British Columbia Boreal Caribou Health Research Program

Final Report:
(November 1, 2013 – December 31, 2017)

Prepared for:

The British Columbia Oil and Gas Research and Innovation Society (OGRIS)
Victoria, BC

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March, 2018

Executive Summary

Wildlife health is determined by the cumulative effects of biological, environmental and socioeconomic pressures acting on individuals and populations. As such, "health" may be considered an indicator of vulnerability that reflects the capacity of wildlife to cope with and respond to natural and anthropogenic challenges (1-4). Understanding and tracking the health status of species-at-risk may, therefore, provide valuable information for wildlife management and conservation assessments and decision making (4-7).

Boreal caribou in northeast British Columbia (*Rangifer tarandus*, population No.14, DU06 (8, 9) are red-listed as threatened due to declines in abundance and distribution (10). Since 2010, the *Implementation Plan for the Ongoing Management of Boreal Caribou (Rangifer tarandus pop. 14) in British Columbia* (BCIP) has guided provincial efforts to manage and conserve this species. A three-year Boreal Caribou Health Research Program (BCHRP) was created in the fall of 2013 to address knowledge gaps surrounding the current health status of boreal caribou in northeast BC (NE BC). This program was initiated in collaboration with a concurrent program to deploy radio-collars on boreal caribou, evaluate late winter recruitment, identify causes of mortality, and develop herd health profiles (11).

In BCHRP Year 1, a comprehensive herd health assessment model was developed and applied across seven boreal caribou herd ranges in the region (12)[reviewed]. Biological samples collected from n=163 live-captured and n=12 dead adult female caribou were used to evaluate exposure to, or infection with, selected bacterial, viral, and parasitic diseases along with other indices of health related to chronic physiological stress, serum biochemistry, and trace minerals. Evidence of notable and novel threats to caribou included the bacterium *Erysipelothrix rhusiopathiae*, the protozoan *Neospora caninum*, and mild to extreme hair loss associated with winter tick (*Dermacentor albipictus*) infestations. A preliminary investigation of selected health biomarkers (eg., serum amyloid A and haptoglobin) that may show potential as simplified health assessment or monitoring tools was also performed in BCHRP Year 1 (12). A summary of health results obtained from the live-captured caribou in BCHP Year 1 and statistical analysis comparing the health parameters by herd in winter 2012-2013 was submitted to *Journal of Wildlife Diseases* in January 2018 and is currently in review (13).

Throughout 2014 and 2015, a continued radio-collar monitoring, maintenance, and replacement program in the study region (14, 15) provided a unique opportunity for a short-term, longitudinal investigation of boreal caribou herd health in NE BC. Biological samples were collected from n=41 live-captured caribou and n=12 dead caribou in 2014 and from n=33 live-captured caribou and n=3 dead caribou in 2015. Live-captures occurred in winter 2013/2014 and 2014/2015 and included n=1 and n=13 re-captured caribou respectively. In BCHRP Year 2, these samples were evaluated using the health assessment criteria established in Year 1 (12)[reviewed]. An enhanced investigation of *E. rhusiopathiae* and winter tick was also initiated and tests for blood borne pathogens and trace minerals of caribou were added to the health assessment model.

Findings in BCHRP Year 2 confirmed that health and disease may have important implications for the long-term sustainability of boreal caribou in NE BC (16)[reviewed]. For example, continued study of *E. rhusiopathiae* uncovered new evidence supporting the hypothesis that this pathogen may have played a role in the unusual pattern of caribou mortality recorded in NE BC in 2013 including: the identification of a moribund caribou most likely clinically affected by the bacterium (16)[reviewed]. Tick counts on hide samples collected from

dead caribou and the development of a classification system for tick associated hair loss (16)[reviewed] in live caribou also revealed that mild to severe infestations with *D. albipictus* are widespread in the study area. In addition, variation in bone marrow fat levels across study years (16)[reviewed] and potential trace mineral deficiencies were identified. Investigative approaches and standardized protocols developed as part of the BCHRP in Years 1 and 2 for live-captured and dead caribou can be used as a model for similar health studies in caribou herds elsewhere as well as for other ungulate species.

In BCHRP Year 3 (April 1, 2016 -March 31, 2017) we continued with our enhanced study of *E. rhusiopathiae* in free-ranging boreal caribou from NE BC. We tested the serum from n=24 boreal caribou collared in winter 2015-2016 and from n= 56 archived boreal caribou historical serum samples collected from 2000-2010. We refined the cut-off value for the *E. rhusiopathiae* serology test using results from a vaccine based experimental study on captive reindeer held at the University of Calgary's Faculty of Veterinary Medicine and serology results from other woodland caribou herds in North America. This work was supported by an ongoing collaboration with caribou researchers in Alberta [Foothills Research Institute (fRI) Caribou Program, Hinton, AB]. We also began analyzing the results comparing health across herds and associations between factors including road and seismic line density, moose density, year, age, mortality, reproductive status, and health parameters collected in BCHRP Years 1-3. The analyses conducted in Year 3 were in collaboration with Drs. Matt Mumma and Mike Gillingham from the University of Northern British Columbia, who provided the data on moose density and anthropogenic features. In Year 3, we also created maps showing the spatial distribution of each pathogen in boreal caribou using ArcGIS.

New findings for Year 3 include additional evidence that *E. rhusiopathiae* may have played a role in the high mortalities observed in winter 2012-2013. Seropositivity to *E. rhusiopathiae* significantly increased from winter 2012-2013 to 2013-2014. In addition, from winter 2012/2013 to winter 2014/2015, 7 recaptured caribou seroconverted from negative to positive indicating that an exposure to this bacteria occurred during the high mortality period. Another key finding from Year 3 was that hair cortisol concentration was positively associated with the likelihood of mortality within the next year. Exposure to *Besnoitia tarandi* was highest in the Chinchaga herd and was significantly associated with anthropogenic landscape features, including level of disturbance and road and seismic line density. Hair loss from winter tick was more common in boreal caribou in late winter (Feb. 16 – Apr. 30) than early winter (Dec. 1-Feb. 15) and was associated with habitat disturbance. Trace mineral deficiencies were suspected for copper and selenium.

This report summarizes the results for BCHRP Years 1, 2, and 3, and presents the activities from Year 3 in detail.

Acknowledgements

For valuable input and assistance in the development, application, and administration of the BCHRP the boreal caribou health program working group would like to thank: D. Culling, B. Culling, M. Watters, K. Haman, C. Nelson, J. Cook, R. Cook, M. Lejeune, M. Mumma, M. Gillingham, T. Forde, W. Hutchins, M. Ball, J. Rubin, A. Mathieu, M. Tryland, I. Nymo, E. Breines, E. Hareide, G. Alvarez García, J. De Buck, U. Kanavets, T. Orro, J. Blake, K. Parker, K. Beckmen, J. Dau, R. Lindsay, A. Dibernardo, C. Brisson, O. Surujballi, J. Copps, S. Tan, P. Barbeau, T. Leighton, D. Godson, V. Misra, D. Ojkic, A. Carlsson, A. Oksanen, G. Verocai, L. Finnegan, D. MacNearney, K. Graham, G. Stenhouse, T. Larsen, D. Janz, M. Cattet, N. Larter, S. Wilson, B. Thomson, G. Sargent, F. Mavrot, J. Wang, A. Schneider, and the Treaty Eight First Nations of British Columbia and the Boreal Caribou Research and Effectiveness Monitoring Board (REMB) of the British Columbia Government's Boreal Caribou Initiative Plan (BCIP). We sincerely thank the BC Oil and Gas Research and Innovation Society (OGRIS), BC Ministry of Forests, Lands, and Natural Resources Operations (FLNRO), National Sciences and Research Council of Canada (NSERC), and Nunuvut Harvesters Association for funding our research and their continued support.

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1.2 Confirm the cause of death or ill health for the unusual pattern of mortality of collared female caribou in 2013.

1.3 Use BCHRP Year 1 findings to establish the first comprehensive herd health baselines for boreal caribou in NE BC and compare the prevalence/seroprevalence of pathogens and other health determinants among herds.

1.4 Determine the statistical associations between caribou fitness (survival and reproduction) with demographic and temporal factors, physical characteristics, chronic physiological stress, trace mineral status, moose density, anthropogenic features, and infection with or exposure to specific pathogens.

1.5 Determine the statistical associations between infection with or exposure to selected pathogens (e.g., E. *rhusiopathiae*, hair loss from winter tick) with demographic and temporal factors, physical characteristics, chronic physiological stress, trace mineral status, moose density, anthropogenic features, and infection with or exposure to specific pathogens.

1.6 Explore the use of serum amyloid A and haptoglobin as bio-indicators of caribou health.

1.7 Develop standardized sampling recommendations for continued monitoring of the health status of live-captured and dead boreal caribou in NE BC.

1.8 Provide ongoing health related recommendations for boreal caribou management and conservation decision making in NE BC.

1.9 Explore the role of the pathogenic bacterium *E. rhusiopathiae* in boreal caribou health and as a factor in the relatively high number boreal caribou mortalities observed in NE BC between April-September 2013.

2. Methods

2.1 Sample Collection and Storage

2.1.1 Sample Collection: Live-captured Caribou

Health based sampling of caribou in winter from 2012–2016 was integrated into an ongoing monitoring program tracking adult caribou survival and juvenile caribou recruitment across seven herds in in NE BC (Fig. 1). This program along with a detailed description of the study area, capture and handling protocols, boreal caribou habitat, and herd ranges in NE BC,

are reported in detail elsewhere (11, 14, 15). Other biological data closely related to the health status of individual caribou (e.g., body size measurements, age, lactation status, presence of a calf at heel) were also recorded at the time of capture.

A standardized suite of biological samples including: hair, feces, and blood were collected from caribou live-captured with net-guns each year from 2012–2016. The occurrence of ectoparasites (e.g., ticks, warbles), other health related anomalies, and any associated pathology were documented, and ectoparasite samples (e.g., winter tick voucher specimens) were collected as encountered. Whole blood was collected and blood smears (minimum of two smears per individual) were made for n=30 animals in winter 2014/2015 and n=3 animals in winter 2015/2016. The pregnancy status of caribou was determined after capture by measuring serum progesterone and/or pregnancy specific protein B (PSPB). In 2014/2015, nasal swabs were collected from all live-captured caribou. Specific details regarding the diagnostic tests used and laboratories samples were submitted to are presented in the boreal caribou health manuscript submitted for publication (13)

From 2012–2016, 239 individual adult caribou were sampled from all seven herds (Table 1). One yearling female was captured in winter 2012/2013 and was later recaptured as an adult. The result from the yearling was excluded from the analysis. Twenty-two caribou were sampled in more than one year because they had to be recaptured for radio-collar replacement throughout the duration of the study (Table 1). All of the recaptured caribou were previously captured in 2012/2013 except three caribou. These three caribou (n = 1 each from Calendar, Chinchaga, and Maxhamish) were initially captured in winter 2013/2014 and then recaptured in winter 2015/2016.

Table 1. Total number of adult female caribou that were radio-collared and sampled from winter 2012/2013 to winter 2015/2016[a].

	Total Number of Adult Female Caribou Captured by Year (Number Recaptured)				
Herd	Winter 2012-2013	Winter 2013-2014	Winter 2014-2015	Winter 2015-2016	**Total**
CAL	27	5	2 (2)	3 (2)	37 (4)
CHIN	37	13	10 (3)	6 (2)	66 (5)
MAX	24	8 (1)	8 (1)	5 (2)	45 (4)
SNK	56	11	8 (3)	8 (2)	83 (5)
PKR	7	1	2 (2)	2	12 (2)
PPH	9	0	2 (1)	0	11 (1)
FNL	3	3	1 (1)	0	7 (1)
Total	163	41 (1)	33 (13)	24 (8)	261(22)

[a] CAL = Calendar; CHIN = Chinchaga; MAX = Maxhamish; SNK = Snake-Sahtaneh; PKR = Parker; PPH = Prophet; FNL = Fort Nelson.

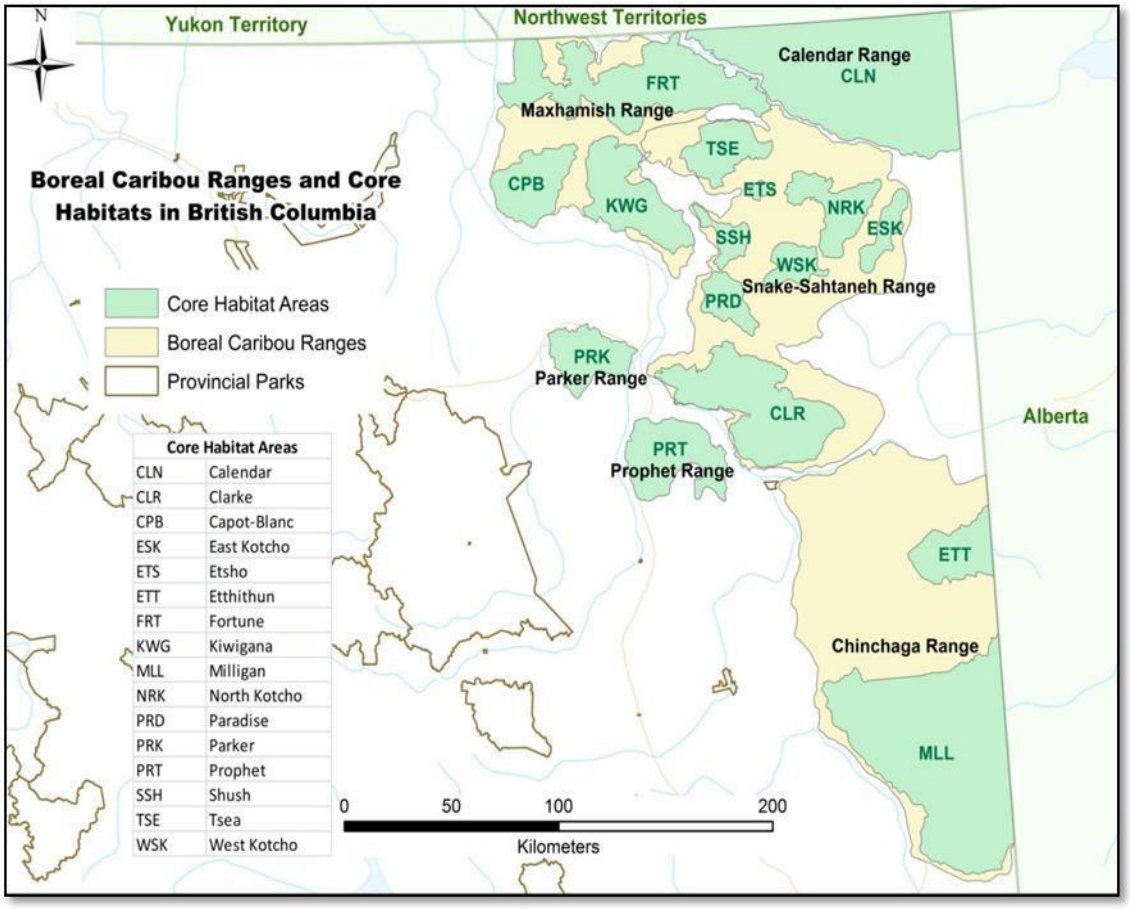

Figure 1. Boreal caribou herd ranges and core habitat areas in northeast British Columbia, Canada. Modified from: British Columbia Ministry of the Environment. (2010). Science update for the boreal caribou (*Rangifer tarandus*, pop. 14) in British Columbia. Victoria, BC. 54 pp.

2.1.2 Sample Collection: Caribou Mortalities

Upon detection of a suspected caribou mortality event, through transmitted GPS/satellite data or VHF signal status from radio-collars, comprehensive ground-based mortality site investigations were conducted as soon after death as possible (17). In BCHRP Year 1, where available and when stage of decomposition permitted, biological samples (e.g., long bones, lower jaws, tissue samples, and hair) were collected for health based analyses. In BCHRP Year 2, any and all caribou tissues remaining at mortality sites were collected and submitted to the University of Calgary, Faculty of Veterinary Medicine (UCVM) and/or the Canadian Wildlife Health Cooperative (CWHC) Calgary, AB for health based evaluation.

2.1.3 Sample Storage

The majority of biological samples from live-captured and dead caribou collected in winter from 2012–2016 were stored frozen (-20◦C) and shipped to the UCVM and/or the CWHC, Calgary, AB for in-house or referred health and diagnostic testing. Exceptions included: live winter ticks (voucher specimens) collected from n=9 and n=15 caribou with hair loss in winter 2013/2014 and 2014/2015 respectively and an opportunistic subset of n= 5 fecal samples collected in 2015 (n=1 Calendar herd range, n=2 Chinchaga, n=2 Snake-Sahtaneh) and submitted cooled/fresh (at 4^{o}C) for parasite analysis.

2.2 Boreal Caribou Health Assessment Model, BCHRP Years 1 and 2

In BCHRP Year 1, a comprehensive Boreal Caribou Health Assessment Model was developed to evaluate archived biological samples collected from live-captured and dead boreal caribou in winter 2012–2013. Pathogens and other determinants of caribou health were incorporated into the model based on a detailed review of the published peer review literature related to the health of *Rangifer* and other cervids, emerging techniques in wildlife health research, and input from biologists, wildlife veterinarians, and other stakeholders working with caribou in NE BC and elsewhere (12).

Background information along with a detailed list of pathogens and other health determinants evaluated as part of the Boreal Caribou Health Assessment Model in BCHRP Year 1 are reviewed in the BCHRP Year 1 Synthesis Report (12) (available online: www.bcogris.ca). Based on preliminary results obtained in Year 1, the health assessment model was modified slightly in BCHRP Year 2. Changes to the model included a more rigorous classification of winter tick infestations and additional analyses to more thoroughly evaluate the potential occurrence of blood borne pathogens in and the trace mineral status of boreal caribou in NE BC. Pathogens and other health determinants evaluated in Years 1 and 2 are presented in Table 2.

Table 2. Overview of pathogens, parasites, and non-infectious health determinants evaluated in Years 1 and/or 2 of the British Columbia Boreal Caribou Health Research Program.

Viral Pathogens
Alphaherpesvirus
Pestiviruses
Bacterial Pathogens
Brucella suis biovar 4
Erysipelothrix rhusiopathiae
Miscellaneous bacterial infections (mortalities)
Muscle, Lung, Neurotrophic, and Gastrointestinal Macroparasites
Protostrongylid nematodes (e.g. *Parelaphostrongylus andersoni)*
Gastrointestinal parasites (primary focus abomasal nematodes, e.g. *Ostertagia gruehneri*)
Trematodes (primary focus giant liver fluke, *Fascioloides magna)*
Tissue Inhabiting Protozoans
Neospora caninum
Toxoplasma gondii
Besnoitia tarandi
Miscellaneous Blood Borne Pathogens and Vector Borne Nematodes
Trypanosoma sp.
Anaplasma sp.
Babesia sp.
Setaria sp.
Onchocerca sp.
Ectoparasites
Dermacentor albipictus (winter tick)
Hypoderma tarandi (warbles)
Cephenemyia trompe (nasal bots)
Non-Infectious Health Determinants
Serum biochemistry
Complete blood counts (CBCs) with white blood cell differentials
Hair cortisol concentration (physiological stress)
Haptoglobin (acute phase protein)
Serum amyloid A (SAA) (acute phase protein)
Bone marrow fat content
Trace mineral levels (serum)

2.3 Diagnostic Tests

The BCHRP's health testing efforts in Year 1 and Year 2 were based out of the UCVM and the CWHC in Calgary, AB, Canada. The BCHRP partnered with a network of academic and commercial laboratories in Canada, the United States, and Europe to employ the most up to date and innovative methods of evaluating the health of caribou. The primary goal of our testing strategy was to identify less costly, less invasive, less complicated and more efficient and accurate indicators of caribou herd health. General strategies used for

health testing are described in detail in the BCHRP Year 1 Synthesis Report (12) and specific diagnostic tests employed for pathogen and health screening in BCHRP Year 1 are presented in Bondo et al. (18).

In BCHRP Year 2, the same diagnostic tests used in Year 1 were employed with some modifications for specific pathogens (see Section 3.0). In the capture period associated with BCHRP Year 1 (winter 2012–2013), *Dermacentor albipictus* (winter tick) infections were recorded (and tick voucher specimens collected) as incidental findings while radio-collars were deployed on caribou in the field. Standardized photographs of all animals captured in all years of the program (2013–2015) were used in BCHRP Year 2 to formally classify the occurrence and severity of winter tick infestations and the effects on boreal caribou in NE BC (see Section 3.4.7). In Year 2, trace mineral levels (Mn, Fe, Co, Cu, Zn, Se, Mo) were also measured in serum from n= 211 caribou captured in winter 2012/2013 (n=136), 2013/2014 (n=41) and 2014/2015 (n=33) using High-Performance-Liquid-Chromatography (HPLC) (in house assays: University of Guelph Animal Health Laboratory, Guelph Ontario). N=57 blood smears from n=27 caribou captured in 2014/2015 and n=15 captured in 2012/2013, but died by winter 2015/2016, were screened by University of Guelph Animal Health Laboratory, Guelph Ontario for evidence of infection with blood borne pathogens or parasites (e.g., *Trypanosoma, Anaplasma, Babesia*) and/or vector borne nematodes (e.g., *Setaria, Onchocerca*). As in Year 1, all diagnostic tests in BCHRP Year 2 were performed by trained laboratory technicians, wildlife veterinarians, or board certified veterinary specialists.

In Year 3, serum samples from winter 2012–2013 (n=24) and historical samples from 2003–2010 (n=56) were examined for evidence of exposure to *E. rhusiopathiae* using the same laboratory methods as Years 1 and 2. We also tested serum from captive reindeer used in the *E. rhusiopathiae* vaccination trial and cultured several tissues and organs of one of the reindeer for the presence of *E. rhusiopathiae* (see section 3.3.2.2.1). This reindeer declined in health and was euthanized and necropsied at the end of the study. Serum samples from Years 1 and 2 were re-tested to refine the cut-off value for the *E. rhusiopathiae* serology test. The methods used to determine the cut-off for the test are described by Mavrot et al. (19).

In Year 3, the historical samples and samples from Year 2 were tested for *Neospora caninum* and *Besnoitia tarandi* using the same methods as described in Year 1. The nasal swabs from Year 2 were tested for evidence of infection with alphaherpesviruses, and the results were compared with the serology results from the same individuals when possible.

2.4 Statistical Modelling

Prevalence or seroprevalence and 95% confidence intervals were calculated for binary and categorical data. Serology results were reported as binary data (positive/negative). To analyze continuous data, one-way ANOVA, Welch's ANOVA, Kruskall-Wallis, or Mann-Whittney U tests were used where appropriate and calculated in SPSS (IBM, Version 24.0).

