EFFECTS OF WINTER TICK (*DERMACENTOR ALBIPICTUS*) ON BLOOD CHARACTERISTICS OF CAPTIVE MOOSE (*ALCES ALCES*)

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ABSTRACT: Eighteen moose calves were raised in captivity. Seven and 6 moose were infested with approximately 21,000 and 42,000 larval winter ticks (*Dermacentor albipictus*), respectively. Five moose were uninfested controls. Blood was collected between October 1982 and April 1983 and analyzed for 17 hematologic and biochemical parameters. Tick infestation level (0, 21,000, and 42,000 ticks) and tick activity (inactive *versus* active) produced different patterns of response in packed cell volume, gamma-globulin, and lactate dehydrogenase. Although there was significant variation in these blood parameters in relation to level of tick infestation and activity of ticks, the magnitude of the tick effects was small. Sex of moose did not affect these 3 blood parameters. Packed cell volume, hemoglobin, red blood cell counts, and serum glutamic oxalacetic transaminase values from calves in this study were much lower than values reported previously for North American cervids. Although there was limited impact of ticks on hematologic and biochemical parameters of well fed captive moose, it may be important to consider infestation with winter ticks when using blood parameters to assess nutritive condition of wild moose.

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Hematologic and biochemical parameters of cervids can be influenced by many factors including methods of restraint and handling, nutrition, season, and disease (see Seal *et al.* 1981). Previous studies of hematology and serum biochemistry of moose (*Alces alces*) have established comparative data (Franzmann and Bailey 1977) and have investigated relationships with nutrition (LeResche *et al.* 1974) and condition (Franzmann and LeResche 1978). However, the usefulness of hematologic and biochemical values as indicators of health is dependent on understanding the influence of additional variables on blood physiology (Franzmann 1985).

The winter tick, *Dermacentor albipictus* has been implicated as a serious pathogen of moose in the wild (Samuel and Barker 1979) and in captivity. Winter ticks on captive moose have been associated with extensive hair loss (McLaughlin and Addison 1986, Glines and Samuel 1989, Welch *et al.* 1990), reduced internal fat reservoirs (McLaughlin and Addison 1986), extensive grooming and rubbing (Samuel 1991), and reduced growth in young moose (Addison *et al.* 1994). Glines and Samuel (1989) reported hypo-albuminemia and transitory anemia in 1 of 3 infested captive moose. Studies with other tick - host systems have demonstrated negative relationships between hematologic parameters and numbers of ticks that engorged (O'Kelly *et al.* 1971, Rechav *et al.* 1980).

The primary objective of this study was to examine effects of winter ticks on blood parameters of captive moose calves during winter. Reference data for hematology and serum biochemistry of uninfested calves

are also reported.

METHODS

Eighteen newborn moose calves were collected when 1 to 3 weeks of age in May 1982 and were raised in captivity (see Addison *et al.* 1983) in Algonquin Provincial Park, Ontario (45°33'N, 78°35'W). One male and 1 female moose from the same tick treatment groups (see below) were placed in 6 adjacent pens (29.6 x 16.5 m). The remaining 6 moose (4 males and 2 females) comprising members of each of the 3 tick treatment groups were placed in a remaining larger pen (29.6 x 35 m). All moose were fed a pelleted ration containing 16% crude protein, 2.5% crude fat and 16% crude fibre (United Cooperative of Ontario, Mississauga, Ontario, Canada). The food was provided *ad libitum* except for the night before blood collections when the quantity of food was reduced by 10 cups/moose. This was done to improve the attractiveness to food that in turn facilitated collection of blood with a minimum of pursuit of the moose.

In September, moose were assigned to 1 of 3 tick treatment groups (TICKGR): *control* (no ticks) - 2 females, 3 males; *medium* (21,000 ticks) - 3 females, 4 males; and *high* (42,000 ticks) - 3 females, 3 males.

