0	6	9,786	
M-80-5	Υ	X	3/26	0.0	0.0	29.4	33, 3	21.0	16.2	0.0	. 13	13,707	
M-80-8	A	۲. ۲	3/26	0.0	0.0	25.9	24.2	27.4	21.9	0.4	4	4,526	•/
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A       F       9/29       7.3       20.0       68.8       2.8       0.7       0.4       0.0         3       A       F       1/29       7.3       10.0       75.7       4.2       1.6       1.1       0.0         3       A       F       11/5       0.8       0.9       95.9       0.0       1.5       1.0       0.0         4       F       11/4       6.2       24.1       69.2       0.1       0.1       0.1       0.0         5       C       F       12/       0.0       0.5       99.2       0.0       0.2       0.1       0.0         5       C       F       1/13       0.0       0.0       94.5       4.0       1.3       0.0         5       C       F       1/2       0.0       0.0       94.5       4.0       1.3       0.0         6       A       M       1/2       0.0       0.0       93.6       2.7       0.1       0.0         6       F       1/28       0.0       0.0       93.6       2.7       0.7       0.0         6       F       1/28       0.0       0.0       4.26       20.3	A         F         9/29         7.3         20.0         68.8         2.8         0.7         0.4         0.0         6,235           A         F         9/29         7.3         10.0         75.7         4.2         1,6         1.1         0.0         6,501           3         A         F         11/5         0.8         0.9         95.9         0.0         1.5         1.0         0.0         6,501           4         A         F         11/4         6.2         24.1         69.2         0.1         0.1         0.0         13,155           5         C         F         12/         0.0         0.5         99.2         0.1         0.1         0.0         6,5380           6         A         W         12/         0.0         0.5         99.2         0.0         0.0         6,5,380           6         A         M         1/2         0.0         0.5         99.2         0.1         0.0         6,5,380           6         A         H         1/2         0.0         0.0         99.2         0.1         0.0         00,3554           6         F         1/28         0.0	1980-81												
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$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	M-80-13	A	<b>[</b> 24	11/5	0.8	0.9	95.9	0.0	1.5	1.0	0.0	13 155	
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$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	. M-80-15	U U	н	12/	0.0	1.1	98.1	0.1	0.5	0.1	0.0	64.380	
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C F 1/28 0.0 0.0 93.6 4.2 1.8 0.4 0.0 A F 1/28 0.0 0.0 93.0 3.6 2.7 0.7 0.0 C F 2/27 0.0 0.0 45.9 22.2 22.4 9.4 0.0 A F 4/1 0.0 42.6 20.3 25.3 11.7 0.0 C M 3/30 0.0 12.8 21.9 28.3 35.6 1.4 average	C F $1/28$ 0.0 0.0 93.6 4.2 1.8 0.4 0.0 59,807 A F $1/28$ 0.0 0.0 93.0 3.6 2.7 0.7 0.0 17,037 C F $2/27$ 0.0 0.0 45.9 22.2 22.4 9.4 0.0 57,778 A F $2/27$ 0.0 0.0 42.6 20.3 25.3 11.7 0.0 39,048 A F $4/1$ 0.0 0.0 8.3 6.7 46.8 36.9 1.2 12,456 C M $3/30$ 0.0 0.0 12.8 21.9 28.3 35.6 1.4 21,755 average 48,096 median 50,403	81-2	A	Ψ	1/2	0.0	0.0	98.0	0.7	0.9	0.4	0.0	87,680	
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average median	average 48,096 median 50,403	81-8	C	X	3/30	0.0	0.0	12.8	21.9	28.3	35.6	1.4	21,755	
median	median 50,403			• .		·,						ave		•
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APPENDIX I (cont.)	(cont	<b>.</b>	· · · · · · · · · · · · · · · · · · ·	•	•			•	ş		
MOOSE NO.	AGE	SEX	DATE mo/day	- Li	EL %	UN of est	UN EN % of estimated total	M otal	UF	EF	ESTIMATED TOTAL NUMBER OF TICKS
W-81-0	-	f r									
	4	4	4/c	0.0		0.0		65.1	26.5	3.6	2.002
M-81-10	A	<b>آلند</b> .	5/4	0.0	0.0	0.0	·	0.8 73.9	17 4	7 0	
M-81-11	. 4	N		. (							J, 400
	C C	IJ	67/C	0°0	0.0	0,0	0.0 ζ 0.0	0.0	0.0	0.0	0
		1					•	•		•	
-		,									
L .											
A=adult. C=cálf	C=calf					•					•

A=adult, C=calt

 $^2$ The average and median do not include hides from September, May or June.