Univariable models were constructed in STATA to analyze the associations between binary data and each fixed effect. If the fixed effects were categorical or binary, exact logistic regression was used. If the fixed effects were continuous, firth logistic regression was used because the models would not converge using exact logistic regression.

Only GPS/satellite collared caribou were included in the analyses for disturbance, moose density, seismic line density, and road density. We identified seasonal home ranges for each individual using the method of Walker et al. (20). For each season, we determined the 90th centile of movement distances between consecutive locations for each individual caribou. Each used location was buffered by the 90th centile of movement distances for the corresponding season (birthing, early summer, late summer, early winter, and late winter). Seasonal home ranges were constructed by merging the resulting polygons for each individual by season. We identified the densities of linear features for each seasonal home range using road and seismic line layers (21, 22). We identified the proportion of disturbed habitat by buffering roads and seismic lines and cutblocks (<40 yrs), and burns (<40 yrs), identified via forestry and fire layers (21), at a 500m radius (23). We determined male and female moose densities for each seasonal home range using estimates from previously conducted moose surveys (24, 25). For each pathogen, we included anthropogenic feature and moose density data in the analysis for the season in which transmission or exposure was most biologically plausible. If no season of interest was identified, anthropogenic feature and moose density data were included in the models for animal movements during the entire year. Female and male moose density were highly correlated ($R^2 > 0.80$), so we only included female moose density in the analysis.

Linearity between the log odds of each outcome and continuous independent variables were determined individually by examining a lowess curve and the significance of a quadratic term and its main effect in each univariable model. Independent variables that had nonlinear relationships with the outcome variable based on a significant quadratic term and visual assessment of the lowess curve were categorized into 3 quantiles or modelled as a quadratic relationship if appropriate. A variable was considered to be a confounding variable if it was a non-intervening variable and its removal from the model resulted in $\geq 30\%$ change in the coefficients of any statistically significant variable (26). Results were statistically analyzed at the herd level for only the Calendar, Chinchaga, Maxhamish, and Snake-Sahtaneh herds because effective sample size was too small for the Parker, Prophet, and Fort Nelson herds. The significance level for all analyses was set to $\alpha \leq 0.05$. Trends were indicated if $\alpha \leq 0.10$.

3.0 Results, Discussion, and Recommendations (Pathogens)

3.1 Terminology

3.1.1 Prevalence and Intensity

Prevalence refers to the proportion (%) of a sample found to have a specified condition (e.g. a specific parasite). Intensity refers to the number of parasites/parasite eggs of the same species detected from a parasite positive host.

3.1.2. Seropositive and Seronegative

An animal is considered to be seropositive when there is evidence that it has produced antibodies against a specific pathogen. In order to be seropositive an animal must have encountered a pathogen and remained alive for a sufficient time after exposure to produce antibodies specific against that pathogen (seroconversion). An increase in the number of

seropositive animals may be detected after an outbreak of infectious disease and may provide evidence that a particular pathogen was involved. However, it should be noted that being seropositive does not necessarily mean an animal is suffering from disease related to the pathogen in question, just that they have been exposed and produced an immune response. Animals classified as seronegative have no/below cut-off levels of antibodies specific to the pathogen in question indicating that they have not been exposed to the pathogen or may have been exposed but did not have sufficient time to produce specific antibodies against that pathogen (seroconvert) prior to live-capture and blood collection (or death). Alternatively, they may have been exposed previously but their immunity may have waned.

3.1.3 PCR and Culture Positive and Negative

A PCR (polymerase chain reaction) positive result means that DNA of interest (DNA indistinguishable from that of the pathogen of interest) was detected in a tissue sample while a PCR negative result means that DNA of interest was not present or was present below the detection limits of the assay. Inhibition may also have occurred. The presence of live pathogens in a tissue sample is not required to obtain a PCR positive result, only their DNA is required. The success of PCR may be adversely affected by inhibitors in decomposing tissue samples and other by factors related to sample storage and handling.

For an animal to be culture positive live pathogens must be recovered (grown) from the tissue sample in question. In culture negative animals the pathogen of interest is not recovered from the tissue sample in question. Culture positive tissue samples may provide evidence that a particular pathogen caused disease in or the death of an infected animal; however, further testing (e.g., histopathology) is often required to establish a diagnosis. The success of culture protocols relies on the presence of live pathogens in tissue samples and depending on the organism in question, may be reduced by environmental exposure, putrefaction, and sample storage conditions (e.g., freezing). As such, animals suspected to have died from a pathogen but are culture negative are not necessarily free of that pathogen.

3.2 Viruses

3.2.1 Alphaherpesvirus

Alphaherpesvirus is a virus that can cause both subclinical effects and overt disease in captive and free-ranging *Rangifer* including: no symptoms, keratoconjunctivitis, ulceration of the nasal, oral, and genital mucosa (+/- secondary bacterial infection), and severe respiratory disease, along with abortions, neonatal morbidity and mortality (27). The overall prevalence of exposure to alphaherpesvirus among adult female boreal caribou captured in NE BC each winter ranged from 60–63% (Table 3).

Based on the confidence intervals, the overall prevalence of alphaherpesvirus in boreal caribou from northeast B.C. appears higher than what was found in woodland caribou from the NWT (38%; 95% CI, 28-48; (28), but generally within range of the prevalence found in woodland caribou in Alberta (52%; 95% CI, 43-61; (29) and Saskatchewan (55%; 95% CI, 38-71; (30).

Table 3. Prevalence of exposure to alphaherpesvirus recorded in live-captured, adult female boreal caribou from six herds in northeast British Columbia in winter 2012/2013, 2013/2014, and 2014/2015 [a].

Herd	Alphaherpesvirus Prevalence and Sample Size			
	2012/2013	**2013/2014**	**2014/2015**	**2015/2016**
Calendar	61% (n=16/26)	80% (n=4/5)	100% (n=1/1)	ND
Chinchaga	69% (n=25/36)	67% (n=8/12)	43% (n=3/7)	ND
Maxhamish	57% (n=12/21)	62% (n=5/8)	62% (n=5/8)	100% (n=1/1)
Parker	83% (n=5/6)	0 (n=0/1)	100% (n=1/1)	ND
Prophet	13% (n=1/8)	ND	100% (2/2)	ND
Fort Nelson	100% (3/3)	67% (2/3)	(ND)	ND
Snake-Sahtaneh	65% (n=36/55)	50% (n=5/10)	50% (n=3/6)	ND
Overall (95% CI)	**63%(55-71)**	**62% (45-77)**	**60%(39-79)**	**NC**
Overall Sample Size	**(n=98/155)**	**(n=24/39)**	**(n=15/25)**	**(n=1/1)**

[a] (CI) Confidence interval; (ND) No data - designates herds where no individuals were captured in the sampling year; (NC) Not calculated – overall prevalence was not calculated in 2015/2016 because only 1 caribou was tested in this year.

The enzyme-linked immunosorbent assay (ELISAs) employed to screen caribou for exposure to alphaherpesvirus in BCHRP Year 1 (LSIVet Bovine IBR gB Blocking ELISA (Life Technologies Inc., Paris, France) was discontinued by the manufacturer in 2014. An alternative ELISA (SERELISA BHV-1 gB Ab Mono Blocking, Synbiotics Europe, SAS, France) was used in BCHRP Year 2. Like the original test, the assay employed in Year 2 had been previously validated in *Rangifer* (31). To confirm that results from both assays were comparable, we evaluated n=41 serum samples from BC boreal caribou collected in winter 2013/2014 with both kits. Results agreed in 95% (n=39/41) of the samples tested, so we combined the results of these tests for the statistical analysis, and excluded the results (n= 1 from each of Chinchaga and Snake-Sahtaneh) with discordant results.

Exposure to alphaherpesvirus was significantly associated with age. Old and mature adults were significantly more likely to be seropositive to alphaherpesvirus than young adults (All $P < 0.05$); however, mature and old adults did not significantly differ in their exposure to the virus ($P = 0.203$). Mature and old adults may have been more exposed to alphaherpesvirus than young adults because this virus, similar to herpesviruses in other species, is likely a lifelong infection in boreal caribou. Our results from 16 recaptured caribou supports this hypothesis because all recaptured caribou that tested positive on their first capture date (n=8) also tested positive 1-3 years later, and we found no evidence of any caribou being seropositive after testing seronegative on its first capture (Table 4).

Table 4. Alphaherpesvirus serology results from 16 recaptured female boreal caribou from 2012-2016. Test results from caribou that seroconverted (seronegative to seropositive) from their first and second captures are highlighted in yellow. Results of those that tested seropositive twice are in red and those that tested seronegative twice are in green.

Animal ID	Herd	Age[a]	2012/2013	2013/2014	2014/2015	2015/2016
SK005	Maxhamish	OA	Positive	Positive		
SK007	Maxhamish	YA; MA	Negative		Negative	
SK014	Parker	MA	Positive		Positive	
SK016	Parker	YA; MA	Positive		Positive	
SK020	Snake-Sahtaneh	MA	Positive		Positive	
SK033	Chinchaga	MA	Positive		Positive	
SK036	Chinchaga	MA	Negative		Negative	
SK066	Maxhamish	JUV: YA	Negative			Positive
SK079	Snake-Sahtaneh	YA; MA	Negative		Positive	
SK097	Snake-Sahtaneh	YA; MA	Positive		Positive	
SK100	Snake-Sahtaneh	YA; MA	Negative		Positive	
SK110	Snake-Sahtaneh	YA; MA	Negative		Negative	
SK126	Calendar	YA; MA	Negative		Positive	
SK136	Calendar	YA; MA	Negative		Negative	
SK146	Calendar	MA; OA	Positive			Positive
SK161	Prophet	MA; OA	Positive		Positive	

[a] YA – young adult; MA – mature adult; OA – old adult

Because age might be a potential confounding variable, and alphaherpesvirus is likely a life-long infection in boreal caribou, we only used young adults in all subsequent statistical analyses for this virus (Table 5).

Table 5. Univariable analyses for associations between exposure to alphaherpesviruses and health and fitness parameters in young adult female boreal caribou. Significant results are high-lighted in yellow and results with a trend are in green.

Parameter	Variable Type	Sample Size	P-Value
Survived to next winter	Binary	88	0.574
Year	Categorical	88	0.231
Pregnancy	Binary	87	0.109
Calf at heel	Binary	87	0.409
Residual antler velvet	Binary	80	0.131
Abnormalities	Binary	88	0.510
Herd	Categorical	58	0.401
Hair loss	Binary	88	0.099
Besnoitia tarandii	Binary	81	0.025
Erysipelothrix rhusiopathiae	Binary	81	0.521
Neospora caninun	Binary	82	0.562
Dorsal-spined larvae	Binary	83	0.185
Cobalt	3 quantiles	74	0.407
Copper	3 quantiles	74	0.799
Iron	3 quantiles	74	0.056
Manganese	3 quantiles	74	0.532
Selenium	3 quantiles	74	0.873
Zinc	3 quantiles	74	0.481
Haptoglobin	Continuous	81	0.033
Serum Amyloid A	3 quantiles	88	0.008
Hair cortisol concentration	Continuous	86	0.494
Disturbance	3 quantiles	38	0.339
Road density	3 quantiles	38	0.339
Seismic line density	3 quantiles	38	0.669
Moose density	3 quantiles	37	> 0.999

In young adults, there was a trend in the association between exposure to alphaherpesvirus and hair loss and iron (Table 5). Young adults without hair loss were more likely to have been exposed to the virus than those with hair loss, and young adults with iron levels in the middle quantile (3.4–4.5 ppm) were more likely to be seropositive for alphaherpesvirus than those in the lowest quantile (1.7–3.3 ppm) (P = 0.046).

Young adults seropositive to alphaherpesvirus were significantly more likely to be seropositive to *Besnoitia tarandii* than those that were seronegative to the virus (P = 0.025).

There was a significant association between exposure to alphaherpesvirus and serum amyloid A and haptoglobin (Table 5). Young adult caribou with SAA levels in the lowest quantile (0–3 0ug/ml) were more likely to have antibodies to alphaherpesvirus than those with levels in the middle quantile (31–81.4 ug/ml) (P = 0.003). There was a negative linear association between exposure to alphaherpesvirus and haptoglobin (Fig. 2).

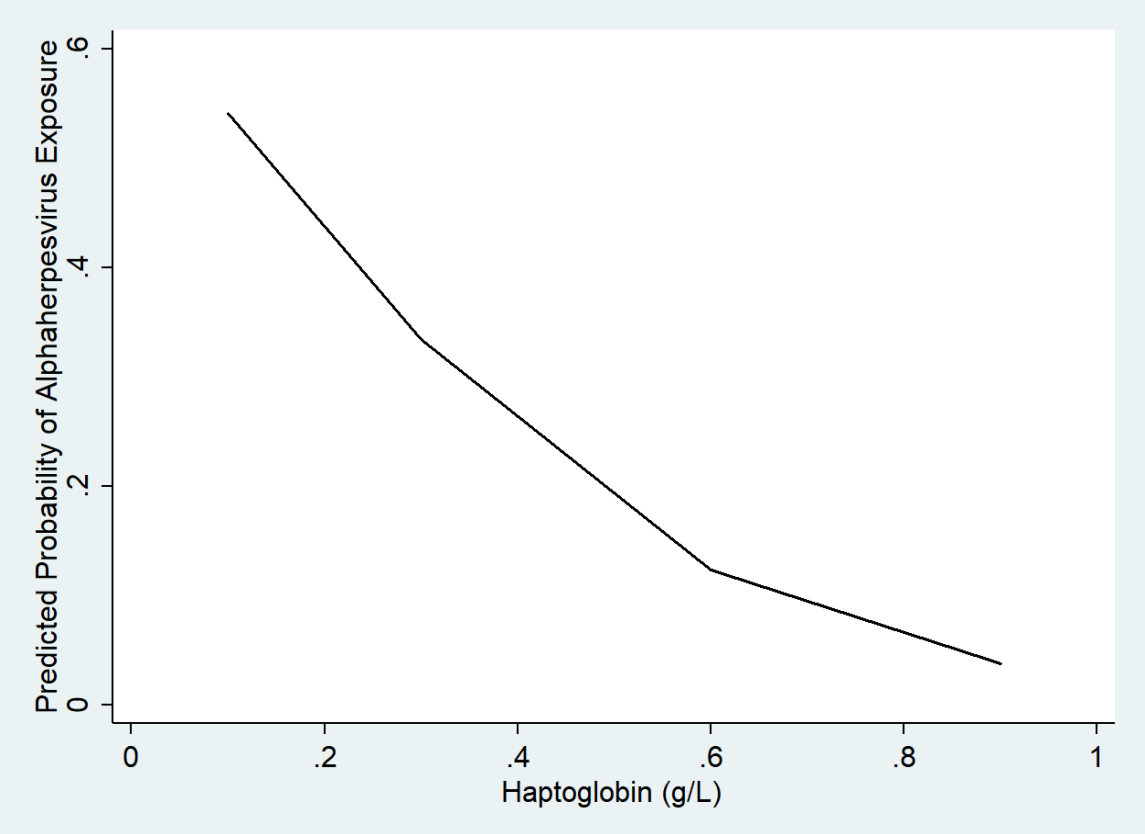

Figure 2. Association between haptoglobin levels and the probability of adult female boreal caribou in NE BC being exposed to alphaherpesvirus.

Herpesviruses are characterized by latency (non-clinical, persistent infections which can reactivate). Clinical disease, viral shedding, and transmission to susceptible caribou may be triggered by physiological stress in persistently infected animals (32). Two nasal swabs from each of 18 boreal caribou captured in 2014–2015 were tested for alphaherpesvirus using PCR at the Norwegian School of Veterinary Sciences, Tromsø, Norway. Four animals tested positive for the virus using PCR (Table 6).

Table 6. Paired serological and nasal swab results from 18 female boreal caribou captured in winter 2014-2015 [a].

Animal ID	Herd	Age	Serology Result	Nasal Swab PCR Result
SK007	Maxhamish	MA	Negative	Negative
SK009	Fort Nelson	OA	Positive	Negative
SK014	Parker	MA	Positive	Negative
SK016	Parker	MA	Positive	Negative
SK020	Snake-Sahtaneh	MA	Positive	Negative
SK026	Chinchaga	MA	Not tested	Positive
SK033	Chinchaga	MA	Positive	Positive
SK036	Chinchaga	MA	Negative	Negative
SK079	Snake-Sahtaneh	MA	Negative	Negative
SK118	Calendar	MA	Positive	Negative
SK126	Calendar	MA	Positive	Positive
SK136	Calendar	MA	Negative	Negative
SK161	Prophet	OA	Positive	Negative
SK219	Snake-Sahtaneh	OA	Positive	Positive
SK220	Snake-Sahtaneh	MA	Positive	Negative
SK222	Chinchaga	OA	Positive	Negative
SK223	Chinchaga	MA	Positive	Negative
SK224	Chinchaga	MA	Negative	Negative

[a] YA – young adult; MA – mature adult; OA – old adult.

The precise alphaherpesvirus found in boreal caribou is unknown at the present time. A sequences and homology search in GenBank, conducted by the Norwegian School of Veterinary Sciences on the positive nasal swabs, indicated alphaherpesvirus for the four nasal swabs. The alphaherpesvirus resembled CvHV2 (cervid herpesvirus 2), BoHV1(bovine herpesvirus 1) and other closely related alphaherpesviruses (e.g., bubaline and suid). These PCR results support the serology screenings that an alphaherpesvirus is circulating in boreal caribou in NE BC. Future molecular work is needed to determine the type of alphaherpesvirus infecting boreal caribou in this region; however, we expect it to be CvHV2.

Alphaherpesvirus appears to be endemic and distributed widely across all of the boreal caribou ranges (Fig. 3)

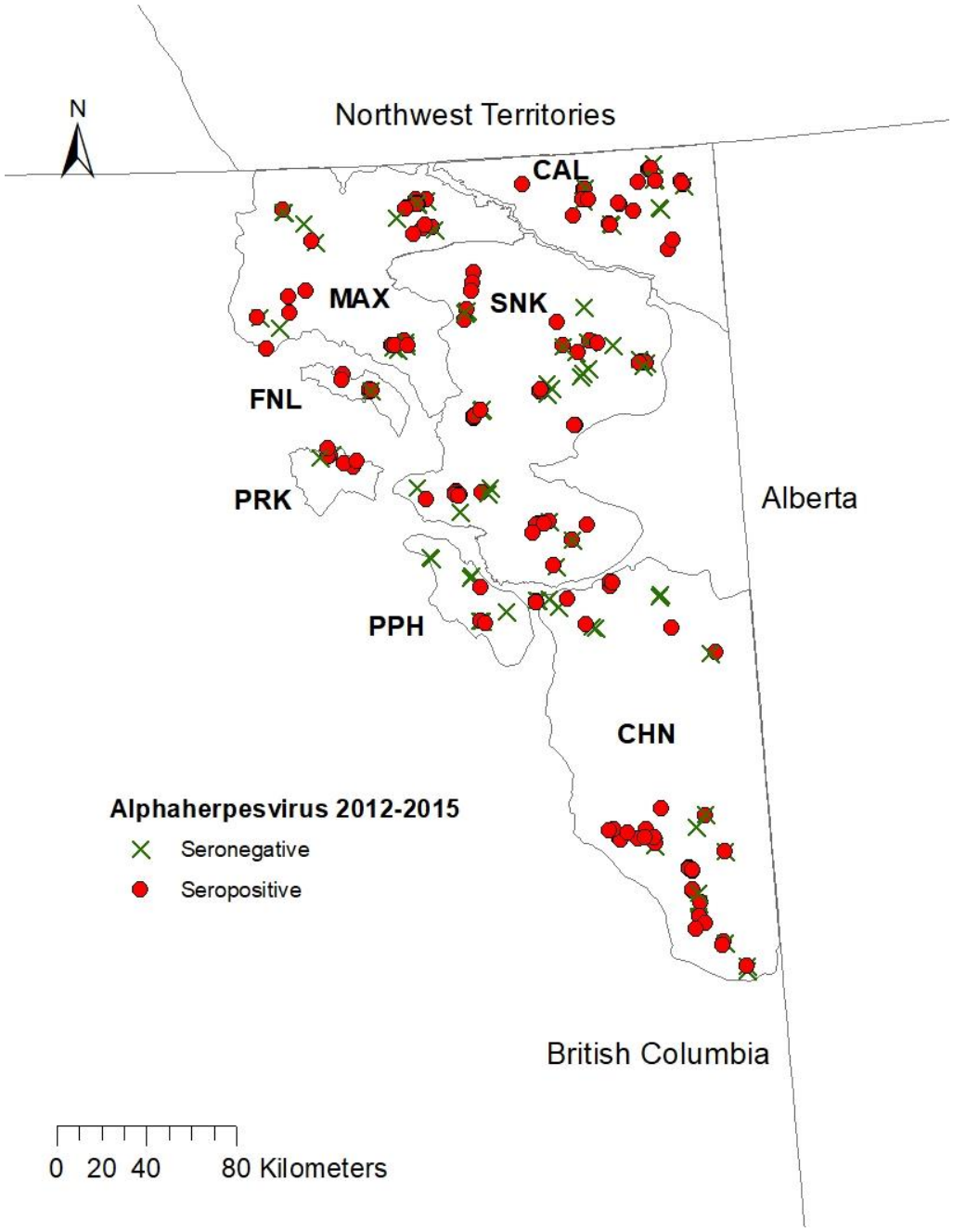

Figure 3. Spatial distribution of exposure to alphaherpesvirus in adult female caribou in northeast B.C. in winter 2012–2013, which was the winter prior to the high mortality.

3.2.2 Pestiviruses

In ruminants, pestiviruses can cause immunosuppression, respiratory and gastrointestinal disease as well as infertility, abortions, and neonatal morbidity and/or mortality (33-35). Pestiviruses such as Bovine viral diarrhea virus-1 (BVDV-1), Bovine viral diarrhoea virus-2 (BVDV-2), and Border disease virus (BDV) can cause respiratory, reproductive disease and mortality in ruminants (36).

Antibodies to pestiviruses using serological tests based on BVDV-1 strains isolated from cattle have been found in *Rangifer* spp. in North America and Europe (37). There have been no published reports on the isolation of pestiviruses from wild or semi-domesticated *Rangifer* spp. However, *Rangifer* experimentally infected with BVDV-1 developed clinical illness and developed laminitis (35), and a pestivirus was isolated from an Eurasian tundra reindeer (*R. t. tarandus*) that died in a German zoo (38).

In BCHRP Year 1, an ELISA (Synbiotics SERELISA BVD Kit**,** Synbiotics Corporation, Lyon, France) was used to test boreal caribou serum collected in winter 2012/2013 and 2013/2014 for exposure to pestiviruses. Only one serum sample tested seropositive (Table 7). However, the serostatus was classified as 'doubtful' in 32% of caribou captured in 2012/2013 and in 37% of caribou captured in 2013/2014 (Table 7). Only one recaptured caribou was tested for exposure to this virus over multiple years, and she tested seronegative in both winter 2012/2013 and 2013/2014.