Larvae of *D. albipictus* were collected in the field during September and October 1982 as described by Addison and McLaughlin (1988) and identified. Moose were haltered and on a short lead and were fed browse during the application of larvae. This was continued for an additional 30 minutes to allow larvae time to reach the hide without being licked off. Infesting began on 17 September and continued until 12 October with most moose receiving half their final infestation by 30 September. Larvae were applied to most moose on 4 to 8 days with daily infestations varying from 880 to 21,429 larvae. Control moose were sprayed with acaricide (Dursban M., Dow Chemical of Canada Ltd., Sarnia, Ontario, Canada) twice in November and powdered with rotenone in December, January, and February to control accidental infestation with larval ticks. Four of the 5 control moose were maintained in pens separate from infested moose. A small number of ticks (<50/moose) were recovered from these 4 control moose at the end of the experiment in April 1983. The control moose housed with infested moose had no ticks on it at the end of the experiment.

The moose pens were sheltered from strong winds by being located within a mixed hardwood-deciduous forest with an abundance of trees within and adjacent to the pens. Minimum winter temperatures were -25°C to -30°C. Shelter within a shed was available to all moose but rarely used.

Growth (surface area) and development (life stage) of ticks were determined monthly throughout the winter (Addison and McLaughlin 1988) and were used to define 2 tick activity periods (ACTIVE): inactive - 20 October 1982 to 24 January 1983; active - 2 February 1983 to 13 April 1983.

Blood was collected into 3 to 5, 15 ml evacuated blood tubes (Vacutainer, Becton Dickinson and Co., Mississauga, Ontario, Canada) by use of 20-gauge, 38 mm needles inserted into the saphenous vein of 16 standing moose on the following dates: 20 October; 1, 10, 18 November; 2, 13, 21, 29 December 1982; 11, 24 January; 2, 14, 28 February; 9, 21, 29 March; and 6, 13 April 1983. Approximately 10 ml of blood were collected in vials with anticoagulant and another 10 ml in vials without anticoagulant. Blood smears were prepared and examined intermittently for evidence of blood parasites. Blood was usually collected between 0800 to 1000 hr although sampling was occasionally not completed until 1100 to 1200 hr. Moose were haltered and tethered

during collection of blood only when required. Beginning on 29 December, the following data were recorded during 10 blood collection periods as indicators of handling stress for each moose: use of halters, reluctance to being haltered, tugging when haltered, and number of needles used. Blood was collected in a similar manner from the remaining 2 moose until they were killed in mid-February. Moose were killed by initial immobilization with 300 mg of xylazine hydrochloride (Rompun, Haver-Lockhart Laboratories, Mississauga, Ontario, Canada) with subsequent administration of T-61 (N-[2-(methoxyphenyl)-2-ethylbutyl-(1)]-g-hydroxy-butyramide and 4,4'-methylene-bis-(cyclohexyl-trimethylammonium iodide)) (Hoechst Canada Inc., Montreal, Quebec, Canada) into the jugular vein. The remaining 16 moose were killed 15-29 April 1983. Moose were killed for examination for gross lesions, collection of tissues for histologic study and to determine the proportion of the infesting doses to be recovered as adult ticks.

Analysis of fresh blood was started within 2 to 3 hr after collection. Red blood cell counts (RBC), white blood cell counts, hemoglobin concentration (HEM), mean corpuscular volume, and packed cell volume (PCV) counts were determined electronically (Coulter Counter (Model S+IV, Coulter Electronics, Burlington, Ontario, Canada). Blood urea nitrogen, serum glutamic oxalacetic transaminase (SGOT), lactate dehydrogenase (LDH), amylase (AMY), calcium, phosphorus, albumin (ALB), and total serum protein fractions were determined using an automatic chemical analyzer (Abbott VP system, Abbott Laboratories, Mississauga, Ontario, Canada) using methods and chemical reagents as recommended by the manufacturer. Protein electrophoresis was used to separate globulin into alpha-1-globulin (A1G), alpha-2-globulin (A2G), beta-globulin (BG), and gamma-globulin (GG) fractions and then stained and quantified (Cliniscan, Helena Canada, Mississauga, Ontario, Canada).