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 $^3{
m It}$  was obvious from visual examination that this hide had no ticks therefore the hide was not digested

and examined further.

#### APPENDIX II

# RAISING MOOSE CALVES

Over the past several years, Dr. W.M. Samuel, graduate students and technicians have raised many neonatal cervids at the University of Alberta Biomedical Animal Centre in Ellerslie; Alberta. From this experience, basic procedures and guidelines have evolved that are followed when raising cervids at the facilities in Ellerslie. Fundamental to success in raising wild animals of any kind is a genuine love and respect for the animals, coupled with meticulous observation and record Keeping. The importance of keeping records cannot be overstressed when large numbers of animals are being raised at one time.

Previous to this study, 12 moose calves had passed through the care of the Ellerslie facilities. The records from these animals provided a very basic knowledge of the • behavior of healthy and sick calves and the success of past management procedures and veterinary treatments. Based on this knowledge, modifications of past procedures were made and the resulting system was followed.

Basic Procedures

In 1979, 10 orphaned moose calves were obtained through the kind cooperation of Fish and Wildlife officers around the province. In addition, five calves were captured, with the use of a helicopter, from Rochester, Alberta. As each

animal was brought into the barn, a detailed written history of the animal was compiled, including when the animal was first found, what, how much and how often the calf had been fed and whether the animal was injured. The animal was examined closely for wounds, a rectal temperature was taken (38.5°C is normal) and the calf was examined with a stethoscope. Although probably unnecessary, the naval was swabbed with iodine. New calves were isolated from the others for 3-5 days to detect any incoming diseases.

The calves were kept in box stalls with concrete floors(5 calves/stall) in a small barn. Each box stall had access to an outdoor concrete pen and calves were allowed outside during the day. Because the calves were initially restricted to the barn and concrete pens, a bucket of dirt was kept in each stall. Dirt appears to be essential for cervids to develop their rumen fauna.

Over the years several rules have evolved to minimize the transmission of disease. Anyone entering or leaving the barn must wear rubber boots and walk through a footbath containing disinfectant. People entering stalls to feed/handle calves must wear coveralls and wash their hands in Hibitane before feeding the calves. Sick animals are isolated in a separate pen, termed "sick bay". Anyone entering "sick bay" must wear a separate pair of boots and coveralls and wash their hands in Hibitane before entering and upon leaving "sick bay". Each animal had its own labelled milk bottle. This served two purposes: it minimized disease transmission and facilitated accurate records of individual milk intake. Between feedings the milk bottles were sterilized in an automatic dishwasher. If an animal refused to feed, the milk was kept in the refrigerator until the next feeding. If the calf refused to feed twice, the milk was thrown away and the bottle was sterilized. New milk mix was offered for the third feeding.

Because these moose were to be handled extensively later on, they were habituated to habiers. This was done in several ways. Calves were haltered and lured with a banana up and down the corridor in the barn. They were led to and from the paddocks on halters, often lured forward with a banana or a milk bottle. We quickly realized that moose are stubborn and usually refuse to be led on a halter. However, I found that they could be coaxed forward if I stood beside the animal and scratched its back or legs. As the calves grew, it became necessary to halter and tie them to the sides of the pens during feeding.

Extensive records were kept on each animal. Information recorded included: date, time, amount of milk offered to the animal, amount of milk taken, the milk mix formula, what the feces looked like, any medication administered and any comments on behavior that might be important. The comments included whether the animal ate browse, dirt, hay; if it seemed listless or playful; whether the animal drank milk enthusiastically; if it had been in the paddock and how it reacted to the halter. Each animal was weighed twice a week. Feeding Schedule

Calves were fied a milk formula of whole unpasteurized cows milk and evaporated milk in a 1:1 ratio. For the first month, 4oz. of bovine colostrum was included in each feeding. The milk was warmed to body temperature before offering it to the calves.

The schedule we followed in the summer of 1980 is shown in table I. This schedule was flexible. Whether an animal proceeded to the next feeding regime was decided by its weight gains and feeding performance.