Table 7. Pestivirus seroprevalence and sample sizes for boreal caribou captured in winter 2012/2013 and 2013/2014.

Herd[a]	**Pestivirus Seroprevalence and Sample Size**					
	Positive Result		**Doubtful Result**		**Negative Result**	
	2012/2013	**2013/2014**	**2012/2013**	**2013/2014**	**2012/2013**	**2013/2014**
CAL	0 (n=0/27)	0 (n=0/5)	52% (n=14/27)	0 (n=0/5)	48% (n=13/27)	100% (n=0/5)
CHN	0 (n=0/36)	0 (n=0/13)	14% (n=5/36)	77% (n=10/13)	86% (n=31/36)	23% (n=3/13)
MAX	4% (n=1/23)	0 (n=0/8)	26% (n=6/23)	0 (n=0/8)	70% (n=16/23)	100% (n=8/8)
PRK	0 (n=0/7)	0 (n=0/1)	14% (n=1/3)	0 (n=0/1)	86% (n=2/3)	100% (n=3/3)
PPH	0 (n=0/9)	(ND)	11% (1/9)	ND	89% (n=8/9)	ND
FNL	0 (0/3)	0 (0/3)	33% (n=1/3)	0 (n=0/3)	67% (n=2/3)	100% (n=3/3)
SNK	0 (n=0/56)	0 (n=0/11)	43% (n=24/56)	45% (n=5/11)	57% (n=32/56)	11% (n=6/11)
Overall (95% CI)[b]	**1% (25-40)**	**0 (0-9)**	**32%(25-40)**	**37% (22-53)**	**67% (59-74)**	**63% (47-78)**
Overall Sample Size	**(n=1/161)**	**(n=0/41)**	**(n=52/161)**	**(n=15/41)**	**(n=108/161)**	**(n=26/41)**

[a] Cal – Calendar; CHN – Chinchaga; MAX – Maxhamish; PRK – Parker; PPH – Prophet; FNL – Fort Nelson; Snake-Sahtaneh – SNK; CI – confidence interval; ND - no data - designates herds where no individuals were captured in the sampling year.

To further understand the "doubtful" results obtained from the ELISA test used in Year 1, a virus neutralization (VNT) assay for pestiviruses (Prairie Diagnostic Services Inc., Saskatoon, SK) was used in BCHRP Year 2 to test a subset of serum samples (n=62). The subset of serum samples tested with the VNT included the 1 serum sample that previously tested positive in winter 2012-2013, all previously tested samples from 2013/2014, and 20 samples from 2014-2015 (n=7, Chinchaga; n=7, Maxhamish; n=1, Prophet; n=5, Snake-Sahtaneh) that had not been previously tested with the ELISA test from Year 1.

In the VNT, serial dilutions of caribou serum (possibly containing anti-pestivirus antibodies) were incubated with an infectious pestivirus of cattle, BVDV. Susceptible cells from cattle were then added to the virus/serum mix and, after a second incubation, were examined for evidence of virus associated damage. If serum samples contained antibodies against pestiviruses, then no (or limited) virus caused cell damage was observed.

No seropositive boreal caribou (n=0/62) were identified using the VNT, including the positive sample and all samples testing "negative" or "doubtful" using the ELISA in Year 1.

These results suggest that the prevalence of pestiviruses in boreal caribou is low. However, antigenic variation between pestiviruses of caribou and those found in domestic cattle (used as the source of antigen or as a positive control in many diagnostic tests, including the ones we used in the ELISA and VNT tests) may have adversely influenced antibody binding properties and the reliability of the test results obtained using.

Phylogeny indicated that the pestivirus isolated from the reindeer from the German zoo was most closely related to BDV-2 (39, 40). It is, therefore, probable that the pestivirus circulating in reindeer is BDV-like, specific and endemic to their populations, and not yet described (41).

Given the apparent low prevalence of exposure and the uncertainty surrounding test results, exposure to pestiviruses was not examined further as part of the BCHRP. However, our "doubtful" results from the ELISA test may indicate that a pestivirus is circulating in this population of boreal caribou and we could not detect it based on the domestic animal tests used. More research is necessary to identify the specific pestiviruses in caribou and reindeer and to evaluate and validate further diagnostic tests for this potential pathogen in *Rangifer*. The impacts that pestiviruses might have on boreal caribou are unknown and need further study. To help establish a baseline for this virus, it is recommended that archived boreal caribou be tested further for pestiviruses once other diagnostic tests for pestiviruses become available for *Rangifer*.

3.3 Bacteria

3.3.1 *Brucella suis* biovar 4

Brucella suis biovar 4 is a bacterial pathogen of caribou and reindeer found in caribou herds in some parts of northern Canada and Alaska (42-44). In at least barren ground caribou, infection with *B. suis* biovar 4 is subclinical or may be associated with severe chronic disease including: bursitis and arthritis plus a variety of reproductive disorders leading to reproductive failure and neonatal morbidity or mortality (45, 46).

In winters of 2012/2013, 2013/2014, and 2014/2015, there was no evidence that any of the adult female boreal caribou in NE BC had been previously exposed to *Brucella* spp. (Table 8). There also was no evidence that any of the n=17 caribou, all of which were first captured in winter 2012/2013 and then recaptured in winter 2014/2015, had seroconverted.

In total, n=222 individual boreal caribou were screened for evidence of exposure to *Brucella* in BCHRP Years 1 and 2. The sensitivity of the ELISA employed in this study was 100% and the specificity 99.3% (27). The current population estimate for boreal caribou in the study area is 728 individuals (47). As a result, we can be 95% certain that *Brucella suis* biovar 4 does not occur in boreal caribou from NE BC at a prevalence of greater than 1.0–1.2% at the present time. This is important baseline knowledge.

Table 8. *Brucella* seroprevalence and sample sizes for boreal caribou captured in winter 2012/2013, 2013/2014, 2014/2015, and 2015/2016.

Herd[a]	***Brucella* Prevalence and Sample Size**			
	2012/2013	**2013/2014**	**2014/2015**	**2015/2016**
Calendar	0 (n=0/26)	0 (n=0/5)	0 (n=0/1)	ND
Chinchaga	0 (n=0/36)	0 (n=0/13)	0 (n=0/7)	ND
Maxhamish	0 (n=0/21)	0 (n=0/8)	0 (n=0/8)	0 (n=0/1)
Parker	0 (n=0/6)	0 (n=0/1)	0 (n=0/1)	ND
Prophet	0 (n=0/8)	(ND)	0 (n=0/2)	ND
Fort Nelson	0 (0/3)	0 (0/3)	(ND)	ND
Snake-Sahtaneh	0 (n=0/55)	0 (n=0/11)	0 (n=0/5)	0 (n=0/1)
Overall (95% CI)[a]	**0 (0-0.02)**	**0 (0-0.09)**	**0 (0-0.14)**	**NC**
Overall Sample Size	**(n=0/155)**	**(n=0/41)**	**(n=0/24)**	**(n=0/2)**

[a] CI - confidence interval; ND – No data - designates herds where no individuals were captured in the sampling year; NC – not calculated because sample size was too small.

Given the potential impact of *B. suis* biovar 4 for caribou (as well as other ungulates and the livestock industry) the BCHRP working group currently recommends continued monitoring of live and dead caribou and initiating targeted serological testing of herds if clinical signs associated with this disease (e.g., swollen joints, reproductive failure) are observed. To date, this pathogen has not been detected clinically or through surveys of boreal caribou in NE BC.

3.3.2 *Erysipelothrix rhusiopathiae*

3.3.2.1 Overview of *Erysipelothrix rhusiopathiae* findings in BCHRP Year 1

Infection with *Erysipelothrix rhusiopathiae* is a known cause of chronic disease, subacute illness, and acute or per-acute death in domestic and wild ungulates (48, 49). Abortions caused by *E. rhusiopathiae* have also been recorded in some species (50). *Erysipelothrix rhusiopathiae* is a pathogen of emerging importance for northern ungulates and has been identified as the agent most likely to be responsible for large scale disease outbreaks and mortality events (which may be associated with recent population level declines) in free-ranging muskoxen in the Canadian Arctic (51). This bacterium has also been recorded as the probable cause of severe disease or death in free-ranging deer and moose (52, 53) and may have been responsible for a historical (1930's) outbreak of severe/fatal disease in semi-domesticated Scandinavian and Russian reindeer (54). *Erysipelothrix* spp. has also been recovered from the carcasses of free-ranging bison found dead in the Northwest Territories (55).

In BCHRP Year 1, *E. rhusiopathiae* was identified in the tissues of n=5 dead boreal caribou (12). PCR and/or culture positive animals represented 63% (n=5/8) of boreal caribou mortalities examined in 2013 from which usable tissue samples (for health and disease testing) were obtained. This included n= 4 radio collared caribou found dead and an un-collared yearling male caribou found dying (see section 3.3.2.1.1) in NE BC between April and September 2013 (12)[reviewed].

Using a proprietary ELISA under development at UCVM (56), evidence of exposure to *E. rhusiopathiae* was also identified in the serum of boreal caribou captured in NE BC in winter 2012/2013 and 2013/2014 (12). To our knowledge, these findings represented the first records of this pathogen in free-ranging caribou in North America. The relatively high number of PCR and/or culture positive caribou mortalities, the rare finding of a moribund caribou most likely clinically affected by the bacterium (see Section 3.3.2.1.1) along with a concentration of unusual caribou deaths occurring in spring and summer of 2013 (Figs. 4, 5, and 6) and lack of mortalities due to suspect disease detected in winter 2014-2016 (Fig. 7) suggests that *E. rhusiopathiae* played a role in caribou mortalities recorded in NE BC in 2013.

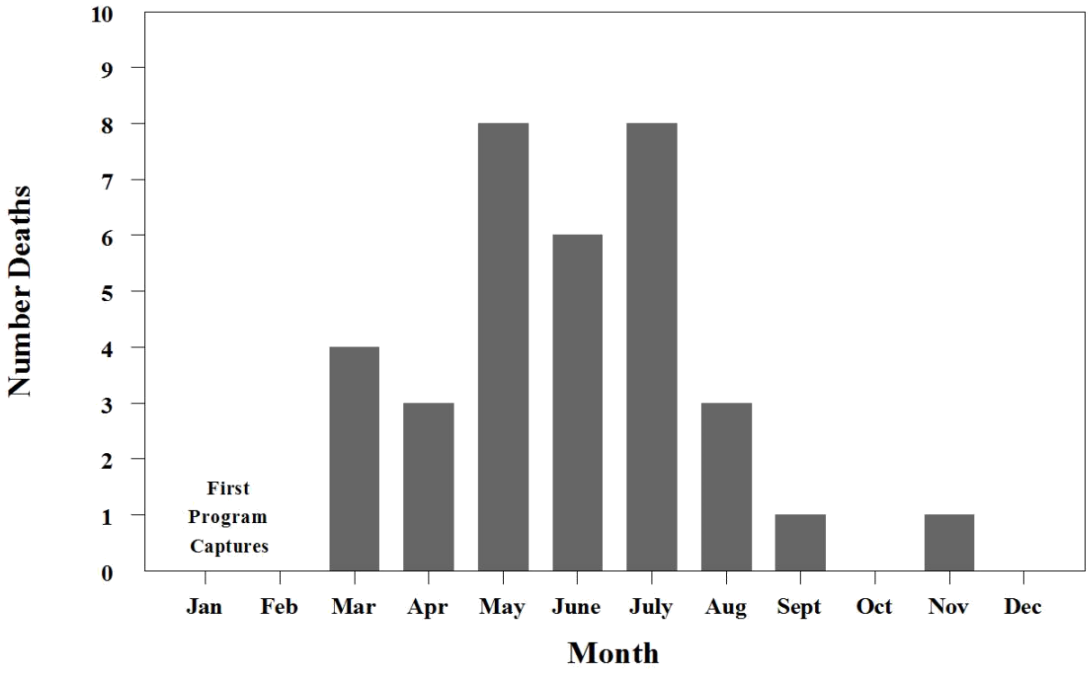

Figure 4. Distribution of known boreal caribou mortalities recorded in northeast British Columbia between March and December 2013. The majority of boreal caribou deaths recorded in 2013 were concentrated in late spring and summer. Many mortalities occurring in this period were not caused by predation (see section 3.3.2.1.1).

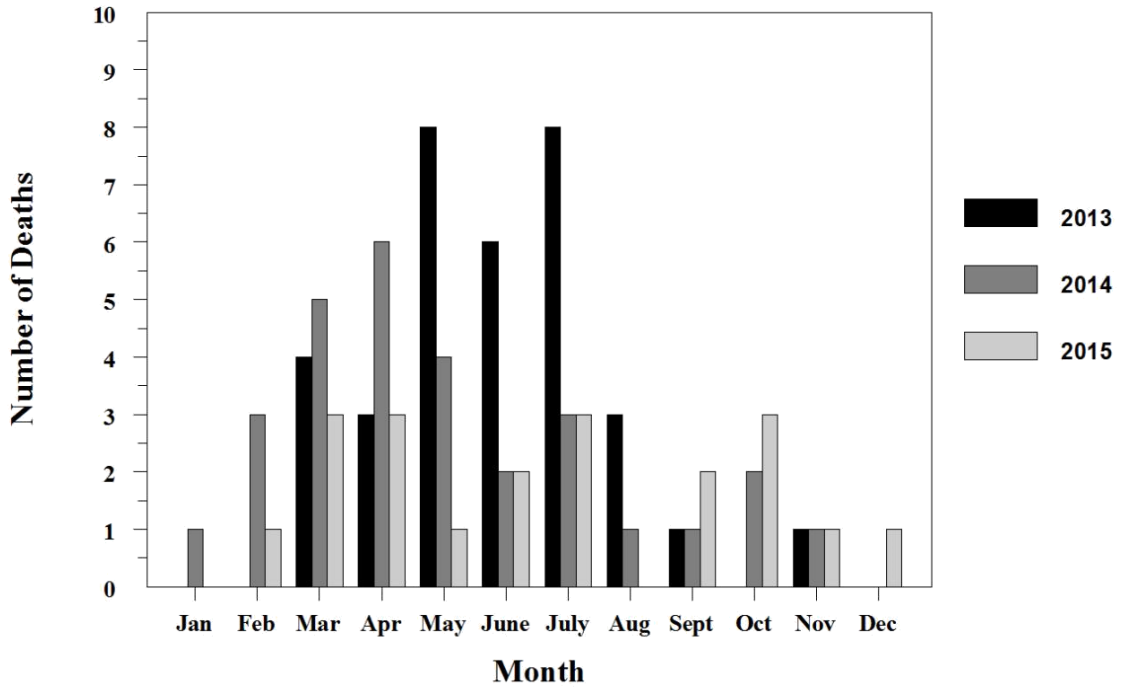

Figure 5. Distribution of mortalities recorded in radio-collared, adult female boreal caribou from northeast British Columbia between March 2013 and December 2015. N=12+ unusual caribou mortalities possibly related to infectious disease (Fig. 4) were recorded in 2013 vs. n=1 in 2014 and n=0 in 2015. The changing pattern and presentation of caribou mortalities in this time period appeared to follow changes in the pattern of exposure to the bacterial pathogen *Erysipelothrix rhusiopathiae* (Table 9).

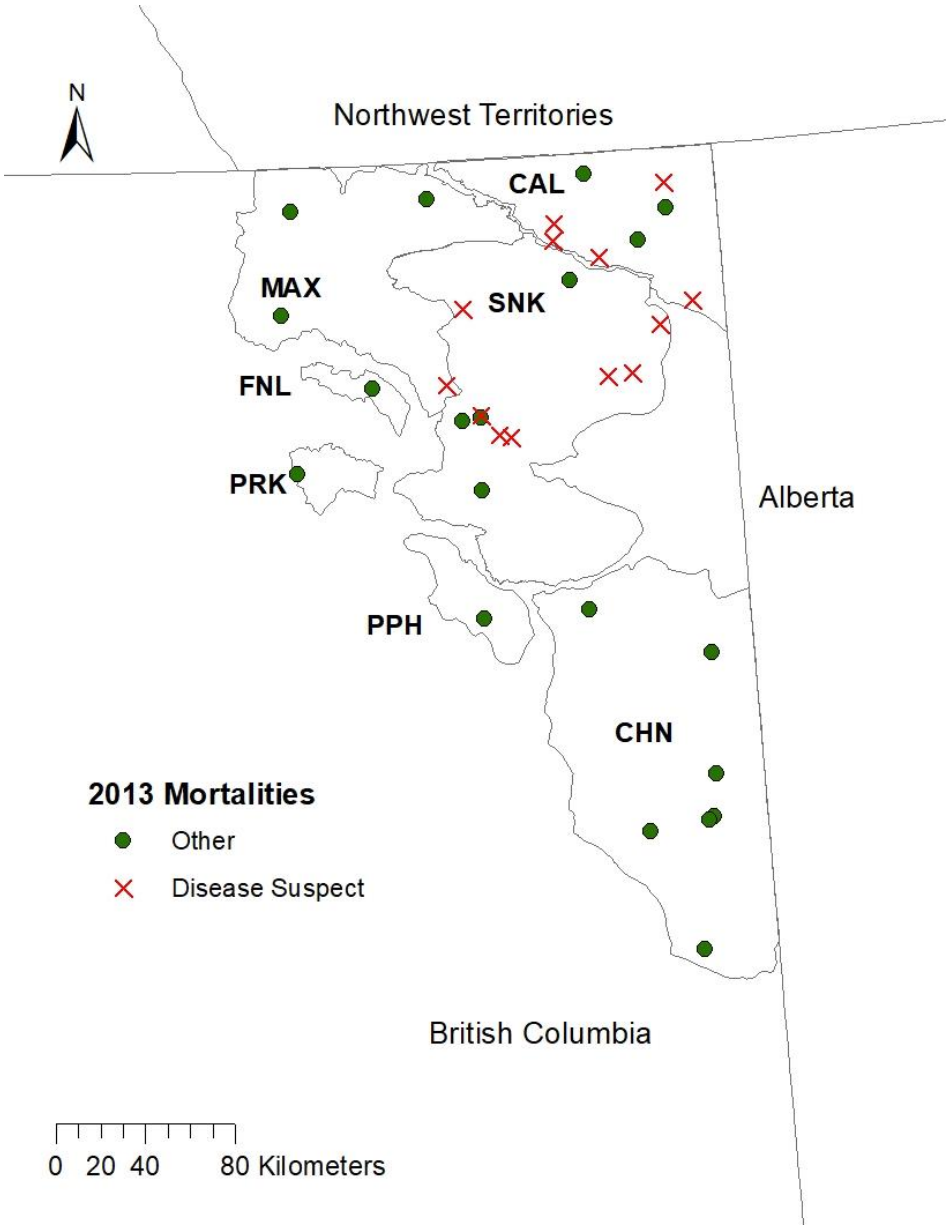

Figure 6: Spatial distribution of disease suspect mortalities of radio-collared adult female boreal caribou that died in 2013.

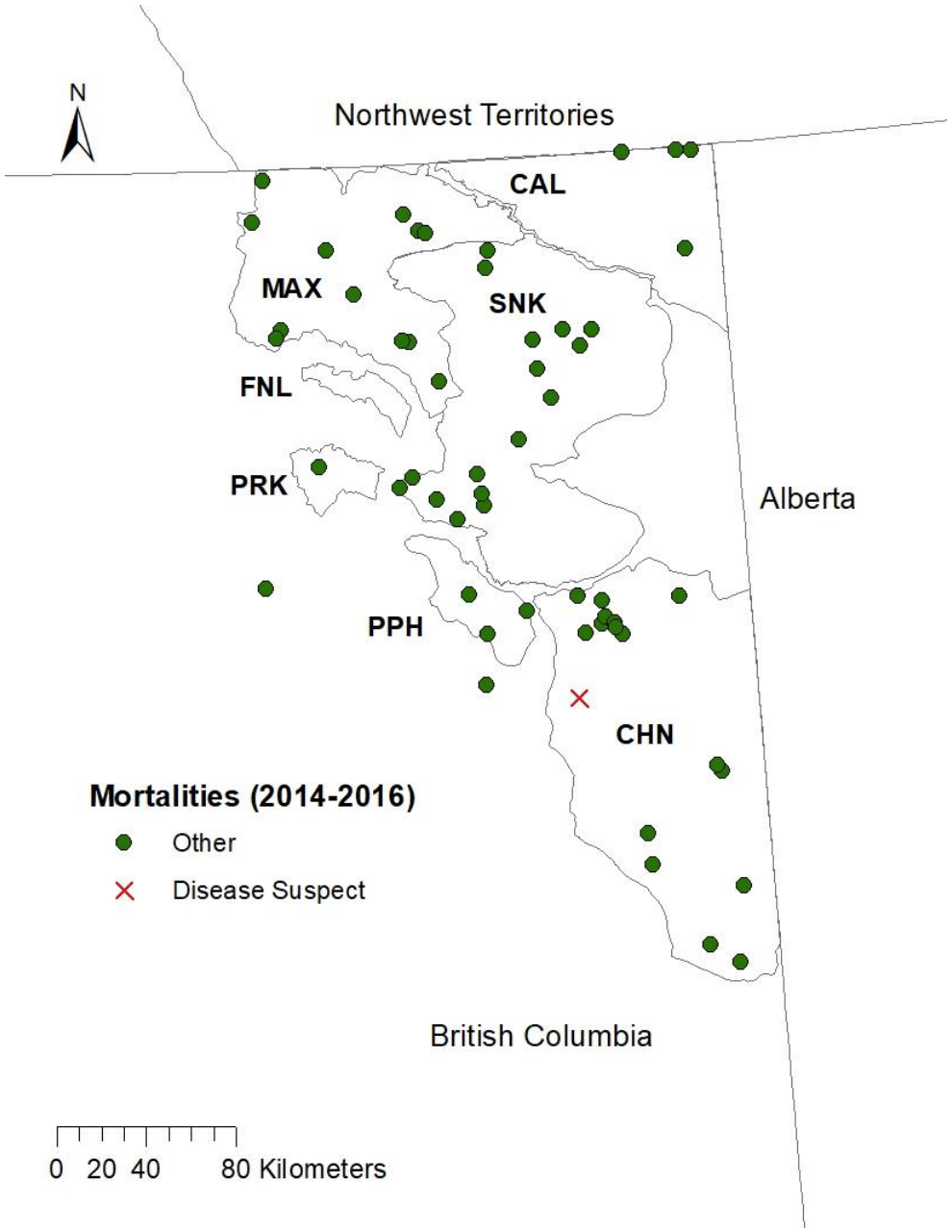

Figure 7. Spatial distribution of disease suspect mortalities of radio-collared adult female boreal caribou that died from 2014-2016.