Hematologic and biochemical data were screened for outliers (sample values > 3 SD from) and tested for normality (Kolmogorov-Smirnov test). Outliers were removed only if we could identify an obvious problem in their collection or if a series of values for a particular moose were identified as outliers. Seven LDH values ($\bar{x}$ = 715, SD = 66.02) from 1 moose collected between 20 October and 21 December were eliminated. All remaining data conformed to the assumption of normality ($P > 0.05$).

A repeated measures analysis of variance was conducted (SAS 1987) on each blood parameter to determine if there was a significant effect on results due to repeated sampling of blood from the same moose. Repeated measures effect was not significant ($P > 0.15$).

The correlation matrix of data for all 17 variables was subjected to common factor analysis (varimax rotation). This was done to identify a subset of orthogonal variables to use in subsequent analyses without violating the assumption of independence (see Wong 1968). We assumed that variables on a given factor which had loading scores with magnitude > 50 were collinear, and that the variable with the highest factor loading score was representative of the group. PCV, GG, and LDH were orthogonal because they had the highest loadings on factors 1, 2, and 3 respectively. These 3 variables showed the following correlation patterns: PCV collinear with RBC and HEM; GG collinear with A2G, A1G, BG, and ALB; and LDH collinear with AMY.

Since hematologic and biochemical data were determined from single samples drawn from an individual moose, we used multiple analysis of variance (MANOVA) (SAS 1987) to test the main effects (TICKGR,

ACTIVE, SEX), and the interaction effect (TICKGR x ACTIVE), on PCV, GG, and LDH (dependent variables). Multiple comparisons were conducted using Duncan's multiple range test ($\alpha = 0.05$) (Steel and Torrie 1980).

RESULTS

Moose stood freely at their feeders on 120 (74%) of 162 collections of blood when moose behavior was recorded. On the remaining 42 occasions moose were haltered. They stood still while being haltered 14 times, and walked slowly for <3 minutes before haltering on 28 occasions. Moose tugged at the halter on only 3 individual collections of blood. One needle was required to collect all blood on 89 (55%) of 162 recorded bleedings, 2-3 needles during 64 bleedings, and 4-6 needles during 9 bleedings.

No blood parasites were observed on blood smears. In addition there were no gross or histologic lesions thought to be associated with parasitic or infectious diseases other than the presence of winter ticks.

Multiple analysis of variance (MANOVA) indicated that number of ticks (TICKGR, Wilks' Lambda $P < 0.0001$) and tick activity (ACTIVE, Wilks' Lambda $P < 0.0001$) had significant main effects on 1 or more of: PCV; GG; LDH. The sex of moose calves did not have an effect on any of the dependent variables (SEX, Wilks' Lambda $P = 0.0954$). No interaction effect of TICKGR and ACTIVE was apparent (Wilks' Lambda $P > 0.1912$).

The variable GG was not affected by level of infestation (Table 1). Moderately infested moose (21,000 ticks) had higher PCV than control moose, while heavily infested moose (42,000 ticks) had lower PCV than the control group. The concentration

Table 1. Results of Duncan's multiple range tests on selected blood parameters from captive Ontario moose calves collected between October, 1982 and April, 1983.

Tick infestation level[1]	Control	Medium	High
Packed cell volume (%Vol)	34.7[2]	35.9	33.7
Lactate dehydrogenase (IU/l)	346.6	373.1	363.9
Gamma-globulin (g/dl)	0.646	0.659	0.687

[3]

Tick activity[4]	Inactive	Active
Packed cell volume	34.1	35.8
Lactate dehydrogenase	389.4	329.9
Gamma-globulin	0.710	0.608

[1] Control = 0 ticks, Medium = 21,000 ticks, High = 42,000 ticks

[2] mean value

[3] underlined means are not different ($P > 0.05$)

[4] Inactive (20 Oct to 24 Jan), Active (2 Feb to 13 April)

of LDH was higher in infested than in control moose but did not differ between moderately or heavily infested moose (Table 1).