Weaning moose calves is a delicate operation and must be done slowly. We started weaning our calves in midadune. Before weaning was begun, the calves were given daily access to aspen and willow browse. While milk intake was being decreased, they were given increasing access to solid foods. They were allowed into grassy paddocks during the day and fresh aspen (and sometimes willow) branches were put in their pens in the afternoon.

The animals were to be weaned onto a modified MRC 'special' ration (Schwartz et al., 1980) containing 25% aspen sawdust. In order to get the animals used to eating aspen sawdust, sawdust was initially mixed with the dirt. The sawdust was obtained by cutting aspen logs with a chainsaw greased with vegetable oil. Over time the proportion of sawdust\_to dirt was increased until there were two separate containers in each pen, one with sawdust and one with dirt. The final weaning process involved mixing deer pellets with the sawdust until a mix of approximately 1/4 sawdust, 3/4 deer pellets was obtained. This mixture was later substituted with the MRC ration. The transition from dirt to sawdust to sawdust-pellet mixture was completed by early August. This allowed the calves time to get used to the mixture before weaning was final.

Table II gives the average weights and weight gains of the calves before and during weaning. It is important to note that three animals gained less than 4.54 kg/wk during weaning and weighed less than 68.18 kg when weaned. Two of these animals began scouring after they were weaned and subsequently died. At the time it was not obvious that these scours were related to the weaning process but in hindsight I feel these animals had not completely developed their rumen fauna and were susequently unable to handle a strictly solid diet. The third animal had been ill with foot rot and other non-specific illnesses for much of the weaning process. This calf was kept on a limited milk diet until September 12. In order to wean her onto moose pellets, a special banana-pellet mash was offered. Although this animal lived until the following May, she was never a sturdy animal. This observation on weight gains and health may be useful when deciding whether a calf is ready to be weaned or not.

#### Illnesses and Treatments

The normal rectal temperature of a moose calf is 38.5 C but if the moose has been in the sun, the temperature can be as high as 39.2 C. This must be taken into account when deciding whether an animal has a fever or not.

The calves suffered specific and non-specific ailments. The first signs of malaise were anorexia and listlessness. It is not abnormal for a calf to skip one feeding but animals that missed one feeding were watched carefully. Usually a calf that missed one or more feedings showed other signs of illness and was immediately isolated in "sick bay". Animals exhibiting anorexia and fever were given ethacillin I.M. for 3-5 days. Non-specific malaise of this nature usually was accompanied by mild diarrhea. Kaopectate was used for mild diarrhea. If the condition didn't improve within three days, then the antibiotic was switched to Trivetrin. Animals that remained ill for several days were given daily vitamin and mineral injections I.M. The two calves that scoured and eventually died were given various treatments. To prevent dehydration, they were given electrolyte solutions orally and at various times treated with Biosol-M and Kaopectate, Donnagel P.G., Lomatil tablets and Cotazvn tablets.

The calves that were captured by helicopter, for several days, refused to suckle milk. We fed them forcibly for two days then used a stomach tube to force 16 oz. of
milk into them. After using the stomach tube, four of the five calves began to suck from the milk bottle. The fifth animal appeared constipated and was given Bloat-aid. Within a day he was suckling as well. Calves were never stimulated to defecate or uninate.

Calves that were bloated were given Bloat-go or Bloat-aid and kept walking until the bloat had subsided. A few calves developed warts on their face and ears. These were not treated and eventually went away.

One animal had foot rot. We clipped her hooves, swabbed them with iodine and kept clean bandages on for one week. After the bandages were removed, her hooves were swabbed with bleach and later with coppersulfate until the hooves were hard. She was also given ethacillin for 3 1/5 days. She recovered from the foot rot but later suffered hoof abnormalities.

One calf developed a fluid-filled swelling of the tarso-metatarsal joint. He was treated with ethacillin and streptomycin. The fluid was drained and the swelling disappeared shortly after.

One calf died suddenly and was sent to the O.S. Longman Building for post mortem examination. The examination indicated a calcium or phosphorus deficiency as well as either a chlamydial or sarcocystis-like infection. This prompted us to analyze the milk mixture and to add calcium and phosphorus supplements to the milk. The analysis showed that the milk mixture contained adequate amounts of calcium and phosphorus (table III).