3.3.2.1.1 Case Report example: *Erysipelothrix rhusiopathiae* in an un-collared yearling male caribou from northeast British Columbia

On March 30, 2013 an un-collared yearling male boreal caribou from the Snake-Sahtaneh herd range of NE BC was observed (from the air) alive but moribund in close proximity to a group of caribou containing collared animals. The caribou is believed to have died sometime between March 30, 2013 and April 2, 2013 when the mortality site and carcass were examined in detail. The carcass was lying on snow and the surrounding vegetation was disturbed with evidence that the animal had been thrashing around the site prior to death (Fig. 8). Despite some scavenging, the carcass was relatively intact and there was no indication of predator attack (ante mortem wounding etc.). These findings are consistent with death caused by a health-related issue. A necropsy was performed and samples collected for microbiological and histological analysis.

Erysipelothrix rhusiopathiae was cultured from multiple organs (femur bone marrow, lung, liver, and skeletal muscle) in ~ pure growth on selective media. Some bacteria associated with putrefaction were also cultured in low numbers from the same tissues using non-selective media (e.g. *Escherichia coli* and *Clostridium sp*.). The identity and genetic profile of selected *E. rhusiopathiae* colonies from each tissue was then confirmed using PCR, 16S sequencing, and/or Illumina MiSeq platform for sequencing (Nextera XT sample preparation kit-generating 250 base pair paired-end reads) (57, 58) (also see Section 3.3.2.2.2). Gross and histopathological examination of tissues revealed severe (likely fatal) aspiration pneumonia and indicated that the caribou was in fair condition (some internal fat reserves, 60.6 % femur marrow fat, mild hepatic atrophy). Liver levels of trace minerals and selected toxins (Vitamin A, Vitamin E, Be, Mg, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Sr, Mo, Cd, Sn, Sb, Ba, Tl, Bi, Pb, and Hg) were all within normal limits. All considered, these findings suggest that *E. rhusiopathiae* may have played a role in the death of this animal.

In free-ranging wildlife, moribund animals are rarely observed due to their quick removal by predators and scavengers. Blood obtained from the heart of this caribou tested negative for exposure to *E. rhusiopathiae* which may indicate that the disease process was relatively acute in this individual (see Section 3.3.2.2.1). Acute (or peracute) death has been recorded muskoxen (51), moose (52), and reindeer (54) infected with *E. rhusiopathiae*. as well as other caribou mortalities observed in NE BC in spring and summer, 2013 (Fig. 9). For example, in addition to n=5 dead or dying caribou tested and culturing positive for *Erysipelothrix rhusiopathiae*, a retrospective analysis of all caribou deaths in 2013 identified an additional n=7 (minimum number) boreal caribou mortalities that were relatively intact and exhibited no signs of predation (Fig. 9). Together, these findings also suggest that *E. rhusiopathiae* may have been a factor in the relatively high number of caribou mortalities recorded in NE BC in 2013.

Figure 8. An un-collared yearling male boreal caribou (*Rangifer tarandus*) found moribund in the Snake-Sahtaneh herd range of northeast British Columbia in March, 2013. Photo credit: D. Culling and B. Culling, Diversified Environmental Services Inc., Fort St. John, BC.

Figure 9. Examples of unusual mortalities observed in boreal caribou from northeast British Columbia in spring and summer, 2013. Unfortunately, no tissue samples were available from most of these cases due to logistical considerations encountered in the field. Photo credit: D. Culling and B. Culling, Diversified Environmental Services Inc., Fort St. John, BC.

3.3.2.2 *Erysipelothrix rhusiopathiae* findings BCHRP Years 2 and 3

3.3.2.2.1 Serology

The ecology of *E. rhusiopathiae* in free-ranging caribou is poorly understood and this bacterium was one of the primary research topics explored in BCHRP Years 2 and 3 due to Year 1 findings. Changes in serum antibody titres and/or the prevalence of exposure over time can help to increase understanding of the ecology of a specific pathogen and may help to identify the cause of infectious disease outbreaks in wildlife. In BCHRP Year 1, we employed a proprietary ELISA developed at by Wendy Hutchins and the Kutz Lab at UCVM to test and compare the prevalence of exposure to *E. rhusiopathiae* in boreal caribou captured in NE BC in 2012/2013 and 2013/2014.

In BCHRP Year 2, we refined our *Erysipelothrix* ELISA for use in caribou and established working cut-off values that more accurately identified seropositive and seronegative individuals. As part of this work, modifications were made to the type and ratio of reagents used in the testing process. In BCHRP Year 3, we worked with Dr. Fabien Mavrot to further refine the cut-off values for the *Erysipelothrix* ELISA test based on a vaccination trial using captive reindeer and on a concurrent analysis of a large number (n>200) of serum samples from other caribou populations in North America being tested for exposure to *Erysipelothrix*, which was part of a separate research project in the Kutz lab at UCVM. In order to determine the cut-off value using this method, all samples previously collected in winter 2012–2013, 2013–2014, and 2014–2015 were tested again and then evaluated against refined cut-off values to more accurately determine the serostatus of the individual animals.

Using the new cut-off values determined in BCHRP Year 3, we found that the overall prevalence of exposure to *E. rhusiopathiae* across six boreal caribou herd ranges in NE BC in 2012/2013 was 14% (Table 9). Seroprevalence in boreal caribou was significantly associated with year (Table 10) and was lower in winter 2012/2013 than in 2013/2014, 2014/2015, and 2015/2016. Although exposure to *E. rhusiopathiae* in caribou was significantly associated with season (Table 10), the association was confounded by year and no longer significant when year was included in the model.

Table 9. *Erysipelothrix rhusiopathiae* seroprevalence and sample sizes of boreal caribou captured in winter 2012/2013 and the three winters following the high mortality.

Herd[a]	***E. rhusiopathiae* Seroprevalence and Sample Size**			
	2012/2013	**2013/2014**	**2014/2015**	**2015/2016**
Calendar	16% (n=4/25)	20% (n=1/5)	100% (n=1/1)	0 (n=0/1)
Chinchaga	3% (n=1/36)	31% (n=4/13)	20% (n=1/5)	75% (n=3/4)
Maxhamish	14% (n=3/21)	62% (n=5/8)	57% (n=4/7)	33% (n=1/3)
Parker	40% (n=2/5)	100% (n=1/1)	100% (n=1/1)	100% (n=2/2)
Prophet	25% (n=2/8)	ND	100% (n=1/1)	ND
Fort Nelson	0 (0/2)	33% (1/3)	ND	ND
Snake-Sahtaneh	17% (n=9/54)	50% (n=5/10)	40% (n=2/5)	14% (n=1/7)
Overall (95% CI)	**14% (9-21)**	**42% (27-59)**	**50% (27-73)**	**41% (18-67)**
Overall Sample Size	**(n=21/149)**	**(n=17/40)**	**(n=10/20)**	**(n=7/17)**

[a] CI – confidence interval; ND – No data; n = total sample size.

Similarly, there was a significant quadratic relationship between testing positive for *Erysiplelothrix* and iron levels, a marginally significant relationship between *E. rhusiopathiae* and zinc levels, and a linear association between testing positive for *E. rhusiopathiae* and haptoglobin levels (Table 10). However, when year was included in each of the models, year confounded the other variables and the associations were no longer significant.

Exposure to *E. rhusiopathiae* was not significantly associated with herd (Table 10) and was distributed across the boreal caribou ranges in winter 2012/2013 (Fig. 10) and the years following the high mortality (Fig. 11).

Table 10. Univariable analyses for associations between exposure to *E. rhusiopathiae* from winter 2012/2013 to 2015/2016 and health and fitness parameters in female adult boreal caribou. Significant associations are high-lighted in yellow and trends are in green.

Parameter	**Variable Type**	**Sample Size**	**P-Value**
Survived to next winter	Binary	227	> 0.999
Year	Categorical	27	< 0.001
Pregnancy	Binary	219	0.652
Calf at heel	Binary	219	0.771
Age	Categorical	227	0.137
Residual antler velvet	Binary	212	0.653
Abnormalities	Binary	232	0.431
Herd	Categorical	134	0.261
Hair loss	Binary	227	0.847
Besnoitia tarandii	Binary	209	0.426
Alphaherpes virus	Binary	207	0.169
Neospora caninun	Binary	216	>0.999
Dorsal-spined larvae	Binary	200	0.591
Cobalt	3 quantiles	186	0.441
Copper	3 quantiles	187	0.750
Iron (squared)	3 quantiles	186	0.011
Manganese	Continuous	186	0.994
Selenium	3 quantiles	186	0.307
Zinc	3 quantiles	185	0.051
Haptoglobin	Continuous	200	< 0.001
Serum Amyloid A	3 quantiles	210	0.477
Hair cortisol concentration	3 quantiles	208	0.169
Disturbance	3 quantiles	38	0.339
Road density	Continuous	102	0.141
Seismic line density	3 quantiles	102	0.249
Moose density	3 quantiles	115	0.751
Season	Binary	227	0.013

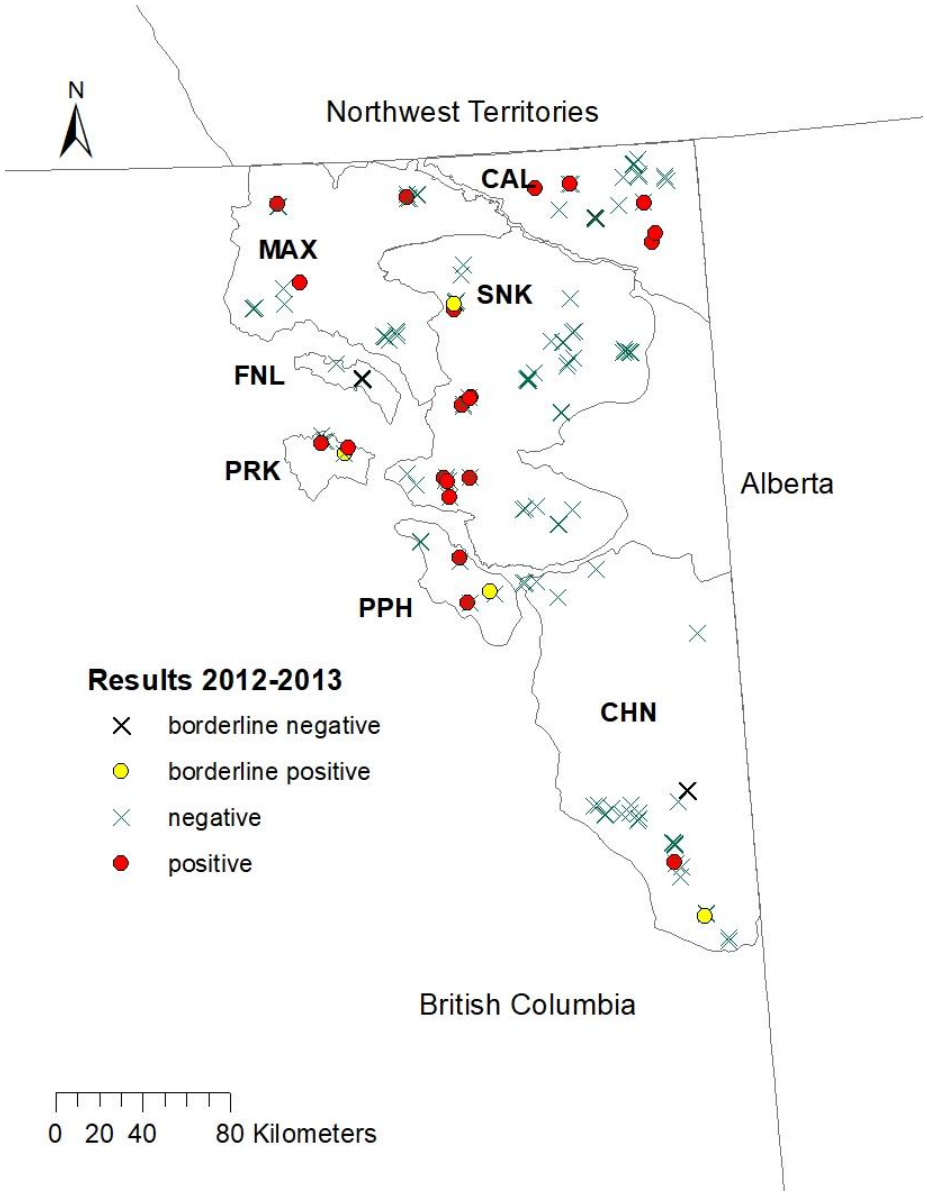

Figure 10. Spatial distribution of exposure to *E. rhusiopathiae* in adult female caribou in northeast B.C. in winter 2012-2013, the winter prior to the high mortality.

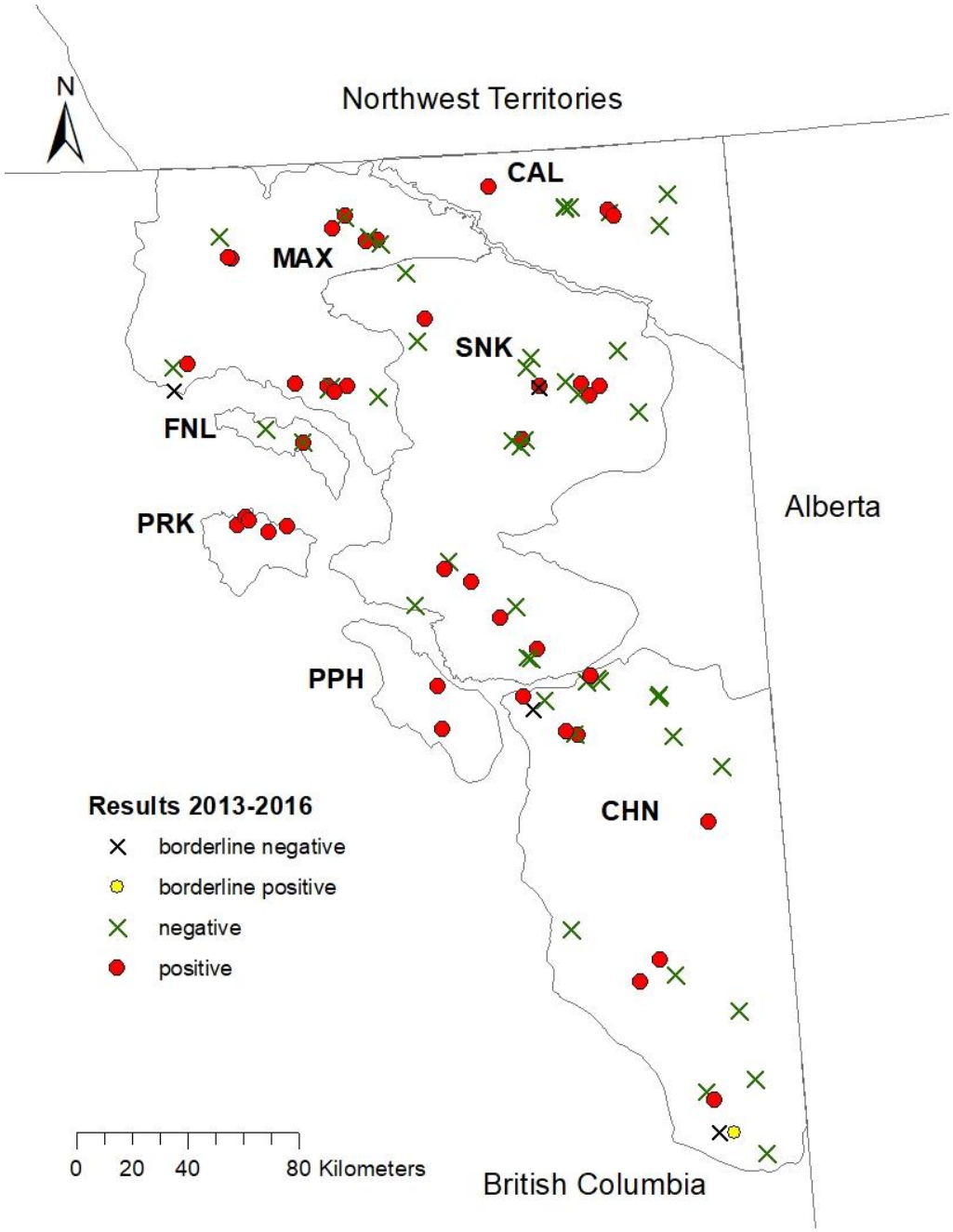

Figure 11. Spatial distribution of exposure to *E. rhusiopathiae* in adult female caribou in northeast B.C. in winters 2013/2014, 2014/2015, and 2015/2016. Exposure in each of these winters was significantly higher than in winter 2012/2013. Repeated captures were excluded from the statistical analysis but are included on the map.

We also examined *E. rhusiopathiae* exposure from archived historical serum collected in winter from 2000–2010 (Table 11). *Erysipelothrix rhusiopathiae* exposure was also significantly lower in 2012/2013 than during 2003–2009, and overall exposure from 2000–2010 did not significantly differ to exposure in 2013/2014, 2014/2015, and 2015/2016 (All $P < 0.05$; Fig. 12).

The high seroprevalence in the historical samples and spatial distribution of exposure (Fig. 13) indicate that *E. rhusiopathiae* is not new to the area and has been present in these herds in the past. These limited data over time do suggest that exposure, or at least seroprevalence, may also be cyclical. A similar cyclicity has been reported for *E. rhusiopathiae* in harbor seals (59). Such cyclicity may indicate cyclicity in exposure to the pathogen or waning of immunity with subsequent outbreaks. Work by Forde et al. (58) demonstrated multiple different strains of *E. rhusiopathiae* is cycling in these herds.

Table 11. Historical *E. rhusiopathiae* seroprevalence and sample sizes of boreal caribou captured from 2000–2010.

Herd[a]	**Historical *E. rhusiopathiae* Prevalence and Sample Size**				
	2000-2004	**2007/2008**	**2008/2009**	**2009/2010**	**Overall**
Calendar	ND	33% (n=5/15)	ND	ND	33% (n=5/15)
Chinchaga	ND	ND	ND	67% (2/3)	67% (2/3)
Maxhamish	ND	ND	50% (n=2/4)	0 (0/3)	28% (n=2/7)
Parker	ND	ND	67% (n=2/3)	ND	67% (n=2/3)
Prophet	ND	ND	50% (n=1/2)	ND	50% (n=1/2)
Fort Nelson	ND	ND	ND	ND	ND
Snake-Sahtaneh	58% (n=7/12)	0 (n=0/1)	20% (n=1/5)	33% (1/3)	43% (n=9/21)
Overall (95% CI)	**58%(28-85)**	**31% (11-59)**	**43% (18-71)**	**33%(7-70)**	**41% (28-56)**
Overall Sample Size	**(n=7/12)**	**(n=5/16)**	**(n=6/14)**	**(n=3/9)**	**(n=21/51)**

a – CI – confidence interval; n - sample size; ND - No data.

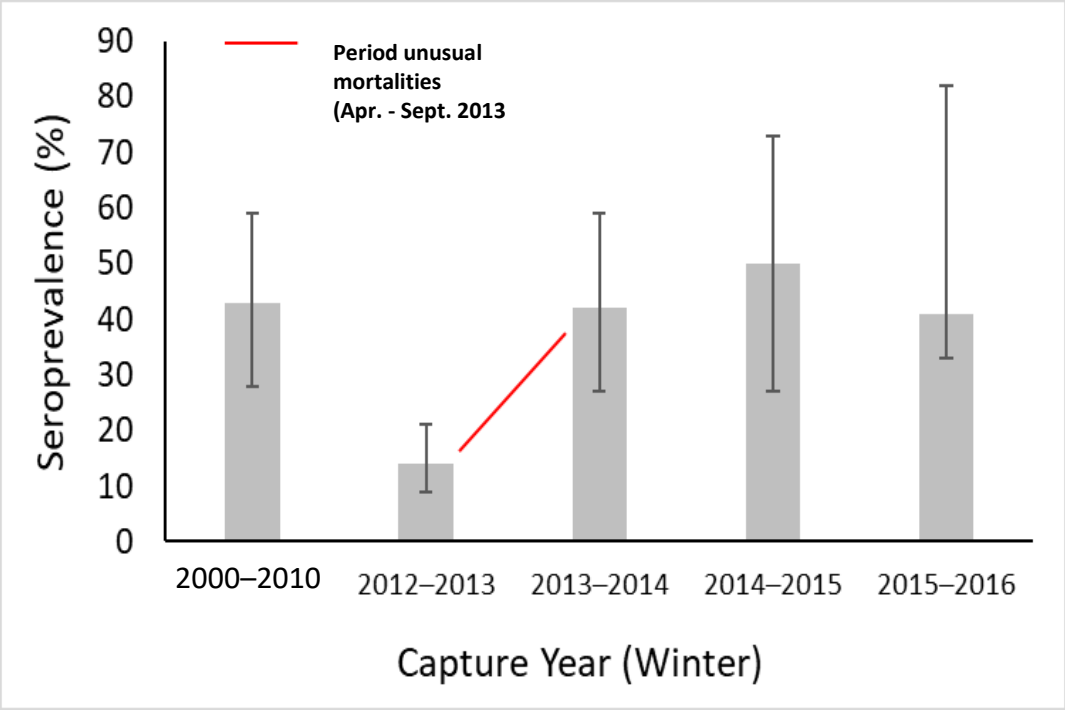

Figure 12. Seroprevalence of exposure to the bacterial pathogen *E. rhusiopathiae* recorded in adult female boreal caribou from northeast British Columbia in winters 2000–2016. Seroprevalence decreased from winter 2012/2103 to 2013/2014, following a period in which higher caribou mortalities (Fig. 4) were observed in the region. *E. rhusiopathiae* seroprevalence did not change from 2013/2014 to 2014/2015 and 2015/2016, and only n=1 unusual caribou mortality was recorded from 2014 to 2015.