All 3 dependent variables varied with tick activity (Table 1). LDH and GG concentrations were lower when ticks were active (control group moose also had lower LDH concentration during the active tick phase ($P < 0.001$)). In contrast, PCV was higher when ticks were more active (PCV did not differ between the times of the inactive and active tick phases for uninfested control moose ($P = 0.0580$)).

Summary data for each dependent variable according to the tick effects (TICKGR and/or ACTIVE) established by MANOVA and Duncan's multiple range tests are presented in Tables 2, 3, and 4. These tables also report summary data for those variables identified by factor analysis to be collinear with each dependent variable. Summary data for the remaining hematologic and biochemical parameters are presented for moose in the control group (Table 5).

DISCUSSION

Effects of immobilization or restraint on some hematologic and biochemical parameters in cervids can be significant (Wesson *et al.* 1979, Seal *et al.* 1981) and should be considered when interpreting blood values. Since moose in the present study were extremely tame and stood quietly while being bled, we suspect the effects of restraint to be minimal. The extent to which blood parameters in our study approach values for unstressed animals remains unknown. However, the lower values of PCV, HEM, RBC, and possibly SGOT in our moose calves as compared to previous studies on adult moose (Franzmann and Bailey 1977, Franzmann and LeResche 1978) and white-tailed deer (*Odocoileus virginianus*) (Seal *et al.* 1981) may reflect the low levels of stress of our moose compared to restrained or immobilized cervids.

Our experiment was designed before we encountered the problem of moose from the uninfested control group having acquired a limited number of ticks. The design did not allow us to assess the potential impact of the acaricides on the blood physiology of the control moose. However, we have assumed that differences in blood assays between treatment groups likely were related to the effects of winter ticks since there was no evidence of occurrence of other potentially influencing parasites and

Table 2. Summary data for gamma-globulin and collinear blood parameters from captive Ontario moose calves infested with winter ticks.

	Tick activity	
Blood parameter	Inactive (20 Oct to 24 Jan)	Active (2 Feb to 13 April)
Gamma-globulin (g/dl)	0.86(0.251)180[1]	0.78(0.261)132
Albumin (g/dl)	4.0(0.437)180	4.2(0.553)132
Alpha-1-globulin (g/dl)	0.48(0.149)180	0.36(0.119)132
Alpha-2-globulin (g/dl)	0.41(0.107)180	0.37(0.125)132
Beta-globulin (g/dl)	0.71(0.160)180	0.61(0.111)132

[1] $\bar{x}$ (SD)n

Table 3. Summary data for packed cell volume and other collinear blood parameters from captive Ontario moose calves infested and not infested with winter ticks.

Tick infestation level[1]	Tick activity[2]	Blood parameter: Packed cell volume(% Vol)	Blood parameter: Red blood cell (10^6/ml)	Blood parameter: Hemoglobin (g/dl)
Control	Inactive	34.3(2.226)45[3]	5.7(0.373)45	12.7(0.817)45
Control	Active	35.3(2.477)40	5.6(0.391)40	12.8(0.850)40
Medium	Inactive	34.8(2.347)63	5.9(0.359)63	13.0(0.908)63
Medium	Active	37.5(2.737)47	6.1(0.427)47	13.6(1.160)47
High	Inactive	33.3(3.342)53	5.6(0.567)53	12.4(1.299)53
High	Active	34.2(2.855)40	5.6(0.574)40	12.4(1.065)40

[1] Control = 0 ticks, Medium = 21,000 ticks, High = 42,000 ticks

[2] Inactive (20 Oct to 24 Jan), Active (2 Feb to 13 April)

[3] $\bar{x}$(SD)n

Table 4. Summary data for lactate dehydrogenase and other collinear blood parameters from captive Ontario moose calves infested and not infested with winter ticks.