Several animals had eye infections. These were treated with antibiotic eyedrops or powder.

dates	no. of	feed	ings/day	amount of offered (oz.)	milk total milk offered /day (oz.)
May 20-23	······································	6		14	84
24-30	2 1	6	• .	16	96
May 31-June 4	•	5		. 22	110
June 5-9		5	2 a	24	120
10-15	1	5		26	130
16-23	•	4		32	128
June 24-July 16	•	<b>`</b> 3	•	36	108
July 17-21		3 .		32	96
22-26		3		30	90
July 27-Aug. 11	•	2		38	76
Aug. 11-18	•	1		38	38
19	· .	0		0	0

TABLE I. Feeding schedule for moose calves raised in summer, 1980. The milk mixture consisted of evaporated milk and whole cows milk in a 1:1 ratio. For the first month, 4 oz. of bovine colostrum was included in each feeding.

•		•	Weight (kg)	
		on arriva	l start of weani (June 16)	ng when weaned (Aug. 18)
		· · · · · · · · · · · · · · · · · · ·	· · · · · · · · · · · · · · · · · · ·	
mean		20.2	32.5	82.8
range		10.2-29.5	28.0-40.5	65.9-103.4
	•	We	ight gain/week	
•		before weaning	during weaning (June 16-Aug. 18)	after weaning (Aug. 19-Oct. 17)
mean		4.4	5.5*	6.8
range		2.4-6.6	2.9-7.9	3.4-7.5

TABLE II. Weight and weight gains of moose calves before, during and after weaning.

\* If sick animals are not included, the mean weight gain was 6.7 kg/wk.

TABLE III. Compositional analysis of two samples of the milk mixture fed to moose calves (evaporated milk to whole milk 1:1).

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assay	sam	ole .	,
	A	В	
calcium (mg/100ml)	100.0	104.5	
phosphorus (mg/100ml)	235	189	
zinc (ppm)	0.044		
butterfat (%)		5.34	
protein (%)	: 	5.21	
lactose (%)		7.74	
Vitamín A (I.U./100ml)		247	
<b>e</b>	·		

## LITERATURE CITED

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Schwartz, C.C., W.L. Regelin and A.W. Franzmann. 1980. A formulated ration for captive moose. Proc. N. Am. Moose Conf. Workshop. 16:82-105.

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reference ranges of the control and pair-fed values (Table IV, Chapter IV). The degree to which the APPENDIX III. Compilation of when physiological values of the tick-infested moose fell outside the (whichever interval is wider) of the reference values. 2) Value is outside values fell outside the range is graded d-3, 1) Value is outside 95% confidence interval or 95% the range of reference values. 3) Value is outside the TI and the TI is wider than the range. tolerance interval (TI)

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MOOSE

	1 I		٩							س	•	
	MCHC	g/100 ml	date value d	NORMAT					<b>ی</b>	3/19 41	NORMAL	. a
	MCH	10-1-/100 ml	date value d	NORMAL				<u> </u>		NORMAL	NORMAL	
	MCV 3	200	date value d	NORMAL		•	1			3/19 59 2	NORMAL	
		.	0	2	2	2	2 -	2		5	2	
INCON	PCV %	٩	алтво	29	28	30	30	. 26		29	56	
¥	đ	101	uare value d	3/19	3/27	4/2	4/23	4/28	•	3/19	2/24	
		17		ŝ	'n	en j	•ش	e	ς.	5	7	<del>, `</del>
	Hb O ml			11.3	10.3	10.4	10.7	11.0	9.6	11.8	19.4	•
	Hb e/100 m1	data waluo		3/19	3/27	4/2	4/16	4/23	4/28	3/19	2/24	•
	,	  ~	, .	с С	e	e	e	e	ŝ	6	<u>си "</u>	
	106/mm3	Value		4.4	4.3	4.5	4.5	4.5	3.9	4.9	8.3	
	106	date value		3/19 4.4	4/2 4.3	4/8	4/16	4/23 4.5	4/28	3/19	2/24	· . ·
•				Mo 23 <sup>2</sup>	•					Mo 41	Mo 35	,

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APPENDIX III.		 