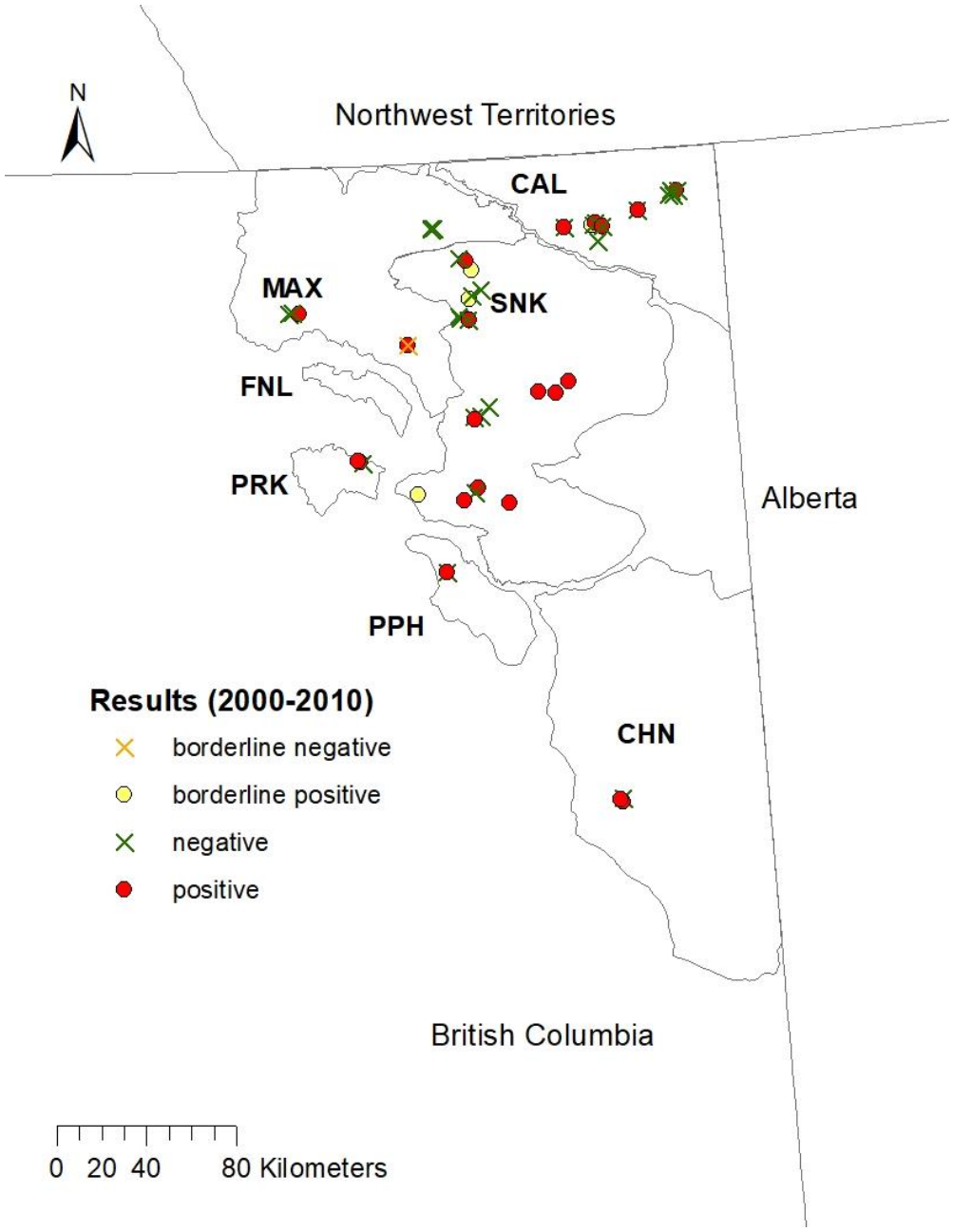

Figure 13. Spatial distribution of historical exposure to *E. rhusiopathiae* in adult female caribou in northeast B.C. in winters 2000-2010.

Evidence of seroconversion to *E. rhusiopathiae* was recorded in caribou first captured in winter 2012/2013 and then recaptured in either winter 2013/2014 or 2014/2015 (Table 12). All recaptured caribou that tested positive on their first capture also tested positive on their 2nd capture two to six years later (Table 12).

Seroprevalence and evidence of seroconversion often increases after an outbreak of infectious disease. Seroprevalence increased in the years after the high mortalities occurred, which offers further support for the potential role of *E. rhusiopathiae* in the caribou mortalities observed in NE BC in 2013. This hypothesis is also supported by the changing pattern and presentation of caribou mortalities observed from 2013 through 2015 (Fig. 5). The number of unusual mortalities (intact carcasses, see Fig. 5) observed declined from n=12+ in 2013 to n=1 in 2014 (health testing results pending) and n=0 in 2015.

In BCHRP Year 2, we also tested blood or "body cavity" fluids obtained from dead caribou for the presence of antibodies against *E. rhusiopathiae*. We identified one caribou (SK106, Calendar herd range) that was seronegative at capture in winter 2012/2013 but was seropositive and culture/PCR positive at death in July, 2013 during the high mortality period. These findings indicate that this caribou most likely survived for a period of weeks after exposure to *E. rhusiopathiae* and may also indicate that the initial exposure event occurred sometime in late spring, 2013. Using heart blood or carcass fluids we also identified seronegative caribou that were culture and/or PCR positive at death [e.g. the un-collared yearling male (Fig. 8), from the Snake-Sahtaneh herd range]. The lack of antibody production in this culture/PCR positive mortality may suggest an acute disease process also occurs in caribou affected by *E. rhusiopathiae*. Acute, subacute, and chronic disease have all been recorded in other ungulates infected with this bacterium (48, 49).

Table 12. *Eryspelothrix rhusiopathiae* serostatus of n=19 recaptured adult female boreal caribou from northeast British Columbia. Four caribou (highlighted in red) were seropositive at first capture and also seropositive at recapture, indicating lasting antibody titres or re-exposure. Six caribou (highlighted in yellow) seroconverted sometime after their first capture in 2012/2013. Caribou that were seronegative upon both captures are in green.

Animal ID	Herd[a]	Age[b]	2007/2008	2012/2013	2013/2014	2014/2015	2015/2016
SK005	MAX	OA		Negative	Positive		
SK007	MAX	YA; MA		Negative		Positive	
SK014	PRK	MA		Negative		Positive	
SK016	PRK	YA; MA		Positive		Positive	
SK020	SNK	MA		Negative		Positive	
SK033	CHIN	MA		Negative		Negative	
SK036	CHIN	MA		Negative		Negative	
SK066	MAX	JUV: YA		Negative			Borderline Negative
SK079	SNK	YA; MA		Negative		Negative	
SK097	SNK	YA; MA		Negative		Negative	
SK100	SNK	YA; MA		Negative		Positive	
SK110	SNK	YA; MA		Negative		Negative	
SK126	CAL	YA; MA		Negative		Positive	
SK146	CAL	MA; OA		Negative			Negative
SK161	PPH	MA; OA		Borderline Positive		Positive	
SK169	MAX	OA			Positive		Positive
SK170	CHIN	OA			Negative		Negative
SK181	CAL	OA			Positive		Positive
SK203	CAL; SNK	MA; OA	Positive		Positive		

[a] CAL – Calendar; CHIN – Chinchaga; MAX – Maxhamish; SNK – Snake-Sahtaneh; PRK – Parker; PPH – Prophet; FNL – Fort Nelson.
[b] YA – young adult; MA – mature adult; OA – old adult.

3.3.2.2.2 *Erysipelothrix rhusiopathiae v*accination trial

In BCHRP Year 3, we conducted an *Erysipelothrix* vaccination trial using 10 captive adult female reindeer housed at the University of Calgary, Spyhill Campus. On January 16, 2017, seven animals were vaccinated intramuscularly with an *Erysipelothrix* vaccine, and three animals were randomly selected as controls and were injected with a sterile saline solution. Prior to being injected with vaccine or saline, blood was collected from each animal on this date. On February 14, (approximately 4 weeks post vaccination), blood was drawn from each animal and tested for antibodies, and a booster or saline shot was administered where applicable. On March 14, (approximately 4 weeks), blood was drawn and tested for antibodies from all animals. The original protocol was to administer the vaccines to all of the animals on the same dates; however, mechanical difficulties with the handling device did not make this possible, and six animals started the trial on Feb. 14, received booster shots on March 15th and finished the trial on March 29. We did not wait 4 weeks to collect the final draw of blood from these animals because we wanted to avoid handling them close to calving. All reindeer were followed up in May to determine if they calved.

As expected, all of the controls (n=3) tested negative before and throughout the trial, and three vaccinated animals tested negative or borderline negative at the start of the trial and positive after they were vaccinated (Table 13). Unexpectedly, three animals tested positive and one animal tested borderline positive at the start of the vaccination trial (Table 13).

To further determine the serostatus of individuals prior to the trial, we tested archived serum collected in June 2014 and October 2016 for exposure to *E. rhusiopathiae.* Animal 3L tested seropositive in 2014 and 2016, and animal 15S tested borderline positive in 2014, positive in 2016, and borderline positive at the start of the trial (Table 13). In 2017, we opportunistically tested serum to *E. rhusiopathiae* from two calves approximately one year old from the same captive herd. One calf tested seropositive and the other seronegative.

All of these animals were born at the facility indicating that exposure is occurring on site.

All of the reindeer in the vaccination trial had calves following the trial except for three, all of which tested positive for *E. rhusiopathiae* at the start of the trial. It was suspected by the pregnancy laboratory results that 2S may have been pregnant and lost her calf.

Reindeer (3L) was an old adult and was intentionally not bred. However, this animal's health and weight declined throughout the duration of the study and was euthanized and necropsied in April 2017. The only abnormal findings found were a kidney cyst. It was concluded that this animal's health declined due to severely worn-down teeth due to old age.

Because this animal was the only one in the herd that tested seropositive to *E. rhusiopathiae* since 2014, it was an intriguing opportunity to determine if this bacterium was infecting the tissues and organs of this individual. Samples of tonsil, lung, liver, kidney, mesenteric lymph nodes, femur bone marrow, and a kidney cyst were collected during the necropsy and tested with PCR for *E. rhusiopathiae.* All of the samples tested negative. These results indicate that this animal was previously exposed to this bacteria but was not currently

infected or carrying the bacteria. Captive animals may have been exposed to a strain of this bacteria in the environment. This is important insight into the epidemiology of this bacteria in free-ranging *Rangifer*.

Table 13. *Erysipelothrix* vaccination trial results from 10 adult female captive reindeer housed at the University of Calgary, Spyhill campus. The vaccination trial results from the control animals are in green, those that sero-converted (seronegative to seropositive) after receiving the vaccine are in yellow, and those that tested seropositive prior to the receiving the vaccine are in red.

	Pre-trial		**Vaccination Trial**				**Had Calf**
Caribou ID	June 18, 2014	Oct. 6-19, 2016	Jan. 16, 2017	Feb. 14, 2017	Mar. 14, 2017	Mar. 29, 2017	
4X [a]	No data	Negative	Negative	Negative	Negative	No data	Yes
3W	Negative	No data	Borderline Negative	Positive	Positive	No data	Yes
5X	Negative	Negative	Negative	Positive	Positive	No data	Yes
4A [a]	No data	Negative	Negative	Negative	Negative	No data	Yes
3X	Negative	No data	No data	Positive	Positive	Positive	No
2Y	Negative	Borderline Negative	No data	Negative	Positive	Positive	Yes [b]
3Y [a]	Negative	Negative	No data	Negative	Negative	Negative	Yes
15S	Borderline Positive	Positive	No data	Borderline Positive	Positive	Positive	Yes
2S	No data	Not tested	No data	Positive	Positive	Positive	No
3L	Positive	Positive	No data	Positive	Positive	Positive	No [c]

[a] Animals used as controls.
[b] Had calf, but it died 24 hours later.
[c] Older animal and calf was not expected.

3.3.2.2.3 Pregnancy detection from blood on filter paper

Curry et al. (43) previously tested the use of blood on filter paper for use in serology. As a component of the reindeer vaccination trial, in BCHRP Year 3, we also investigated whether filter paper eluate could be used to test caribou for exposure to *E. rhusiopathiae* and for pregnancy using Pregnancy Specific Protein B (PSPB) (bioPRYN, ELISA test, Moscow, Idaho, USA). When possible, we collected paired filter paper and serum samples from each of the reindeer on each date of the trial. For exposure to *E. rhusiopathiae*, filter paper and eluate results matched in 26/26 of the paired samples (Table 14). For pregnancy testing, serum test results predicted the calving outcome in 8/10 cases whereas the filter paper eluate predicted the calving outcome in 10/10 cases (Table 15).

Table 14. Comparisons between paired filter paper eluate and serum samples tested for exposure to *E. rhusiopathiae* from 10 captive reindeer in the vaccination trial. Matching results are highlighted in yellow.

	Filter Paper and Serum Comparisons			
Caribou ID	Jan. 16, 2017	Feb. 14, 2017	Mar. 14, 2017	Mar. 29, 2017
4X	No data	Matched	Matched	No data
3W	No data	Matched	Matched	No data
5X	No data	Matched	Matched	No data
4A	No data	Matched	Matched	No data
3X	No data	Matched	Matched	Matched
2Y	No data	Matched	Matched	Matched
3Y	No data	Matched	Matched	Matched
15S	No data	Matched	Matched	Matched
2S	No data	Matched	Matched	Matched
3L	No data	Matched	Matched	Matched

Table 15. Comparisons between paired filter paper eluate and serum samples tested for PSPB from 10 captive reindeer in the vaccination trial. Discordant results are highlighted in yellow.

	Pregnancy Results on Mar. 14, 2017		
Caribou ID	**Serum**	**Filter Paper**	**Calved**
4X	Pregnant	Pregnant	Yes
3W	Pregnant	Pregnant	Yes
5X	Pregnant	Pregnant	Yes
4A	Pregnant	Pregnant	Yes
3X	Pregnant	Not Pregnant	No
2Y	Pregnant	Pregnant	Yes
3Y	Pregnant	Pregnant	Yes
15S	Pregnant	Pregnant	Yes
2S [a]	Pregnant	Not Pregnant	No
3L	Not Pregnant	Not Pregnant	No

[a] Due to low optical density of the serum result, it was suspected that the calf may have been lost, so the serum and filter paper eluate collected on a later date, Mar. 29, 2017, were tested. The results from Mar. 29 did not differ from the samples collected on Mar. 14.

3.3.2.2.4 Molecular analysis, strain typing and tissue culture

The ecology of *E. rhusiopathiae* in caribou is not known and our understanding of the occurrence and potential importance of genetic variation in this pathogen is limited. In BCHRP Year 2, the genetic profiles of selected *E. rhusiopathiae* colonies obtained from 7 culture positive boreal caribou from NE BC were determined by Dr. Taya Forde using Illumina MiSeq platform for sequencing (Nextera XT sample preparation kit-generating 250 base pair paired-end reads) (57, 58) to explore the relationships between isolates from caribou and those obtained from other species. A diversity of isolates were detected. *Erysipelothrix rhusiopathiae* isolates from two boreal caribou were found to be genetically different from those recorded in other domestic and free-ranging animals (both terrestrial and aquatic) (58). Isolates from n=2 caribou found dead in the high mortality period in 2013 were identical [SCEK069 and the un-collared male yearling (Fig. 5) found moribund; both from the Snake-Sahtaneh herd range] which may indicate a common source for infection, the possibility of an emerging pathogen in caribou, or the occurrence of a disease outbreak. However, isolates obtained from the other five culture positive caribou from NE BC were dissimilar indicating that other explanations for the occurrence of *E. rhusiopathiae* in caribou must also be evaluated.

A polyclonal *Erysipelothrix* infection (multiple genetic profiles of different isolates obtained from the same animal) was recorded in the yearling male found moribund in 2013 (Fig. 5) (58). Polyclonal infections have also been recently recorded in other ungulates (60), and it is important to note that every *E. rhusiopathiae* colony obtained from every caribou tissue sample was not sequenced in this study (58). It is thus possible that that a shared isolate (or isolates) common to all caribou may have been missed. Funding permitting, future research should aim to sequence a greater number of *E. rhusiopathiae* colonies obtained from culture of caribou tissues.

In some species, individual animals carry *E. rhusiopathiae* in their tonsils, pharynx, bile, and muscles without developing clinical disease (48, 49). However, clinical disease, the shedding of large numbers of bacteria, environmental contamination, transmission to naive conspecifics, and widespread outbreaks of severe disease may occur if infected "carrier" animals are stressed or their immunity is compromised (48, 49). *E. rhusiopathiae* infections are also known to be transmitted between different species (48, 49). Accordingly, the pattern of genetic variation observed in *E. rhusiopathiae* isolates from boreal caribou could reflect both a disease outbreak caused by a unique caribou strain as well sporadic infections obtained from other host species (e.g., rodents, birds, fish, other ungulates) that may be capable of opportunistically causing disease in caribou when they are stressed. In this context, nutritional or other stressors associated with the harsh winter of 2012/2013 could have been contributing factors as well as other interacting factors such as predation risk, chronic stress, immune status, and sympatric species.

Multiple strains of *E. rhusiopathiae* are known to exist in a variety of species and different virulence factors occurring in different strains are associated with the type and severity of disease seen in infected animals (e.g., chronic vs. per acute fatal) (49). The relationship between genetic variation and the virulence of *E. rhusiopathiae* isolates found in caribou is unknown at the present time.

An alternate explanation for observed variation may be that some isolates of *E. rhusiopathiae* cultured from tissues obtained from carcasses could represent post mortem contaminants. The occurrence and persistence of *E. rhusiopathiae* in the environment is somewhat contentious and while some researchers (most often studying soil or organic material from heavily and repeatedly contaminated domestic livestock enclosures) believe this pathogen may be ubiquitous this is unlikely to be the case in the natural environment. There is also no evidence that this pathogen can replicate outside a host (61, 62). Rather, it is more likely that *Erysipelothrix* persists in nature primarily in carrier animals with a transient existence (of several weeks to months (63) in the external environment secondary to "loading" associated with the shedding of bacteria by asymptomatic carriers, sick animals, and/or the carcasses of animals dying from the disease (48). Bacteria contaminating the environment (e.g., soil, water, fomites) are believed to be of primary importance in the transmission of *E. rhusiopathiae* infections to naive hosts both within a species and between different species (48, 49). Continued research is required to improve our understanding of the occurrence and significance of genetic variation in *E. rhusiopathiae* in boreal caribou.

3.3.2.2.5 *Erysipelothrix* in other caribou, other ungulates from BC and elsewhere

In BCHRP Year 2, we initiated comparative testing for *E. rhusiopathiae* in other western Canadian woodland caribou populations and in other species that may share caribou habitat in NE BC and elsewhere. In collaboration with the Foothills Research Institute (fRI) Caribou Program in Hinton, Alberta we identified *E. rhusiopathiae* in the tissues of mountain and boreal caribou from AB including a caribou found dead and intact (Fig. 14). A collaborative, interagency and organizational approach to evaluate the role of *E. rhusiopathiae* (and other health determinants) as drivers of woodland caribou population dynamics was instrumental in initiating the original BCHRP and continues to be strongly encouraged. Using this approach we recorded exposure to and infection with *E. rhusiopathiae* in serum and/or tissues collected from moose in both BC (64) and AB (65) and white-tailed deer in AB (65). The investigation of exposure to *E. rhusiopathiae* in moose and other sympatric species (e.g., rodents, birds, carnivores, other ungulates) may provide important insight into the occurrence and distribution of potential carriers or sources of this bacterium to caribou. Serum samples from n=4 captive barren ground caribou located in Fort St. John, BC were also tested for exposure to *E. rhusiopathiae* in BCHRP Year 2. All four captive caribou tested negativ

Figure 14. A radio-collared adult female mountain caribou (*Rangifer tarandus*) from the Narraway herd range, Alberta found dead in October, 2013. No evidence of predators or scavengers was identified at the mortality site and infectious disease was suspected as the cause of death. The case presentation shared many characteristics with boreal caribou mortalities recorded in northeast British Columbia in the same year (Fig. 9). *Erysipelothrix rhusiopathiae* was cultured from the tissues of this caribou. Photo credit: L. Finnegan, fRI Caribou Research Program, Hinton, AB.

3.3.2.3 Effects of Weather

In BCHRP Year 3, we downloaded historical daily weather data from Environment Canada (66) for the years, 2011-2017, including daily mean temperature, maximum temperature, minimum temperature, and total snow on the ground. The data were obtained from a weather station in Fort Nelson, British Columbia (Latitude 58.84, longitude -122.57). Results were analyzed according to the following seasons determined to be significant to caribou ecology and movement (67): early winter (Nov. 1–Feb. 15), late winter (Feb. 16–May 15), calving (May 16–July 15), and late summer (July 16–Oct. 31).

Early Winter
The median of daily mean temperature in early winter in 2012/2013 (labeled 2012 in Fig. 15) was colder than all other winters except 2010/2011 and 2011/2012. Likewise, median daily maximum temperature in 2012/2013 was colder than all other years except winter 2010/2011 (Fig. 16). Median daily minimum temperature was only colder than winter 2012/2013 in 2015/2016 and 2016/2017. There was more total snow in 2012/2013 than 2015/2016 and 2016/2017.

Late Winter
Median daily mean temperature and median maximum and minimum temperature in 2012/2013 was colder than winters 2010/2011 and 2016/2017. Median amount of total snow did not differ between late winter 2012/2013 and all of the other years.

Calving and Late Summer
There was no difference in median daily mean temperature or and median maximum and minimum temperature in 2013 than all other years during the calving and late summer periods.

Conclusions
Although the weather data used in the analysis are likely not representative of the actual weather and snow conditions across the entire boreal caribou ranges of northeast B.C., they do provide indication that early winter 2012/2013 in the region may have been more extreme in temperature, snowfall, and possibly ice crusting than other years. Culling and Culling (2014) observed that winter prior to the high mortalities observed in spring 2013 was severe with snow crusting and also observed a heavy snowfall event in the spring of 2013.

It is possible that severe weather with high levels of ice crusting in winter 2012/2013 nutritionally and energetically stressed the caribou and made them more susceptible to *Erysipelothrix* infections. Future research on the transmission and ecology of *Erysipelothrix* is needed to better understand how climate change and severe weather events will affect exposure to this pathogen in caribou.

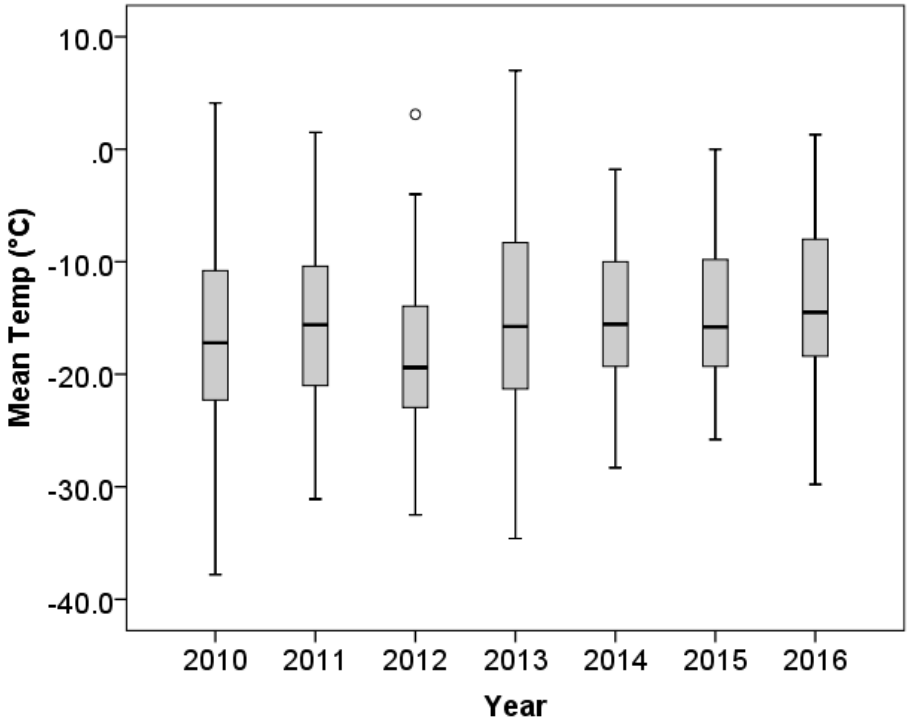

Figure 15. Box-plot of daily mean temperatures in early winter from winter 2010/2011(labelled 2010) to 2016/2017 (labelled 2016). Median daily mean temperature in early winter was lower in winter 2012/2013 (labelled 2012) than winter 2010/2011 (labelled 2010) and 2011/2012 (labelled 2011).