Tick infestation level[1]	Tick activity[2]	Blood parameter: Lactic dehydrogenase (IU/l)	Blood parameter: Amylase (IU/l)
Control	Inactive	383.7(62.565)45[3]	5.7(10.480)50
Control	Active	310.5(39.473)32	37.8(7.254)40
Medium + High	Inactive	396.0(51.642)130	44.7(11.116)130
Medium + High	Active	336.4(56.437)92	38.9(7.633)92

[1] Control = 0 ticks, Medium = 21,000 ticks, High = 42,000 ticks

[2] Inactive (20 Oct to 24 Jan), Active (2 Feb to 13 April)

[3] $\bar{x}$(SD)n

infectious diseases. Furthermore, food quality and quantity, time of day of blood collection, relatively low and consistent apparent stress of blood collection techniques to moose, and other factors such as availability of shelter and quietness of the surroundings were partially to fully controlled within the experimental design.

The degree of correlation among the blood parameters we chose to measure at the outset of our experiment precluded a parameter by parameter analysis of results without violating the assumptions of MANOVA. Therefore, we considered a subset of 3 variables (PCV, LDH, GG) that were statistically independent and together accounted for a high proportion of the variance in our data. Although these variables may not typify blood parameters used to assess clinical responses in tick - host sys-

Table 5. Summary data for remaining blood parameters from captive Ontario moose calves not infested with winter ticks between October, 1982 and April, 1983.

Blood parameter	$\bar{x}$	SD	n
White blood cell (10^3/ml)	6.2	1.628	85
Mean corpuscular volume (m^3)	62.7	2.543	85
Blood urea nitrogen (mg/dl)	36.3	7.116	90
Serum glutamic oxalacetic transaminase (IU/l)	51.9	7.952	90
Calcium (mg/l)	10.0	0.448	90
Phosphorus (mg/l)	8.7	1.200	90
Total serum protein (g/dl)	6.5	0.368	90

assess clinical responses in tick - host systems, they are variables that could be used to evaluate the data in a statistically valid manner.

Presence of ticks in numerous other tick - host systems has resulted in decreased PCV in the host (O'Kelly and Seifert 1970, Barker *et al.* 1973, Dipeolu and Ogunji 1977, Rechav *et al.* 1980, de Castro *et al.* 1985). The lower PCV in heavily infested as compared to control moose in the present study was consistent with this pattern. However, in contrast, the PCV was actually higher when ticks were more actively feeding and moderately infested moose had higher PCV values than did control moose. Taking all of these observations into account, we conclude that the effects of winter ticks on the PCV of moose was minimal under the conditions of the experiment.

LDH concentrations were measured because, in humans, they elevate with exercise (15-40%), in addition to responding to a wide variety of pathological conditions (Schiele 1985). Infested moose are much more active than uninfested moose in late winter - early spring because of the time spent grooming winter ticks (Samuel 1991, E. M. Addison, *unpubl. data*). Thus, we might expect higher LDH concentrations in infested calves than in uninfested calves and higher LDH values in infested calves in late winter, coincident with pronounced grooming, than earlier in the infestation period. The higher LDH concentrations in infested than in uninfested moose would support this thesis. However, the significantly lower LDH values for infested moose in the very active grooming tick phase as compared to the inactive phase would appear to contradict the suggestion of the positive relationship between tick related activity of moose and LDH values. As in the present study, O'Kelly *et al.* (1971) documented significant negative correlations between level of infestation with ticks (*Boophilus microplus*) and concentrations of serum LDH in cattle (*Bos taurus*). LeResche *et al.* (1974) documented lower LDH values during late winter than during early winter in adult moose not infested with ticks. It is possible that the differences in LDH levels during periods of activity and inactivity of ticks was a pattern common to moose and was influenced by factors other than ticks. It remains unclear as to the strength of the association between LDH concentrations and activity of ticks and the processes by which that interaction might be mediated.