	total	protein		81	globulin		b.	albumen	•.	alb/eloh	do		
	date B/	/100 ml	1	<u>8/</u>	g/100 m1		18	g/100 ml		TS/NTD			
	חסרב	Value	•	date	value	ק	date	value	י	date	value	q	
	12/4	6.20	e	10/30	2.11	~	3/26	3.55	2	11/6	2.27	m	
	3/11	6.20	ŝ	11/6	1.96	e	4/2	3.44	5	11/13	2.19	5	
	3/26	5,70	ŝ	11/13	2.07	'n	4/8	3, 53	5	12/4	2,33	່ ຕ	•
	4/2	5.80	Ω.·	12/4	1.86	ŝ	4/16	3.09	2	- 1/15	2.15	5	
	4/16	5.80	e	1/29	2.13	2,2	4/23	2.34	7	2/12	2,16	7	
<b>1</b> 2	4/23	<del>3</del> , 00	ŝ	- 2/12	2.09	Ż	4/28	2.57	.2	4/2	1.46	2	
•	4/28	5.50	n	.3/5	2,03	ς Ω	5/7	3.24	7	4/8	1.23	jen Jen	
	5/7	<b>6.</b> 20	ິ ຕີ	3/19	2,12	2		• •		4/16	1,14	ŝ	
			- <del></del>	·			• .			4/23	0.88	ŝ	
										4/28	0.88	e.	
		Ń	· · · ·							5/7	1,09		
		1		•				:		5/21	1.46	5	
•	1/15	6,00	Ś	12/30	2.12	7	NORMAL	AL		4/8	1,44	5	
	•	•		1/15	1.92	e.							
				4/8	3.07	ς Υ	•				ч. ц.	•	
-	NORMAL	M		11/6	2,97	Ś	1/29	3.88	7	11/6	1.63	5	
	•	•		12/18	2.75	7	2/12	3.66	2	11/13	1.64	7	
		•		2/18	2.93	ů	•	•		2/12	1.47	ę	
	•		<u>_</u>	2/24	3.04	e				2/18	1.32	m	
			-										