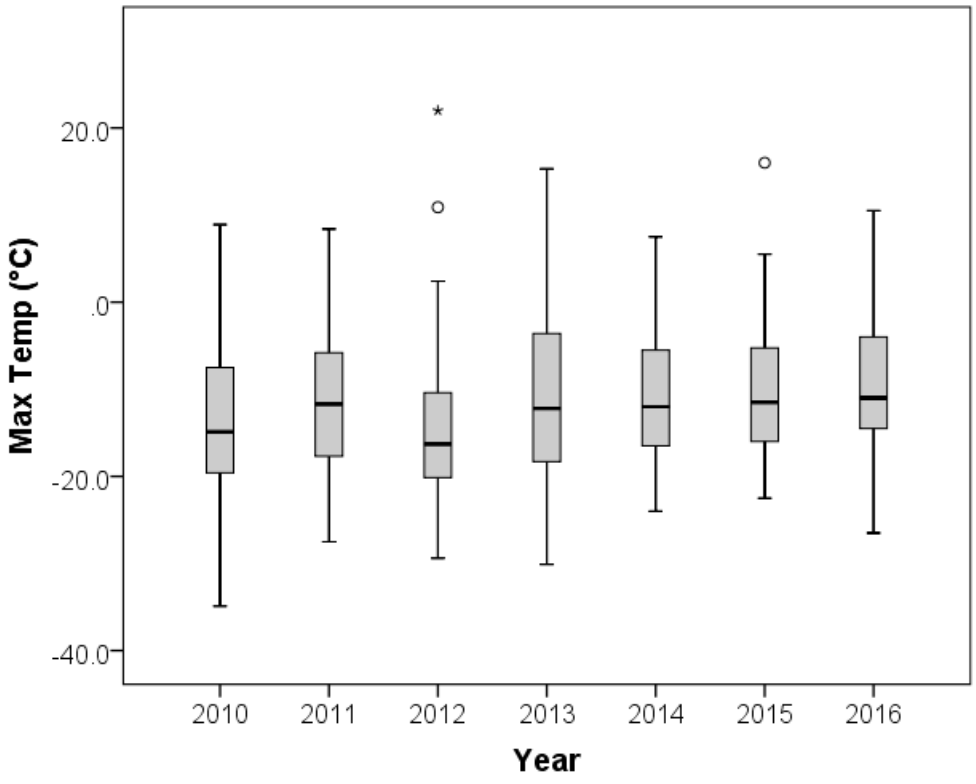

Figure 16. Box-plot of maximum temperatures in early winter from winter 2010/2011(labelled 2010) to 2016/2017 (labelled 2016). Median maximum daily temperature in winter 2012/2013 (labelled 2012) was colder in all other years except winter 2010/2011 (labelled 2010).

3.4 Parasites

3.4.1 Gastrointestinal Parasites (Focus on abomasal nematodes)

Infection with abomasal nematodes such as *Ostertagia gruehneri* has been associated with diminished food intake, weight loss, and reduced pregnancy rates in free-ranging caribou herds (68). In BCHRP Year 1, a pilot study in n=36 caribou captured in winter 2012/2013 found few gastrointestinal parasites. Only 14% (n=5/36) of caribou had Strongylate or Nematodirinae eggs in their feces and the intensity of infection was exceptionally low (< 1 egg /gram feces) in all cases. Likewise, only 8% (n=3/36) of caribou examined in BCHRP Year 1 had cestode eggs (tapeworm; *Moniezia* sp.) in their feces. With the exception of Protostrongylid dorsal spined larvae (DSLs) identified in 35% of caribou (see 3.4.2) and eggs of the caribou pin worm (*Skrjabinema tarandi*) identified in n=1 caribou no other parasites were found in caribou feces in BCHRP Year 1. These findings suggested that the winter gastrointestinal parasite burden of boreal caribou in NE BC may be very low. However, eggs of *Ostertagia* sp. and many other important gastrointestinal parasites (and lungworm larvae, e.g., *Dicytocaulus* sp.) that may be found in caribou feces are adversely affected by freezing or freeze thaw cycles and sample storage prior to analysis would have dramatically reduced these egg/larval counts.

The majority of fecal samples available from caribou live-captured in winter 2013/2014 and 2014/2015 had also been stored frozen for 6-12 months. As a result, fecal floatation testing was not pursued for most samples available in BCHRP Year 2. In winter 2014/2015, fresh (cooled/ 4°C) fecal samples were opportunistically obtained from n=5 boreal caribou from NE BC [n=2 Chinchaga herd range, n=2 Snake-Sahtaneh herd range, n=1 Calendar herd range]. All samples contained strongyle-type eggs but the intensity of infection was low in all cases (mean 3.5 eggs/gram feces, range 0- 8.9 eggs/g feces). No Nematodirinae, *Marshallagia*, or tapeworm (*Moniezia*) eggs and no lungworm larvae were recovered in any sample. Although the prevalence and intensity of abomasal nematodes and other parasite infections may vary seasonally (and with the life history stage of infected hosts) (69), these findings do suggest that the winter gastrointestinal parasite burden of boreal caribou in NE BC is unlikely to be a limiting factor at the present time. The opportunistic acquisition and testing of fecal samples obtained from different age classes of caribou and across seasons would provide additional insight into the prevalence, intensity, and potential impact of gastrointestinal parasites in boreal caribou in NE BC.

3.4.2 Protostrongylid Nematodes

Protostrongylids are nematode parasites found in the lungs, muscle, and nervous system of ungulates. Species reported in *Rangifer* from North America include: *Parelaphostrongylus odocoilei, P. andersoni, P. tenuis, Elaphostrongylus rangiferi* (introduced in Newfoundland only), and *Varestrongylus eleguneniensis* (68-70). For caribou, the consequences of infection with these parasites may range from subclinical/mild to highly pathogenic /fatal depending on the intensity of infection, the age of host, and the species of parasite (68). In BCHRP Year 1, dorsal-spined Protostrongylid larvae (DSLs) were found in 35% of fecal samples collected from boreal caribou captured in NE BC in winter 2012/2013 (Table 16), and the average intensity of infection was 25 DSLs/gram feces (range < 1 - 191 DSLs/ gram feces). Dorsal-spined larvae recovered from seven animals were identified (using PCR) as the caribou muscle worm,

Parelaphostrongylus andersoni. *Varestrongylus eleguneniensis* was not detected in this small sample size; however, this lungworm has been detected in caribou across most of their range in North America (71). *Varestrongylus eleguneniensis* may have been present but not detected in this study because only DSls from seven animals were identified to species using PCR and morphological keys differentiating these larvae (72) were not available at the time of the samples were analyzed. In a separate study, from 2003-2010, DSL of *V. eleguneniensis* were detected by PCR in the feces of 5/14 caribou from the Chinchaga herd, but no caribou from the Calendar (n=17), Maxhamish (n=3), or Snake-Sahtaneh herds (n=12) (71).

In BCHRP Year 2, DSLs were quantified in the feces of all caribou captured in winter 2013/2014 and 2014/2015. In 2013/2014 and 2014/2015, 38% and 33% of all fecal samples contained DSLs, respectively (Table 16). The average intensity of DSL infections recorded in 2013/2014 and 2014/2015 were 8 DSLs/gram feces (range < 1 - 32 DSLs/ gram feces) and 7 DSLs/gram feces (range < 1 - 26 DSLs/ gram feces) respectively. The identity of these DSLs is not known; however, they have been archived for future identification. *Varestrongylus eleguneniensis* and *P. andersoni* can be differentiated based on morphological characteristics of the larvae (72). Thus, further morphological identification of the larvae to determine if *V. eleguneniensis* is present could be done. However, because *P. odocoilei* is also potentially present and cannot be morphologically differentiated from *P. andersoni,* high throughput techniques for molecular identification would be required to rule out this parasite.

Table 16. Dorsal-spined larvae prevalence and sample sizes of boreal caribou captured in winter 2012/2013 and the three winters following the high mortality.

Herd	**Dorsal Spined Larvae Prevalence and Sample Size**			
	2012/2013	**2013/2014**	**2014/2015**	**Overall**
Calendar	48% (n=12/25)	40% (n=2/5)	33% (n=1/3)	45% (15/33)
Chinchaga	51% (n=19/37)	25% (n=3/12)	30% (n=3/10)	42% (25/59)
Maxhamish	32% (n=7/22)	43% (n=3/7)	25% (n=2/8)	32% (12/37)
Parker	29% (n=2/7)	0 (n=0/1)	0 (n=0/2)	20% (2/10)
Prophet	0 (n=0/8)	0 (0/8)	0 (n=0/1)	0 (0/9)
Fort Nelson	33% (1/3)	67% (2/3)	100% (n=1/1)	57% (4/7)
Snake-Sahtaneh	26% (n=14/54)	45% (n=5/11)	50% (n=4/8)	31% (23/73)
Overall (95% CI)	**35%(28-43)**	**38% (23-55)**	**33%(18-52)**	**36%(29-42)**
Overall Sample Size	**(n=55/156)**	**(n=15/39)**	**(n=11/33)**	**(n=81/228)**

[a] CI – confidence interval; n = total sample size.

In BCHRP Year 3, we analyzed the results statistically in a univariable analysis and found that DSL prevalence was significantly associated with age, residual antler velvet, and alphaherpesvirus (Table 17). There was a trend between DSL presence and herd and iron levels (Table 17).

Young adults were significantly more likely to have DSL in the feces than mature (P = 0.02) and old adults (P < 0.001). Although old adults were less likely to have DSL in their feces than mature adults, the association was marginally significant (P=0.074). In addition, caribou with residual antler velvet were more likely to have DSL in the feces than those without residual velvet and those testing seropositive to alphaherpesvirus were less likely to have DSL in their feces than those testing seronegative.

To investigate whether age was a confounding variable, we modeled it with each of the significant and marginally significant variables. Age confounded alphaherpesvirus serostatus, iron, and residual antler velvet, and these variables were no longer significant when included in the model with age.

Table 17. Univariable analyses for associations between dorsal-spined larvae presence and health and fitness parameters in female adult boreal caribou captured in winter 2012/2013 to 2014/2015. Significant associations are highlighted in yellow and trends are in green.

Parameter	**Variable Type**	**Sample Size**	**P-Value**
Survived to next winter	Binary	212	0.362
Year	Categorical	212	0.828
Pregnancy	Binary	210	>0.999
Calf at heel	Binary	205	0.603
Age	Categorical	212	< 0.001
Residual antler velvet	Binary	193	0.047
Herd	Categorical	153	0.064
Hair loss	Binary	212	0.345
Besnoitia tarandii	Binary	193	0.702
Alphaherpes virus	Binary	208	0.032
Neospora caninun	Binary	200	0.695
Erysipelothrix	Binary	200	0.591
Cobalt	3 quantiles	186	0.728
Copper	3 quantiles	187	0.980
Iron	Continuous	186	0.094
Manganese	Continuous	186	0.805
Selenium	3 quantiles	186	0.307
Zinc	3 quantiles	185	0.365
Haptoglobin	3 quantiles	200	0.887
Serum Amyloid A	Continuous	211	0.834
Hair cortisol concentration	3 quantiles	210	0.914
Disturbance	3 quantiles	100	0.566
Road density	3 quantiles	100	0.371
Seismic line density	3 quantiles	100	0.484
Moose density	Continuous	98	0.474
Season	Binary	212	0.115

The results from the recaptured boreal caribou indicate that the presence of dorsal-spined larvae in the feces can change over time. Three caribou tested negative on their first capture, but positive on the next capture and two caribou tested positive on the first captured and tested negative on the next capture (Table 17). One individual tested positive on both captures (Table 17). It is unknown of this animal remained persistently infected or if it became re-infected, which is a limitation of sampling fecal samples from individuals during only one point in time.

Table 18. Presence of dorsal-spined larvae in n=13 recaptured adult female boreal caribou from northeast British Columbia. Test results that were negative on the first capture and positive on the second capture are in yellow, positive on the first captured and negative on the second capture are in orange, positive on both captures are in red, and negative on both captures are in green.

Animal ID	Herd	Age[a]	2012/2013	2013/2014	2014/2015
SK005	Maxhamish	OA	Negative	Negative	
SK007	Maxhamish	YA; MA	Negative		Negative
SK009	Fort Nelson	MA; OA	Negative		Positive
SK014	Parker	MA	Positive		Negative
SK016	Parker	YA; MA	Negative		Negative
SK033	Chinchaga	MA	Negative		Negative
SK036	Chinchaga	MA	Negative		Positive
SK079	Snake-Sahtaneh	YA; MA	Negative		Negative
SK097	Snake-Sahtaneh	YA; MA	Negative		Negative
SK100	Snake-Sahtaneh	YA; MA	Negative		Positive
SK126	Calendar	YA; MA	Negative		Negative
SK 136	Calendar	YA; MA	Positive		Positive
SK146	Calendar	MA; OA	Positive		Negative

[a] YA – young adult; MA – mature adult; OA – old adult.

Dorsal-spined larvae were found throughout the boreal caribou herd ranges in NE BC (Fig. 17).

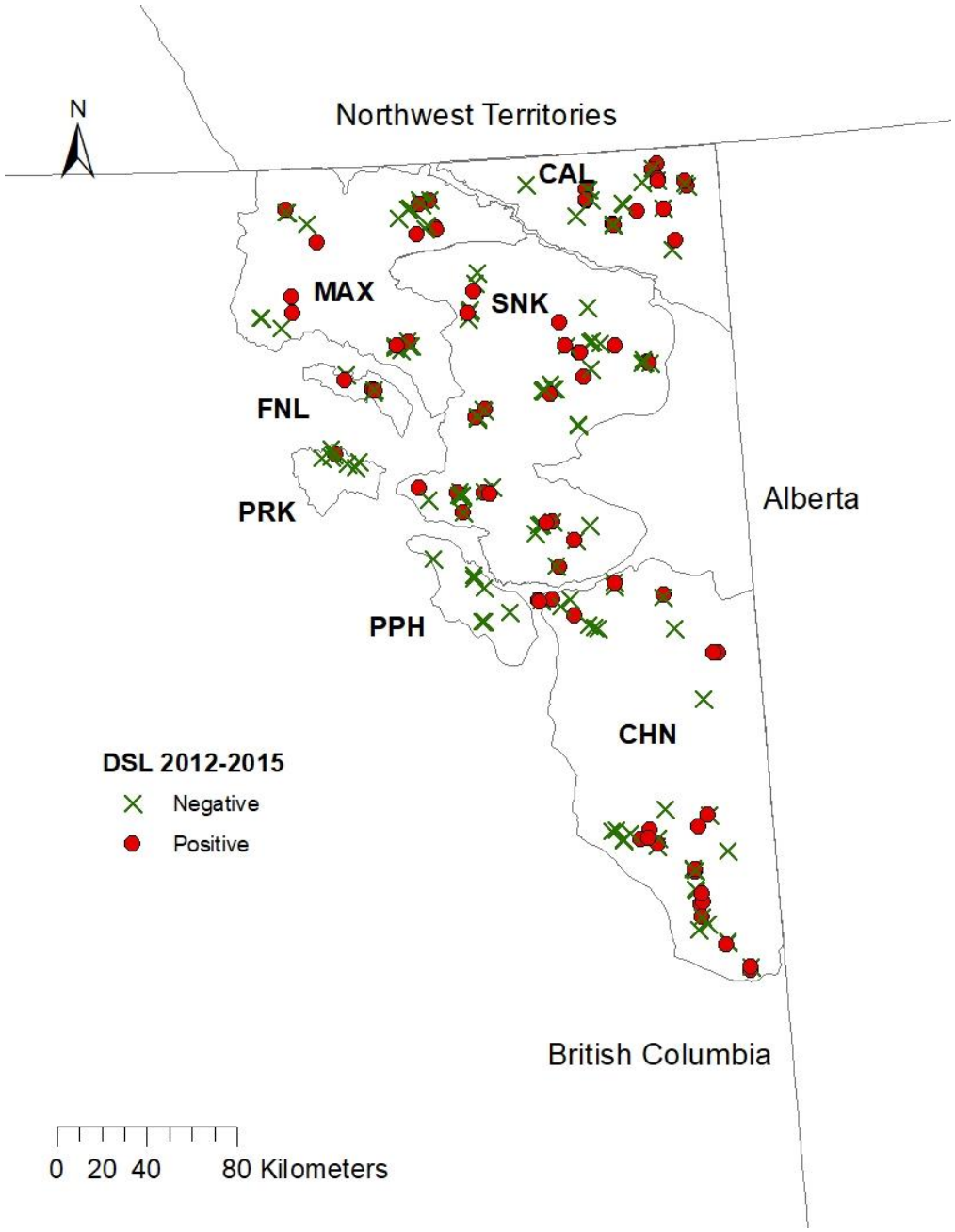

Figure 17. Spatial distribution of dorsal-spined larvae presence in adult female caribou in northeast B.C. in winters 2012/2013 to 2014/2015. Repeated captures are included on the map.

3.4.3 Giant Liver Fluke (*Fascioloides magna*)

Fascioloides magna (the giant liver fluke) is a trematode parasite which may be found in the liver and bile ducts of ungulates including white-tailed deer, elk, moose and caribou (73). Although it causes significant liver pathology in caribou, no reports of clinical disease due to *F. magna* have been reported in this species (74, 75). Infected caribou also shed *F. magna* eggs in their feces and function as a definitive host for this parasite (70, 75). Subclinical effects of *F. magna* infections are probable in caribou and caribou may transmit the parasite to other species (e.g., moose) where it may cause severe to fatal disease (76). This parasite has not been reported in NE BC.

In BCHRP Year 1, no fluke eggs were identified in fecal samples collected from adult female boreal caribou in winter 2012/2013 (Table 18). In BCHRP Year 2, no fluke eggs were identified in fecal samples collected in winter 2013/2014 and 2014/2015 (Table 18).

Table 19. Giant liver fluke prevalence and sample sizes of boreal caribou captured in winter 2012/2013 and the three winters following the high mortality.

Herd[a]	Giant Liver Fluke Prevalence and Sample Size			
	2012/2013	**2013/2014**	**2014/2015**	**Overall**
Calendar	0 (n=0/25)	0 (n=0/5)	0 (n=0/1)	0 (0/31)
Chinchaga	0 (n=0/37)	0 (n=0/12)	0 (n=0/7)	0 (0/56)
Maxhamish	0 (n=0/20)	0 (n=0/7)	0 (n=0/8)	0 (0/35)
Parker	0 (n=0/6)	0 (n=0/1)	0 (n=0/1)	0 (0/8)
Prophet	0 (n=0/7)	0 (0/1)	0 (n=0/0)	0 (0/8)
Fort Nelson	0 (0/3)	0 (0/3)	ND	0 (0/6)
Snake-Sahtaneh	0 (n=0/54)	0 (n=0/11)	0 (n=0/5)	0 (0/70)
Overall (95% CI)	**0 (0-2)**	**0 (0-9)**	**0 (0-15)**	**0 (0-2)**
Overall Sample Size	**(n=0/152)**	**(n=0/39)**	**(n=0/23)**	**(n=0/214)**

[a] CI – confidence interval; n = total sample size; ND – no data.

Assuming test sensitivity of 90% and specificity of 100%, we can be nearly 95% certain that *F. magna* does not occur in boreal caribou from NE BC at a prevalence of greater than 1.2 % at the present time. This is important baseline knowledge. Given the apparent absence of *F. magna* in caribou, continued monitoring of the parasite's occurrence in more typical (and more commonly hunted) hosts (e.g., moose) in NE BC may be a more practical surveillance technique to provide an early warning system for potential risk to caribou in the region.

3.4.4 *Besnoitia tarandi*

Besnoitia tarandi is a protozoan parasite found in caribou throughout their distributional range (68, 77). Pathology caused by *B. tarandi* is related to the presence of intracellular cysts containing bradyzoites which may occur in a wide variety of tissues (78, 79). Clinical disease associated with this parasite most often manifests as hair loss and skin lesions (78, 79). Bone and testicular lesions have also been reported. Reduced mobility, poor body

condition, morbidity, and mortality have all been attributed to this parasite in free-ranging caribou (78-80). Outbreaks of clinical disease may also occur (77-80).

In BCHRP Year 3, we submitted serum samples collected in 2013/2014, 2014/2015, and historical samples (2003-2010) to Complutense University, Madrid, Spain for *B. tarandi* testing using an indirect ELISA with a posteriori Western Blot. The overall prevalence of exposure to *B. tarandi* in adult female boreal caribou captured in NE BC from 2012-2016 was 54% (Table 19) and the historical prevalence was 68% (Table 20).

Table 20. *Besnoitia tarandi* seroprevalence and sample sizes of boreal caribou captured in winter 2012/2013 and the three winters following the high mortality.

Herd[a]	***Besnoitia* Prevalence and Sample Size**				
	2012/2013	**2013/2014**	**2014/2015**	**2015/2016**	**Overall**
Calendar	42% (n=8/19)	25% (n=1/4)	0 (n=0/1)	50% (n=1/2)	37% (n=10/27)
Chinchaga	85% (n=28/33)	45% (n=5/11)	14% (n=1/7)	60% (n=3/5)	66% (n=37/56)
Maxhamish	67% (n=14/21)	29% (n=2/7)	29% (n=2/7)	0 (n=0/5)	45% (n=18/40)
Parker	50% (n=3/6)	0 (n=0/1)	100% (n=1/1)	50% (n=1/2)	50% (n=5/10)
Prophet	67% (n=4/6)	ND	50% (n=1/2)	ND	62% (n=5/8)
Fort Nelson	0 (n=0/3)	0 (n=0/3)	ND	ND	0 (n=0/6)
Snake-Sahtaneh	54% (n=29/54)	73% (n=8/11)	83% (n=5/6)	50% (n=3/6)	58% (n=45/77)
Overall (95% CI)	**60% (51-68)**	**43% (27-61)**	**42% (22-63)**	**40% (19-64)**	**54% (47-60)**
Overall Sample Size	**n=85/142**	**n=16/37**	**n=10/24**	**n=8/20**	**n=120/224**

[a] CI – confidence interval; n= total sample size; ND – no data.