Gamma-globulins sometimes comprise

part of the immunological response to disease and have been elevated in infested hosts in some tick - host systems (O'Kelly and Seifert 1969, Williams *et al.* 1977). For these reasons we anticipated finding higher concentrations of gamma-globulins in infested moose and higher values during late winter when ticks were more active. However, there was not a significant positive correlation between level of infestation or of tick activity and gamma-globulins. Glines and Samuel (1989) also did not find an obvious association between winter ticks and concentration of gamma-globulins in two moose each infested with 31,000 larvae. We conclude that winter ticks, at least at the levels of infestation and nutritive plane provided in the current study and in that of Glines and Samuel (1989), do not stimulate a gamma-globulin immune response in moose.

The magnitude of effects of ticks on hematologic and biochemical characteristics in our study and in that of Glines and Samuel (1989) was small. Glines and Samuel (1989) described a transitory normocytic, normochromic anemia in only 1 of 3 captive moose infested with 31,000 winter tick larvae. More pronounced differences between infested and uninfested moose may have been evident in the present study if moose had been infested with greater numbers of ticks, with continuation of the experiment until more of the winter ticks had completed feeding, or with histologic examination of hemopoetic tissues.

Caution must be exercised in extrapolating the results of this work with captive moose to predict the impact of winter ticks on wild moose exposed to natural conditions. From one perspective it may be difficult to detect effects of winter ticks on hematology and serum biochemistry of wild moose since variability in blood parameters could be increased in wild moose by variability in quality and quantity of food, occurrence of other parasites and disease-causing organisms, and the effects of snow on mobility and access to food. Wild moose are also subject to more variability in levels of stress during blood sampling.

Alternatively, there may be larger more readily detectable differences in hematologic and biochemical parameters associated with winter ticks on wild moose than have been observed with captive moose. Numbers of winter tick larvae used by Glines and Samuel (1989) and in our study were small compared to numbers of larvae acquired by heavily infested wild moose. Of the approximate 42,000 larvae placed on each heavily infested moose in our studies, approximately 2,000-8,000 ticks were recovered when the moose were killed in April. In contrast, in excess of 30,000 to 100,000 winter ticks have been recovered from some moose found dead in the wild in late winter in Alberta (Samuel and Barker 1979) and Ontario (E.M. Addison, *unpubl. data*).

Food of wild as compared to captive moose could also have the effect of increasing tick associated impacts of ticks on hematologic and biochemical parameters. O'Kelly *et al.* (1971) demonstrated that PCV increased on cattle lightly infested with *Boophilus microplus* when on an adequate diet but that poor nutrition resulted in reduced PCV and hemoglobin concentration. Nutrition has also influenced the response of a variety of other hematologic and biochemical parameters of cattle to *B. microplus* (O'Kelly and Seifert 1969, 1970). Our captive calves were provided with a high quality diet (e.g., 16% protein) *ad libitum* throughout winter whereas the quality of browse available to wild moose in winter is much lower (e.g., 5-7% protein) (Oldemeyer 1974, Regelin *et al.* 1987). This possibility of an interactive effect of nutritional plane and ticks on blood parameters is cause to hypothesize that winter ticks could have a significant effect on

blood assays of wild moose, particularly during the period of active feeding by nymph and adult ticks in mid to late winter. We recommend that blood parameters should not be used as indicators of winter condition/nutrition of wild moose without consideration of tick infestation. We also recommend that future studies to determine the impact of winter ticks on wild moose compare hematologic and serum biochemical parameters from heavily infested wild moose with similar data from uninfested or lightly infested moose from the same location in late winter.

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