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date     value     d     date     value     d     date     value     d     date       4/8     0.32     1     12/4     0.43     2     11/6     0.48     2     10/       4/16     0.35     2     4/28     0.95     3     11/13     0.51     2     10/       4/15     0.28     1     2     12/4     0.49     2     11/       4/23     0.28     1     12/30     0.49     2     11/       12/30     0.28     1     23/12     0.49     2     1/       3/19     0.49     2     1//2     0.49     2     1/       3/19     0.49     2     1/     3/12     0.49     2     1/       11/20     0.28     1     NORMAL     NORMAL     NORMAL     10/     12/       11/6     0.28     1     2     2     2     2/     2/       2/24     0.33     2     11/6     0.79     2     2/       2/24     0.33     2     11/6     0.79     2     2/	MOOSE	- od	ㅋㅋㅋ	alpha 2 g/100 ml	beta g/100 ml	gamma g/100 m1	gamma 100 ml	
4/8       0.32       1       12/4       0.43       2       11/13       0.51       2       10/16       0.85         4/16       0.32       1       2       4/28       0.95       3       11/13       0.51       2       10/30       0.85         4/16       0.32       1       12/4       0.50       2       10/30       0.85         4/23       0.228       1       1/29       0.695       2       11/13       0.61         3/5       0.49       2       11/13       0.61       2       11/13       0.68         3/5       0.49       2       11/29       0.69       2       11/13       0.68         3/5       0.49       2       11/29       0.69       2       11/15       0.68         3/19       0.49       2       11/15       0.69       2       11/29       0.86         NORMAL       NORMAL       NORMAL       NORMAL       10/20       2       2/12       0.69         11/6       0.28       1       10/20       2       2/12       0.86         11/6       0.28       1       NORMAL       NORMAL       10/20       0.79       2/12 <th></th> <th>date</th> <th>value</th> <th></th> <th>value</th> <th>date</th> <th>e U</th> <th>•</th>		date	value		value	date	e U	•
4/16       0.35       2       4/28       0.95       3       11/13       0.51       2       10/30       0.85         4/23       0.28       1       12/4       0.50       2       11/13       0.83         1/29       0.50       2       11/13       0.83       3/5       0.49       2       11/13       0.83         3/5       0.49       2       11/13       0.69       2       11/13       0.83         3/5       0.49       2       1/12       0.69       2       1/12       0.83         3/5       0.49       2       1/12       0.69       2       1/15       0.85         3/19       0.49       2       1/12       0.83       3/19       0.49       2       1/15       0.86         NORMAL       NORMAL       NORMAL       NORMAL       NORMAL       10/23       0.86       1/12       0.49       2       1/19       0.79       1/10       0.66       0.79       1/10       0.66       0.79       1/10       0.66       0.79       1/10       0.79       2       1/12       0.79       2/12       1/12       0.79       2/12       1/12       0.79       2/12 <td< td=""><td>o 23 î</td><td>4/8</td><td>0.32</td><td>0.43</td><td>/6 0.48</td><td> </td><td></td><td></td></td<>	o 23 î	4/8	0.32	0.43	/6 0.48			
4/23       0.28       1       12/4       0.50       2       10/30       0.85         3/5       0.49       2       11/13       0.83         3/5       0.49       2       11/13       0.81         3/5       0.49       2       11/13       0.83         3/19       0.49       2       1/12       0.87         3/19       0.49       2       1/29       0.85         3/19       0.49       2       1/29       0.86         3/19       0.49       2       1/29       0.86         3/26       0.47       2       2/12       0.86         3/26       0.47       2       2/12       0.86         3/26       0.47       2       2/14       0.86         3/26       0.47       2       2/12       1.40         NORMAL       NORMAL       NORMAL       10/23       0.88         11/6       0.50       2       2/14       0.68         11/5       10/20       0.50       2       2/12       0.48         11/6       0.28       1       10/23       0.88       1/25         12/24       0.33*       2		// <del>/</del>	6 0.35	/28 0.95	/13 0.51			
11/5     0.50     2     11/13     0.83       3/5     0.49     2     11/13     0.83       3/19     0.49     2     1/129     0.87       3/19     0.49     2     1/29     0.87       3/19     0.49     2     1/29     0.87       3/19     0.49     2     1/29     0.87       3/26     0.47     2     2/12     0.89       3/25     0.47     2     2/12     0.89       3/26     0.47     2     2/12     0.89       3/25     0.47     2     2/12     0.89       3/26     0.47     2     2/12     0.89       3/25     0.40     2     1/29     0.86       11/6     0.28     1     10/23     0.86       11/6     0.28     1     11/15     0.79       2/24     0.33     2     2/18     1.42       2/24     0.33     2     2/18     1.42		4/2	3 0.28	, ( j) ,	/4 0.50 /30 0.49	10/		
3/5     0.49     2     12/4     0.71       3/12     0.49     2     1/15     0.87       3/19     0.49     2     1/15     0.87       3/19     0.49     2     1/15     0.87       3/26     0.47     2     2/12     0.89       3/26     0.47     2     2/12     0.86       3/26     0.47     2     2/12     0.86       3/5     0.86     3/5     0.140     2       NORMAL     NORMAL     NORMAL     10/23     0.86       11/6     0.28     1     10/23     0.86       11/6     0.28     1     1/15     0.79       11/6     0.29     2     2/18     1.42       12/30     0.28     1     1/16     0.79       2/24     0.33     2     2/18     1.42       2/24     0.33     2     2/18     1.42	•		• • •	-	29 0.50			
-3/12     0.49     2     1/15     0.87       3/19     0.49     2     1/15     0.87       3/26     0.47     2     2/12     0.89       3/25     0.50     2     2/18     0.89       3/5     0.50     2     2/18     0.89       3/5     0.50     2     2/18     0.89       3/5     0.50     2     2/18     0.89       3/5     0.50     2     2/19     0.86       10/23     0.88     1/15     0.88       11/6     0.28     1     10/23     0.88       12/30     0.28     1     1/15     0.79       12/34     0.33     2     2/12     1.28       2/24     0.33     2     2/18     1.42	~	•	· · · · · ·		/5 0.49	12/		<b>5</b> .
3/26     0.47     2     2/12     0.85       4/2     0.50     2     2/18     0.89       3/5     0.86       3/5     0.86       3/5     0.86       3/5     0.86       3/5     0.86       3/5     0.86       3/5     0.86       3/5     0.86       3/5     0.86       3/5     0.86       3/5     0.86       3/5     0.86       1/15     0.88       1/15     0.79       1/15     0.79       1/15     0.79       1/15     0.79       1/16     0.79       2/24     0.33       2/24     1.42       2/24     1.42	19 K	Ĵ.	· · · · ·		12 0.49	<u> </u>		<b>9</b>
MORMAL NORMAL NORMAL NORMAL 0.50 2 2/18 0.89 3/5 0.86 5/7 1.40 10/23 0.88 12/4 0.88 1/15 0.79 2 2/12 1.28 1/15 0.79 2 2/12 1.28 2/24 0.33 2 2/24 0.33 2 2/24 1.42	5 <b>u</b>	•	· · ·		/26 0.47	5		
NORMAL NORMAL NORMAL NORMAL NORMAL 10/23 0.86 5/7 1.40 12/4 0.88 12/4 0.33 2 2/24 0.33 2 11/6 0.79 2 2/12 1.28 2/24 1.42 2/24 1.42	•	•	•		/2 0.50	5		
NORMAL NORMAL NORMAL NORMAL NORMAL 10/23 0.88 12/30 0.84 12/30 0.84 12/30 0.84 12/30 0.84 12/30 0.88 11/15 0.79 12/30 0.88 11/15 0.79 2 2/12 1.28 2/24 1.42 2/24 0.33* 2 2/24 0.33* 2 2/24 1.42 2/24 1.42 2/24 1.42 1.42 1.42 1.42 1.42 1.42 1.42 1.								
NORMAL NORMAL NORMAL NORMAL 10/23 0.88 12/4 0.88 12/30 0.84 1/15 0.79 1/29 0.88 4/8 1.42 2/24 0.33 2 2/18 1.33 2/24 1.42	· ·		•.	<u>,</u>		<u> </u>		
35 11/6 0.28 1 NORMAL 11/6 0.79 2 2/12 1.28 1.32 2/24 1.42 2/24 1.42 2/24 1.42 2/24 1.42 2/24 1.42 2/24 1.42 2/24 1.42 2/24 1.42 2/24 1.42 2/24 1.42 2/24 1.42 2/24 1.42 2/24 1.42 2/24 1.42 1.42 2/24 1.42 1.42 2/24 1.42 1.42 2/24 1.42 1.42 2/24 1.42 1.42 1.42 1.42 1.42 1.42 1.42 1.	0 41			NORMAL	NORMAL	/23	:	
35 11/6 0.28 1 10/15 0.79 12/30 0.84 1/15 0.79 1/29 0.88 1/29 0.88 1/29 0.88 1/29 1/29 1.29 2/24 0.33 2 2/18 1.33 2/24 1.42						4		
35 11/6 0.28 1 NORMAL 11/6 0.79 2 2/12 1.28 1.42 2/24 1.42 2/24 1.42 2/24 1.42				,		30	•	
35 11/6 0.28 1 NORMAL 11/6 0.79 2 2/12 1.28 12/30 0.28 1 2/18 1.42 2/18 1.33 2/24 1.42					di si edi	CT /		
35 11/6 0.28 1 NORMAL 11/6 0.79 2 2/12 1.28 12/30 0.28 1 2/14 1.33 2/24 0.33 2 2/24 1.42					<i>ي</i> ن ۱	i œ		
'30     0.28     1       '24     0.33     2       '24     0.33     2		11/6		NORMAL	0.79	2/12	1 28 1	to.
0.33 2 24 1.42		12/3	0.28	•	-			
		2/2	0.33		м., <sup>2</sup>	24/24		
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APPENDIX III (cont.)