Table 21. Historical *Besnoitia tarandi* seroprevalence and sample sizes of boreal caribou captured in winter 2000-2010.

Herd[a]	**Historical *Besnoitia tarandi* Prevalence and Sample Size**				
	2000-2004	**2007/2008**	**2008/2009**	**2009/2010**	**Overall**
Calendar	ND	75% (n=12/16)	ND	ND	75% (n=12/16)
Chinchaga	ND	ND	ND	67% (n=2/3)	67% (n=2/3)
Maxhamish	ND	ND	40% (n=2/5)	67% (n=2/3)	50% (n=4/8)
Parker	ND	ND	33% (n=1/3)	ND	33% (n=1/3)
Prophet	ND	ND	100% (n=2/2)	ND	100% (n=2/2)
Fort Nelson	ND	ND	ND	ND	ND
Snake-Sahtaneh	61% (n=8/13)	100% (n=1/1)	71% (n=5/7)	100% (3/3)	71% (n=17/24)
Overall (95% CI)	**61% (27-56)**	**76% (50-93)**	**59% (33-81)**	**78% (40-97)**	**68% (54-80)**
Overall Sample Size	**(n=8/13)**	**(n=13/17)**	**(n=10/17)**	**(n=7/9)**	**(n=38/56)**

[a] CI – confidence interval; n= total sample size; ND – no data.

The prevalence of exposure to *B. tarandi* from winter 2012/2013 to 2015/2016 was significantly associated with herd, seropositivity to alphaherpesvirus, disturbance, seismic line, and road density (Table 21). The Chinchaga herd was significantly more likely to be seropositive to *B. tarandi* than all of the other herds. Caribou seropositive to *B. tarandi* were also more likely to be seropositive to alphaherpesvirus (Table 21). Exposure to *B. tarandi* was more likely in areas of medium (P= 0.003) and high disturbance (P=0.026) than low disturbance. Likewise, exposure to *B. tarandi* was also more likely in areas of medium and high than low seismic line density. However, exposure to *B. tarandi* was higher in areas of high road density than low road density (P =0.015) but not differ between areas of medium and high road density (P=0.322). There also were significant quadratic associations between exposure to *B. tarandi* and haptoglobin (Fig. 18) and selenium (Fig. 19).

There was a trend with year, seropositivity to *Neospora caninum*, copper, and season (Table 21). Exposure to *B. tarandi* was more likely in winter 2012-2013 than 2013-2014 (P=0.061), 2014-2015 (P=0.061), and 2015-2016 (P=0.088). Caribou exposed to *Besnoitia* were more likely to be seropositive to *N. caninum*, and caribou were more likely to be exposed to *B. tarandi* in early than late winter.

However, it is possible that year, herd, and age could be confounding variables, so we included these variables in the models, one at a time, with each of the significant and marginally significant variables. Year confounded season, and season was no longer significant when year was included in the model (P=0.390). Although year confounded copper and selenium, when included in the models with these variables, the associations with exposure were still marginally significant. Herd confounded disturbance, road density, and seismic line density, but when year was modelled with these variables, interpretation of the significance of the model did not change.

Age did not confound the association between *B. tarandi* and alphaherpesvirus serostatus, and the association with alphaherpesvirus and *B. tarandi* serostatus was still significant when age was included in the model. This indicates that immunity to these bacteria in caribou may be associated, and that co-infections with these bacteria may occur.

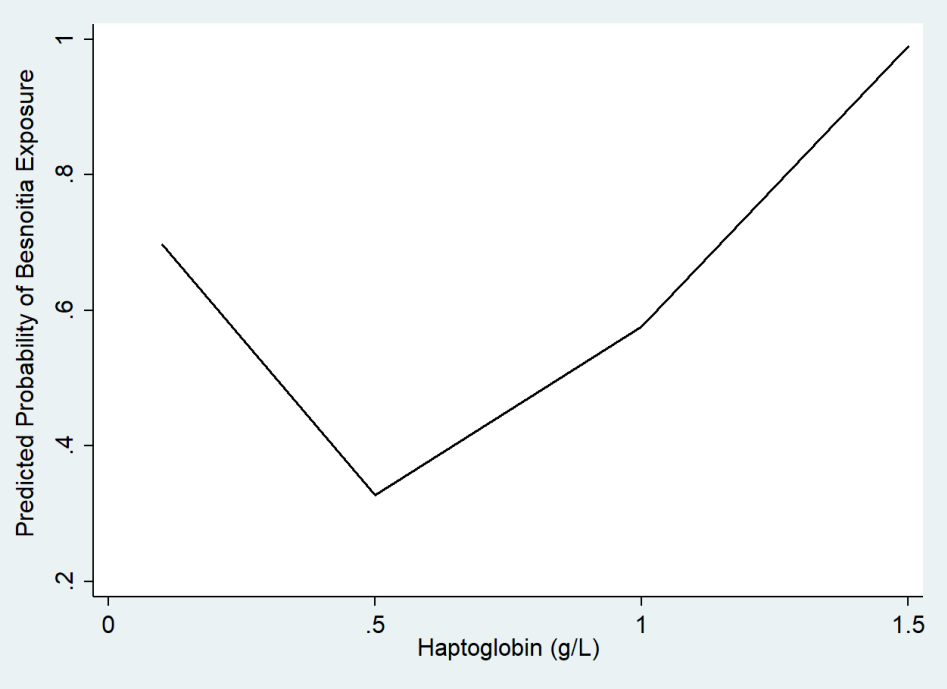

Figure 18. Significant quadratic association between haptoglobin level and probability of *Besnoitia tarandi* exposure in adult female boreal caribou.

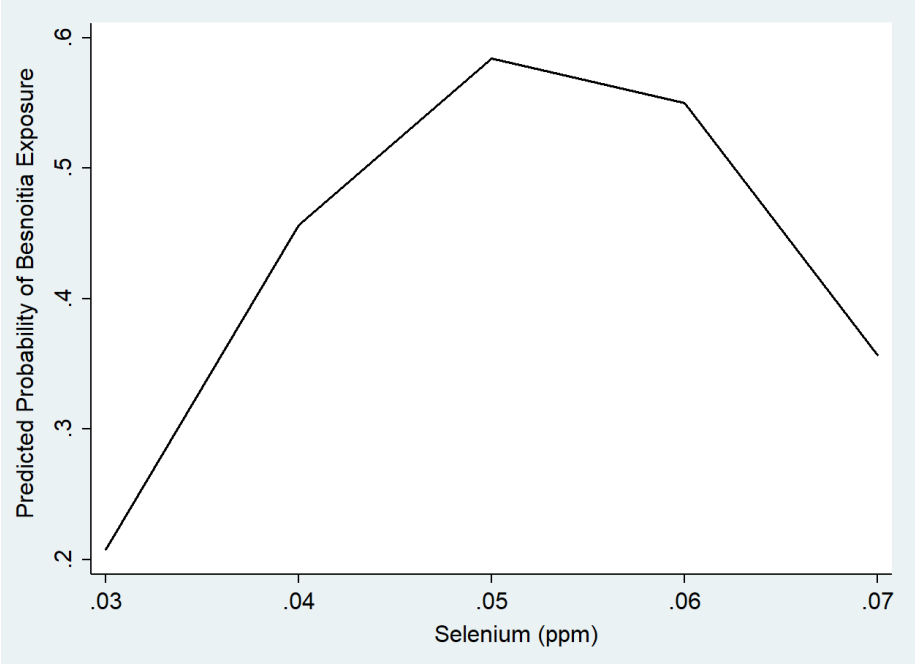

Figure 19. Significant quadratic association between selenium level and probability of B. *tarandi* exposure in adult female boreal caribou.

Table 22. Univariable analyses for associations between *Besnoitia tarandi* seropositivity and health and fitness parameters in female adult boreal caribou captured in winter 2012/2013 to 2015/2016. Significant associations are highlighted in yellow and trends are in green.

Parameter	Variable Type	Sample Size	P-Value
Survived to next winter	Binary	224	>0.999
Year	Categorical	224	0.052
Pregnancy	Binary	219	0.413
Calf at heel	Binary	217	0.873
Age	Categorical	224	0.145
Residual antler velvet	Binary	203	0.451
Herd	Categorical	199	0.042
Hair loss	Binary	224	0.623
Dorsal-spined larvae	Binary	194	0.652
Alphaherpes virus	Binary	203	0.004
Neospora caninun	Binary	224	0.063
Erysipelothrix	Binary	212	0.274
Cobalt	3 quantiles	191	0.684
Copper	3 quantiles	191	0.070
Iron	3 quantiles	191	0.421
Manganese squared	Continuous	191	0.828
Selenium squared	Continuous	191	0.040
Zinc	3 quantiles	191	0.681
Haptoglobin squared	Continuous	201	0.003
Serum Amyloid A	Continuous	211	0.834
Hair cortisol concentration	3 quantiles	210	0.914
Disturbance Late Summer	3 quantiles	101	0.006
Road density Late Sumer	3 quantiles	101	0.036
Seismic line density Late Summer	3 quantiles	101	0.007
Moose density Late Summer	3 quantiles	114	0.447
Season	Binary	212	0.058

From the recaptured caribou, our results indicate that antibodies to *B. tarandi* may last for years after exposure. This is not surprising as exposed animals can remain infected for years. None of the 19 recaptured caribou seroconverted from positive to negative (Table 22). Ten caribou that tested seropositive on the first capture also tested seropositive 1-6 years later (Table 22).

Our results suggest that factors influencing *B. tarandi* transmission (e.g., infections in carnivores or insect vectors) may be relatively prominent in areas with higher anthropogenic disturbance (e.g., seismic line and road density) in NE BC. Exposure to this parasite across all of the herd ranges historically (2000-2010) and from 2012-2016 is shown in Figs 20 and 21, respectively.

Table 23. *Besnoitia tarandi* serostatus of n=19 recaptured adult female boreal caribou from northeast British Columbia. Results where caribou seroconverted (seronegative to seropositive) are in yellow, those that were seropositive on both captures are in red, and those that were seronegative on both captures are in green.

Animal ID	Herd	Age[a]	2007/2008	2012/2013	2013/2014	2014/2015	2015/2016
SK005	Maxhamish	OA		Positive	Positive		
SK007	Maxhamish	YA; MA		Positive		Positive	
SK009	Fort Nelson	MA; OA		Negative		Negative	
SK014	Parker	MA		Negative		Positive	
SK016	Parker	YA; MA		Negative		Positive	
SK020	Snake-Sahtaneh	MA		Positive		Positive	
SK026	Chinchaga	MA		Positive		Positive	
SK033	Chinchaga	MA		Positive		Positive	
SK036	Chinchaga	MA		Positive		Positive	
SK038	Chinchaga	YA; MA		Positive			Positive
SK066	Maxhamish	JUV; YA		Negative			Positive
SK079	Snake-Sahtaneh	YA; MA		Negative		Negative	
SK097	Snake-Sahtaneh	YA; MA		Negative		Negative	
SK100	Snake-Sahtaneh	YA; MA		Negative		Negative	
SK110	Snake-Sahtaneh	YA; MA		Positive		Positive	
SK126	Calendar	YA; MA		Negative		Negative	
SK146	Calendar	MA; OA		Positive		Positive	
SK 161	Prophet	MA; OA		Positive		Positive	
SK203	Calendar; Snake-Sahtenah	MA; OA	Positive		Positive		

[a] YA – young adult; MA – mature adult; OA – old adult.

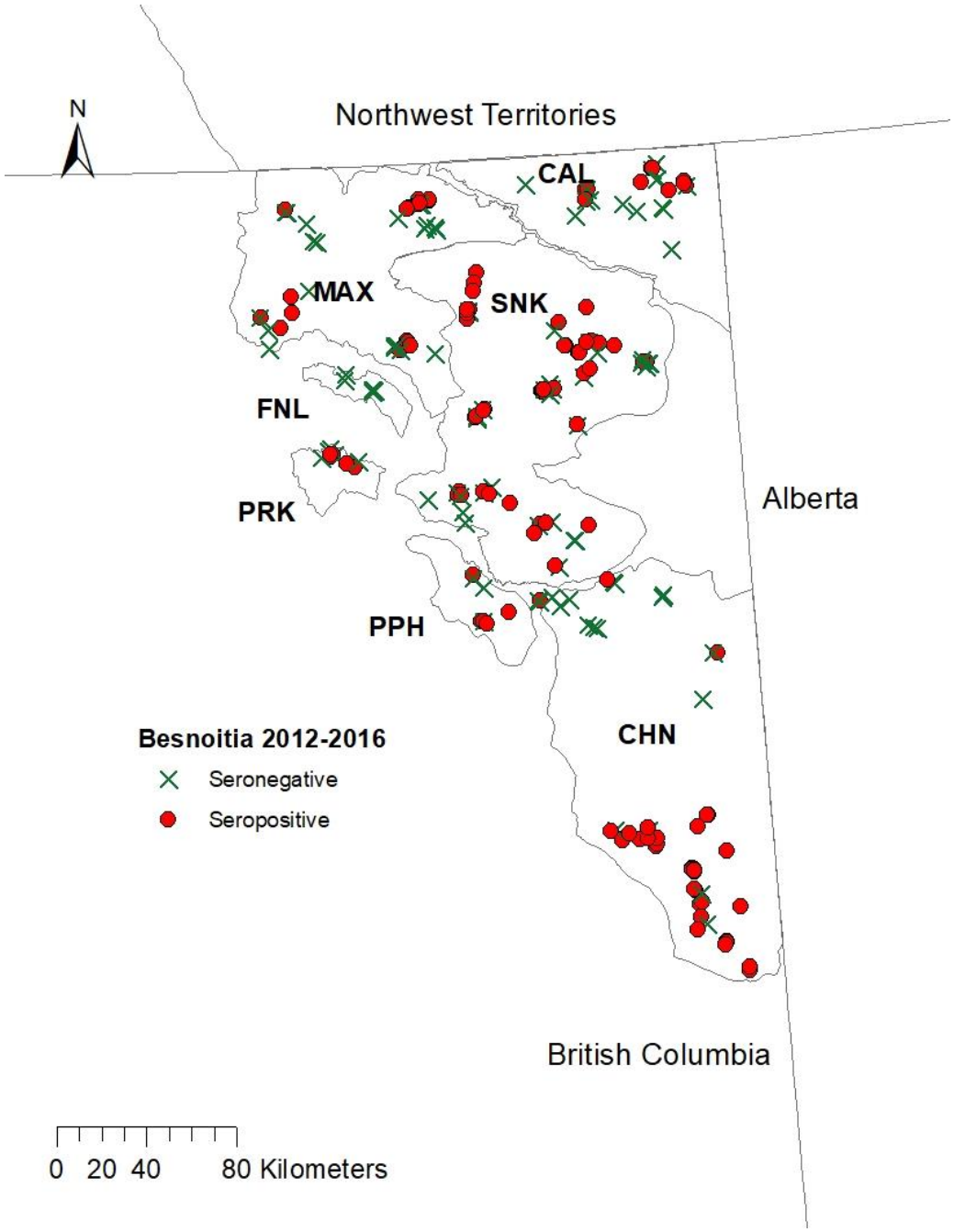

Figure 20. Spatial distribution of *B. tarandi* serostatus in adult female caribou in northeast B.C. in winters 2012/2013 to 2014/2015. Repeated captures are included on the map.

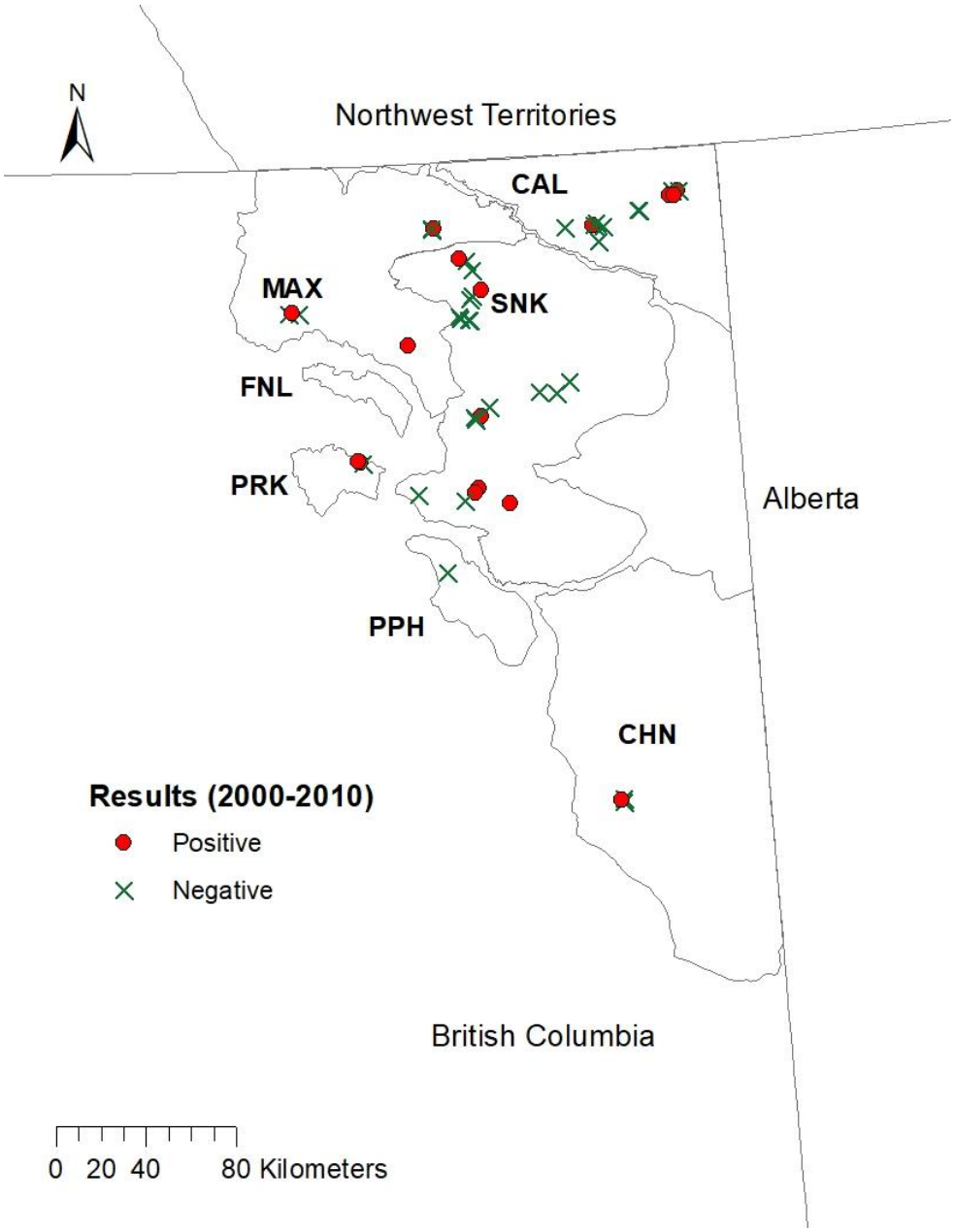

Figure 21. Spatial distribution of historical *B. tarandi* serostatus in adult female caribou in northeast B.C. in winters 2000-2010.

3.4.5 *Toxoplasma gondii*

Toxoplasma gondii is a protozoan parasite with a felid definitive host (in caribou range most likely lynx or cougar in some cases) and a wide variety of intermediate hosts including wild ungulates such as caribou (81-83). This parasite may cause a spectrum of diseases in intermediate hosts ranging from mild/sub clinical to severe/fatal that may include pneumonia, enteritis, and encephalitis along with congenital defects, abortions, still births, and weak neonates (81).

In BCHRP Year 2, serum samples collected from boreal caribou in NE BC were tested for exposure to *Toxoplasma*. No seropositive caribou were detected in any year (Table 24), and no evidence of seroconversion in recaptured caribou was recorded.

Table 24. *Toxoplasma gondii* seroprevalence and sample sizes of boreal caribou captured in winter 2012/2013 and the three winters following the high mortality.

Herd[a]	***Toxoplasma gondii* Seroprevalence and Sample Size**			
	2012/2013	**2013/2014**	**2014/2015**	**Overall**
Calendar	0 (n=0/25)	0 (n=0/5)	ND	0 (0/30)
Chinchaga	0 (n=0/37)	0 (n=0/13)	0 (n=0/7)	0 (0/57)
Maxhamish	0 (n=0/21)	0 (n=0/8)	0 (n=0/8)	0 (0/37)
Parker	0 (n=0/6)	0 (n=0/1)	0 (n=0/1)	0 (0/8)
Prophet	0 (n=0/8)	ND	0 (n=0/2)	0 (0/10)
Fort Nelson	0 (0/3)	0 (0/3)	ND	0 (0/6)
Snake-Sahtaneh	0 (n=0/55)	0 (n=0/11)	0 (n=0/5)	0 (0/71)
Overall (95% CI)	**0 (0-2)**	**0 (0-9)**	**0 (0-15)**	**0 (0-2)**
Overall Sample Size	**(n=0/155)**	**(n=0/41)**	**(n=0/23)**	**(n=0/219)**

[a] CI – confidence interval; n= total sample size; ND – no data.

The sensitivity and specificity of the test used (ID Screen Toxoplasmosis Multispecies Indirect Elisa Kit (Innovative Veterinary Diagnostics, Grabels, France) have not been established in caribou. Assuming test sensitivity of 90% and specificity of 100%, we can be nearly 95% certain that exposure to *Toxoplasma gondii* does not occur in boreal caribou from NE BC at a prevalence of greater than 1.2 % at the present time. If test sensitivity is reduced to 50% we can still be 95% certain that that exposure to *Toxoplasma gondii* does not occur in boreal caribou from NE BC at a prevalence of greater than 2.5 % at the present time. *Toxoplasma gondii* was not evaluated further as part in the BCHRP after Year 2.

3.4.6 *Neospora caninum*

Neospora caninum is a protozoan parasite with a canid definitive host (in caribou range most likely wolf, coyote, or fox) and a ruminant intermediate host (68, 84). This parasite is suspected as a likely cause of abortions and unthrifty calves in free-ranging caribou (68). Importantly, the persistent and trans-generational nature of *N. caninum* infections in ungulates also suggests that this parasite could limit the recovery of caribou populations even if it occurs at

low levels. White-tailed deer, elk, and moose may be important in the maintenance of this parasite in certain areas and its transmission to canid definitive hosts (85). As such, infection with *N. caninum* may represent a form of apparent competition (86) that could adversely affect caribou as the number of alternate intermediate and definitive hosts for this parasite increase in caribou range due to landscape and climatic change.

In BCHRP Year 1, we found evidence that 2% of adult female boreal caribou captured in winter 2012/2013 had been previously exposed to *N. caninum* (Table 25).