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		cour.						··.			
MOOSE	W 103 date	WBC 103/mm <sup>3</sup> ie value	יסין	neŭtro 103, date	neutrophils 103/mm <sup>3</sup> date value	ק	lymphocytes 10 <sup>3/mm3</sup> date value d	eosinophils 10 <sup>3</sup> /mm <sup>3</sup> date value d	basophils 10 <sup>3/mm3</sup> date value d	monocytes 103/mm3 date value	ם ו
	. 100					-					
MO 23	4/23	9.6	2	4./23	6.82	~	2/16 3,17 2	NORMAL	4/28 0,12 2	NORMAL	
Mo 41	4/23	11.3	2	4/23	9.27	7	NORMAL	4/16 0.84 2 4/23 1 02 2	4/28 0.19 2	NORMAL	- 5 -
						<b></b> .		1.07		•	
Mo 35	2/24 12.8	12.8	5	2/16	6, 65	5	NORMAL	NORMAL	NORMAL	2/24 0.38	2
		-					••••••••••••••••••••••••••••••••••••••				
•	cal mg/l	calcium mg/100 ml		bili mg/1	bilirubin mg/100 ml	-	ASAT IU/1	creatinine mg/100 ml	cholesterol mg/100 ml	T.I.B.C. mcg/100 ml	1
	·		»   ·								1
Mo 23	10/23	13.5	8	-11/13	0.0	-	:	10/23 0.3 2	NORMAL	12/4 201	7
ر ر	4/23	8.6	â			, <b>,</b>	5/7 41 .2			12/30 228	5
	4/28	<b>6°</b>		•		•	, ,			1/29 219	2
							•			2/24 201	3
			÷		•     				•	5/21 204	2
Mo 41		<b>6</b>		.4/28	0.6	7	NORMAL	NORMAL	3/5 78 1	NORMAL	•
Mo 35	2/18	9.6		NORMAL	<b>tAL</b>		NORMAL	NORMAL	11/6 . 75 1	NORMAL	
·	-		:				•		2/24 77 1		
		3	- (SQ. 14				· · ·	, <sup>1</sup>	•		
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alk. phos. IU/1 ite value NORMAL NORMAL NORMAL NORMAL NORMAL NORMAL NORMAL NORMAL NORMAL /12 6.0 /24 6.2 /24 6.2 /24 6.2 /24 6.2 /25 5.2 /28 5.6 /28 5.7 /28 5.7 /29 5.7 /
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d = degree to which the value fell outside the reference range.<sup>2</sup>Mo 23 and Mo 41 were male. Mo 35 was female.

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APPENDIX IV. Compilation of when hematological values (as determined by the author) fell outside the reference ranges of the combined control and pair-fed values. The degree to which the values fell outside the range are graded 1-3 as in APPENDIX III.

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value         d         date         value         d         date         value         d         da           8.67         1         3/19         29.5         1         4/1           3/26         27.0         2         4/2         27.5         2           4/2         27.5         2         4/16         29.5         1           4/16         29.5         1         4/2         26.5         2           3/19         26.5         2         3         3         3           8.72         1         2/24         47.0         1         12           9.45         2         11/20         66.0         2         11	MOOSE			RBC 10 <sup>6</sup> /mm <sup>3</sup>	•	•	WBC 10 <sup>6</sup> /mm <sup>3</sup>	/	• • • • •	PCV 2		hen	hemoglobin <pre>o/100 ml</pre>	
23 $3/19$ $4.22$ 2 $4/23$ $8.67$ 1 $3/19$ $29.5$ 1 $3/26$ $4.04$ 2 $4.03$ 2 $4.03$ 2 $4.03$ 2 $4/2$ $4.03$ 2 $4.03$ 2 $4.03$ 2 $4/2$ $27.5$ 2 $4/2$ $4.03$ 2 $4.03$ 2 $4.03$ 2 $4/2$ 1 $4/2$ $4.03$ 2 $4.03$ 2 $4.16$ $2.05$ 2       1 $4/16$ $4.10$ 2 $4.19$ 2       2 $4/28$ $26.5$ 2       2 $41$ $3/19$ $3.44$ 2       1 $2/24$ $4.7.0$ 1 $41$ $3/19$ $3.44$ 2 $2/24$ $10.35$ 2       2       2 $41$ $3/19$ $3.44$ 2 $2/24$ $10.35$ 2       2       2       2       2       2       2       2       2       2       2       2       2       2       2       2       2<			·date	value	ď	date	value	P	date	value	Ч	date	value	P
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	Mo 23		3/19	4.22	2	4/23	8.67	-	3/19	29.5	-	4/2	9.6	
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	·		3/26	4.04	7				3/26	27.0	5			
$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$			4/2	4.03	2	•			4/2	27.5	7			
$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$		• • . •	4/8	4.42	ľ				4/16	29.5			•	
$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$	•		4/16	4.10	7				4/28	26.5	7			
$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$			4/23	4.19	2									
41     3/19     3.44     2       35     2/24     7.3     1     2/12     8.72     1     2/24     47.0     1       35     2/24     7.3     1     2/12     8.72     1     2/24     47.0     1       36     2/24     10.35     2     1     2/24     10.35     2       17     11/20     10.2     2     11/20     9.45     2     11/20     66.0     2	•		4/28	3.73	2								•	
35     2/24     7.3     1     2/12     8.72     1     2/24     47.0     1       17     11/20     10.2     2     11/20     9.45     2     11/20     66.0     2	Mo 41		3/19	3.44	2				3/19	29.0	5			
17         2/24         10.35         2           11/20         10.2         2         11/20         66.0         2			2/24	7.3		2/12	8.72		2/24	47.0		12/4	18.0	Ч
17         11/20         10.2         2         11/20         9.45         2         11/20         66.0         2				•		2/24	10.35	2						
	Mo 17	•	11/20	10.2	5	11/20	9.45	2	11/20	66.0	2	11/20	20.0	2

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