In BCHRP 2, logistical issues prevented further samples being sent to Spain for testing, so 225 samples (including 148 samples from winter 2012-2013, previously tested in Spain) were submitted to Prairie Diagnostic Services (PDS), Saskatoon, Saskatchewan, Canada for diagnostic testing. In BCHRP 3, the winter 2015/2016 samples, historical samples, and samples from 2013/2014 and 2014/2015 (65 of which were tested previously by PDS) were submitted to Spain for diagnostic testing.

Of the 214 samples submitted to both PDS and Spain, results agreed 99% of the time, and there were only two discordant results. These included one caribou from the Chinchaga range captured in winter 2012-2013 that tested seropositive by PDS but "doubtful" by Spain, and one caribou from the Chinchaga range captured in winter 2014-2015 that tested seronegative by PDS but "weak positive" by Spain. Results from both tests were combined, and the caribou with the discordant results were considered to be seropositive when calculating seroprevalence. From 2012-2016, twelve samples were tested only by PDS and 25 samples were tested only by Spain.

Table 25. *Neospora caninum* seroprevalence and sample sizes of boreal caribou captured in winter 2012/2013 and the three winters following the high mortality.

Herd[a]	***Neospora caninum* Seroprevalence and Sample Size**				
	2012/2013	**2013/2014**	**2014/2015**	**2015/2016**	**Overall**
Calendar	5% (n=1/21)	0 (n=0/5)	0 (n=0/2)	0 (n=0/3)	4% (1/31)
Chinchaga	3% (n=1/34)	15% (n=2/13)	0 (n=0/7)	0 (n=0/4)	5% (3/58)
Maxhamish	0 (n=0/21)	0 (n=0/8)	0 (n=0/9)	0 (n=0/6)	0 (0/44)
Parker	14% (n=1/7)	0 (n=0/1)	0 (n=0/2)	0 (n=0/2)	10% (1/12)
Prophet	0 (n=0/8)	ND	0 (n=0/2)	ND	0 (0/10)
Fort Nelson	0 (0/3)	0 (0/3)	ND	ND	0 (0/6)
Snake-Sahtaneh	0 (n=0/55)	0 (n=0/11)	0 (n=0/8)	0 (n=0/8)	0 (0/82)
Overall (95% CI)	**1%(0.2-5)**	**5% (0.6-17)**	**0 (0-12)**	**0 (0-15)**	**2% (0.7-5)**
Overall Sample Size	**(n=2/149)**	**(n=2/41)**	**(n=0/30)**	**(n=0/23)**	**(n=5/243)**

[a] CI – confidence interval; n= total sample size; ND – no data.

The overall prevalence of *N. caninum* in boreal caribou from NE BC, at ~2%, appears to be low and falls within the range previously recorded in other free-ranging caribou herds (43, 87). Seroprevalence in the historical samples from NE BC was 3% overall, and seropositive caribou were identified in the Chinchaga and Prophet herd ranges (Table 25).

Table 26. Historical *Neospora caninum* seroprevalence and sample sizes of boreal caribou captured in winter 2000-2010.

Herd	Historical *Neospora caninum* Seroprevalence and Sample Size				
	2000-2004	**2007/2008**	**2008/2009**	**2009/2010**	**Overall**
Calendar	ND	0 (n=0/16)	ND	ND	0 (0/16)
Chinchaga	ND	ND	0 (n=0/7)	33% (n=1/3)	10% (1/10)
Maxhamish	ND	ND	0 (n=0/5)	0 (n=0/3)	0 (0/8)
Parker	ND	ND	0 (n=0/3)	ND	0 (0/3)
Prophet	ND	ND	50% (n=1/2)	ND	50% (1/2)
Fort Nelson	ND	ND	ND	ND	ND
Snake-Sahtaneh	0 (n=0/13)	0 (n=0/1)	0 (n=0/7)	0 (n=0/3)	0 (0/24)
Overall (95% CI)	**0 (0-24.7)**	**0 (0-19.5)**	**4%**	**11%**	**3% (0.4-11)**
Overall Sample Size	**(n=0/13)**	**(n=0/17)**	**(n=1/24)**	**(n=1/9)**	**(n=2/63)**

[a] CI – confidence interval; n= total sample size; ND – no data.

Although overall prevalence of *Neospora* was low, it is a pathogen that can have an impact on reproductive productivity, a critical demographic indicator for caribou conservation. The overall pregnancy rate for adult female boreal caribou captured from 2012-2015 was 86% (n=204/237). The odds of "not being pregnant" was 11 times greater (95% C.I. 1-138 times greater, P=0.016) in *N. caninum* seropositive caribou than in *N. caninum* seronegative caribou.

Of the caribou that were seropositive on both tests from 2012-2015, one was pregnant and had a calf at heel at the time of capture (captured March 6, 2014), one was pregnant but had no calf at heel (captured February 25, 2013), and one was not pregnant and had no calf at heel (captured January 7, 2013). Of the caribou with discordant results, both were not pregnant and had no calf at heel at the time of capture (January 21, 2013 and March 6, 2014).

Continued monitoring of this parasite in caribou as well as in other ungulate intermediate hosts and canid definitive hosts in NE BC is recommended. The current distributions of exposure to *N. caninum* historically and from 2012-2016 are shown in Figs. 22 and 23, respectively.

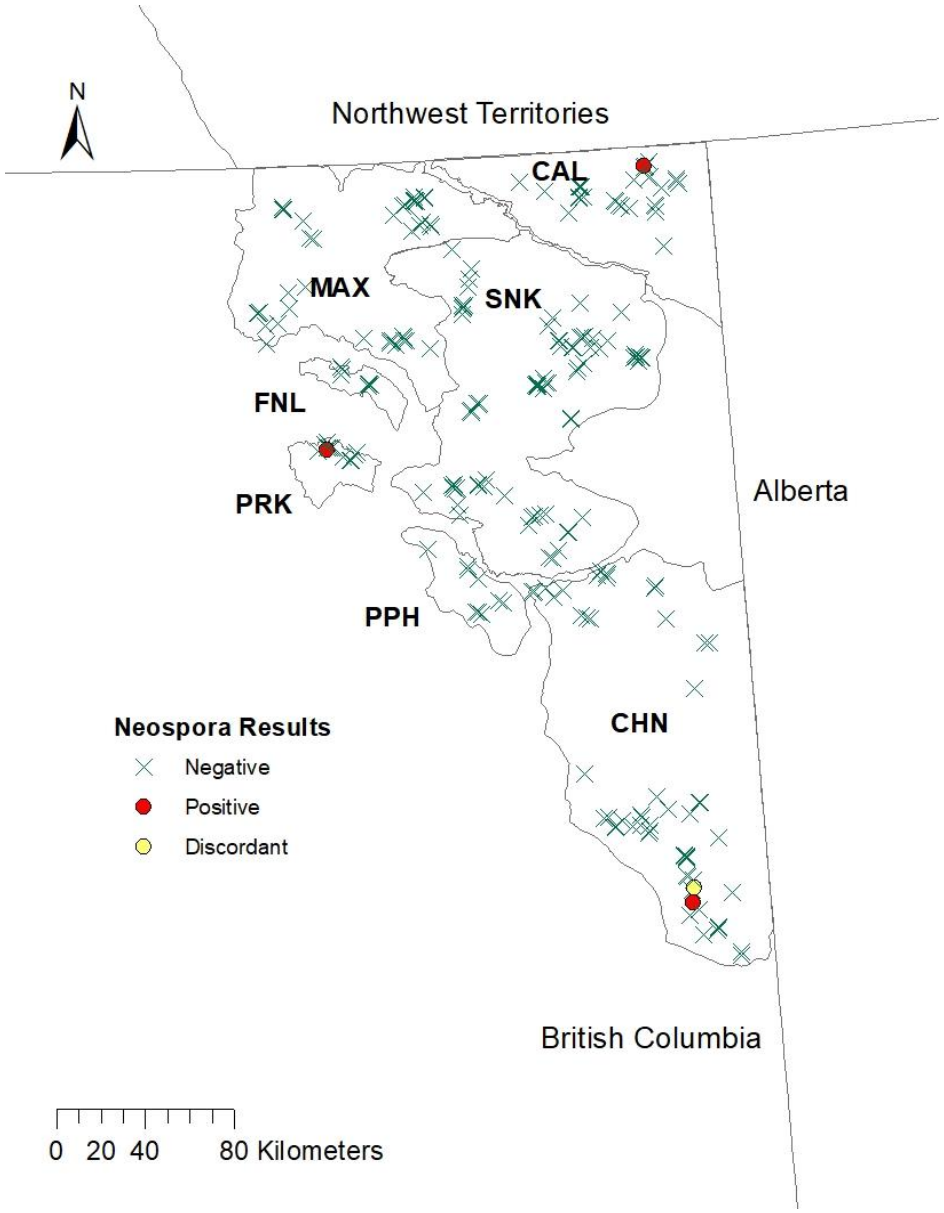

Figure 22. Spatial distribution of *Neospora caninum* serostatus in adult female caribou in northeast B.C. in winters 2012/2013 to 2015/2016. Repeated captures are included on the map. The 2nd discordant result marker is not visible on the map, but is located behind the positive marker in Chinchaga.

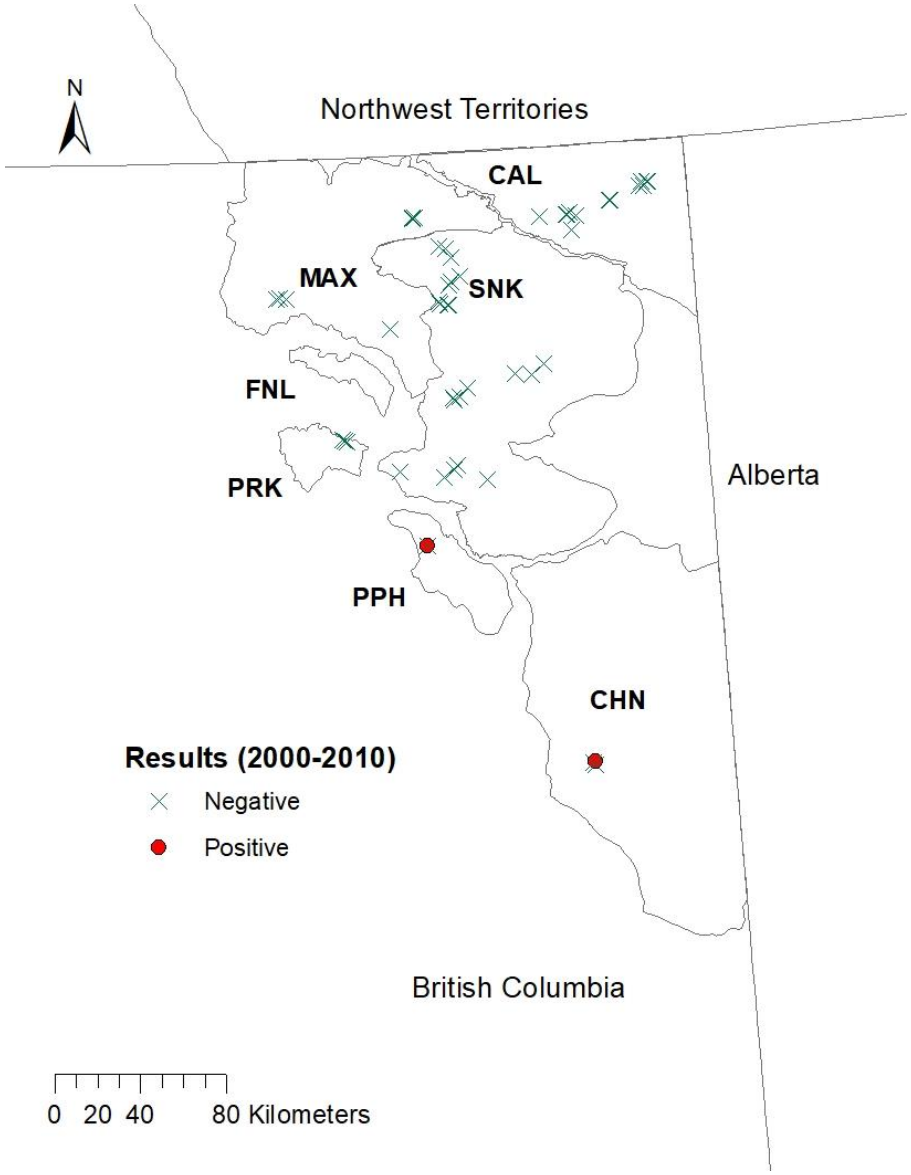

Figure 23. Spatial distribution of historical *N. caninum* serostatus in adult female caribou in northeast B.C. in winters 2000-2010.

3.4.7 Ectoparasites

In BCHRP Year 1, winter ticks (*Dermacentor albipictus*) were recorded as incidental observations in 14% (n=7/164), 22% (9/41), 41% (13/32), and 75% (18/24) of adult female boreal caribou captured in NE BC in winters 2012/2013, 2013/2014, 2014/2015, and 2015/2016 respectively.

In BCHRP Year 2, we used standardized photographs taken of all n=238 individual caribou captured in NE BC in winter 2012-2015 to develop a classification system for tick associated hair loss in live caribou, which is similar to what is used for moose (88) (Fig. 24).

Although the majority of BCHRP caribou captures occurred from late January to early April, almost half of the caribou captured in winter 2012/2013 were captured in December when winter ticks are small and difficult to locate on infested animals. It must be noted that caribou with high tick burdens may not exhibit any hair loss. Therefore, prevalence estimates based on incidental winter tick observations and hair loss should be viewed as a crude index of infestation at the herd or population level only.

We also performed KOH digests to obtain tick counts from hide samples collected from n=5 dead caribou from NE BC in 2014 and 2015. Hide digests revealed evidence of tick burdens [mean 5 ticks/cm^2 (range 0-14 ticks/cm^2, n=5)] in some boreal caribou from NE BC that were higher than those previously recorded in woodland caribou from Alberta (0.005-0.01 ticks/cm^2) (89) and burdens typically recorded in moose (~1-2 ticks/cm^2) (90).

Figure 24. Recommended classification score for *Dermacentor albipictus* (winter tick) related hair loss in boreal caribou from northeast BC. NONE: No hair loss or breakage (not pictured), MILD: Few small to medium sized patches of broken hair or hair loss (pictured top left), MODERATE: Several or large patches broken hair or hair loss with no exposed skin (pictured top right), SEVERE: Several or large patches broken hair or hair loss with small area of exposed skin (pictured bottom left), EXTREME: Several or large patches broken hair or hair loss with large or multiple areas of exposed skin (pictured bottom right). Photo credit and classification score: D. Culling, Diversified Environmental Services Inc., Fort St. John, BC

In Year 1, we did not find a significant difference in mild, moderate, severe, or extreme classification scores for hair loss, so for the analysis, we analyzed the results as to whether hair loss was present or absent. Because hair loss varies according to season in moose (91), we presented the results by early (Dec. 1-Feb. 15) and late winter (Feb. 16-Apr. 30). Across all years, the overall prevalence of winter tick associated hair loss in boreal caribou from NE BC was 72% in early winter (Table 26) and 86% in late winter (Table 27).

Table 27. Prevalence of hair loss related to *Dermacentor albipictus* (winter tick) infestations on adult female boreal caribou live-captured in northeast British Columbia in early winter (Dec. 1- Feb. 15) from 2012/2013 to 2015/2016.

Herd[a]	**Prevalence of Winter Tick Associated Hair Loss (%) and Sample Size in Early Winter**				
	2012/2013	**2013/2014**	**2014/2015**	**2015/2016**	**Overall**
Calendar	ND	ND	ND	ND	ND
Chinchaga	67% (n=20/30)	ND	25%(n=1/4)	ND	62% (21/34)
Maxhamish	65% (n=11/17)	ND	33% (n=2/6)	ND	56% (13/23)
Parker	83% (n=5/6)	ND	ND	ND	83% (5/6)
Prophet	100% (n=6/6)	ND	0 (n=0/1)	ND	`100% (6/6)
Fort Nelson	100% (2/2)	ND	ND	ND	100% (2/2)
Snake-Sahtaneh	83% (n=24/29)	ND	100% (n=3/3)	ND	84% (27/32)
Overall (95% CI)	**76%(65-84)**	**ND**	**43% (18-71)**	**ND**	**72% (62-80)**
Overall Sample Size	**(n=68/90)**	**ND**	**(n=6/14)**	**ND**	**(n=74/103)**

[a] CI – confidence interval; n= total sample size; ND – no data.

Table 28. Prevalence of hair loss related to *Dermacentor albipictus* (winter tick) infestations on adult female boreal caribou live-captured in northeast British Columbia in late winter (Feb. 16-Apr. 30) from 2012/2013 to 2015/2016.

Herd[a]	**Prevalence of Winter Tick Associated Hair Loss (%) and Sample Size in Late Winter**				
	2012/2013	**2013/2014**	**2014/2015**	**2015/2016**	**Overall**
Calendar	85% (n=22/26)	100% (n=5/5)	100% (n=1/1)	100% (n=1/1)	88% (29/33)
Chinchaga	71% (n=5/7)	62% (n=8/13)	100%(n=3/3)	100% (n=4/4)	74% (20/27)
Maxhamish	100% (n=5/5)	100% (n=8/8)	100% (n=2/2)	100% (n=4/4)	100% (19/19)
Parker	ND	100% (n=1/1)	100% (n=1/1)	50% (n=1/2)	75% (3/4)
Prophet	100% (n=2/2)	ND	100% (n=1/1)	ND	`100% (3/3)
Fort Nelson	100% (1/1)	100% (n=3/3)	ND	ND	100% (4/4)
Snake-Sahtaneh	77% (n=20/26)	91% (n=10/11)	100% (n=3/3)	100% (n=6/6)	85% (39/46)
Overall (95% CI)	**82%(71-90)**	**85% (71-94)**	**100% (72-100)**	**94% (71-100)**	**86% (79-91)**
Overall Sample Size	**(n=55/67)**	**(n=35/41)**	**(n=11/11)**	**(n=16/17)**	**(n=117/136)**

[a] CI – confidence interval; n= total sample size; ND – no data.

Table 29. Univariable analyses for associations between hair loss and health and fitness parameters in female adult boreal caribou captured in winter 2012/2013 to 2015/2016. Significant associations are highlighted in yellow and trends are in green.

Parameter	Variable Type	Sample Size	P-Value
Survived to next winter	Binary	240	0.102
Year	Categorical	240	0.157
Pregnancy	Binary	237	0.637
Calf at heel	Binary	232	>0.999
Age	Categorical	240	0.918
Residual antler velvet	Binary	218	0.654
Herd	Categorical	140	0.367
Besnoitia	Categorical	220	0.510
Dorsal-spined larvae	Binary	212	0.390
Alphaherpes virus	Binary	220	0.064
Neospora caninun	Binary	228	>0.999
Erysipelothrix	Binary	227	0.847
Positive serology	Binary	240	0.049
Cobalt	3 quantiles	196	0.405
Copper	3 quantiles	196	0.068
Iron	3 quantiles	196	0.104
Manganese	Continuous	196	0.974
Selenium	3 quantiles	196	0.229
Zinc	3 quantiles	195	0.751
Haptoglobin	3 quantiles	211	0.706
Serum Amyloid A	3 quantiles	223	0.264
Hair cortisol concentration	3 quantiles	220	0.978
Disturbance Late Summer	3 quantiles	108	0.028
Road density Late Sumer	3 quantiles	108	0.114
Seismic line density Late Summer	3 quantiles	108	0.296
Moose density Late Summer	3 quantiles	121	0.884
Season	Binary	240	0.006

Hair loss in caribou was significantly more likely in late than early winter, consistent with the syndrome seen in moose infested with winter ticks (Table 28). Hair loss was also significantly associated with habitat disturbance (Table 28) and was more common in habitats with disturbance in the middle than bottom quantile (P = 0.025). Although hair loss was more common in disturbed habitats in the top quantile than those in the bottom quantile, the association was not significant (P = 0.107).

Hair loss was more common in caribou that were seronegative to alphaherpesvirus, *Besnoitia*, and *Erysipelothrix* than those that tested seropositive to one or more of the pathogens

(Table 28). Caribou testing seropositive to alphaherpevirus were less likely to have hair loss than those testing seronegative. This association was not confounded by age.

There were trends between hair loss and seropositivity to alphaherpesvirus, and copper levels (Table 28).

Season confounded copper, and when season was included in the model, caribou with copper levels in the middle quantile were more likely to have hair loss than those in the top quantile (P=0.045). There was a trend with caribou in the bottom quantile being less likely to have hair loss than those in the middle quantile (P=0.065).

Of 13 caribou initially captured in early winter and then later recaptured in late winter of a subsequent year, 11 worsened, 1 improved, and 1 stayed the same in their tick classification score. Of 4 caribou initially captured in late winter and then recaptured in early winter of a subsequent year, 1 worsened, 1 improved, and 2 stayed the same. These observations are generally consistent with our findings that hair loss is more common in late than early winter.

Of 5 caribou recaptured in the same season (late winter) over multiple years, 2 improved, 2 stayed the same, and 1 worsened in their tick classification scores.

Table 30. Winter tick classification scores of n=22 recaptured adult female boreal caribou from northeast British Columbia. Boxes highlighted in blue indicate early winter captures and boxes highlighted in red indicate late winter captures.

Animal ID	Herd	Age[a]	2007/2008	2012/2013	2013/2014	2014/2015	2015/2016
SK005	Maxhamish	OA		None	Moderate		
SK007	Maxhamish	YA; MA		Moderate		Severe	
SK009	Fort Nelson	MA; OA		Mild		None	
SK014	Parker	MA		Mild		Severe	
SK016	Parker	YA; MA		Moderate		Severe	
SK020	Snake-Sahtaneh	MA		None		Mild	
SK026	Chinchaga	MA		None		Mild	
SK033	Chinchaga	MA		Mild		Mild	
SK036	Chinchaga	MA		None		Mild	
SK038	Chinchaga	YA; MA		Mild			Moderate
SK066	Maxhamish	JUV; YA		Moderate			Severe
SK079	Snake-Sahtaneh	YA; MA		Mild		Moderate	

SK097	Snake-Sahtaneh	YA; MA		Moderate		Severe	
SK100	Snake-Sahtaneh	YA; MA		Mild		None	
SK110	Snake-Sahtaneh	YA; MA		Mild		Mild	
SK126	Calendar	YA; MA		Severe		Moderate	
SK 136	Calendar	YA; MA		Severe		Severe	
SK146	Calendar	MA; OA		None		None	
SK 161	Prophet	MA; OA		None		Mild	
SK 169	Maxhamish	OA; OA		Moderate		Severe	
SK 170	Chinchaga	OA; OA		None		None	
SK 181	Calendar	MA; OA		Moderate		Mild	

[a] YA – young adult; MA – mature adult; OA – old adult.

In moose, hair loss associated with *D. albipictus* infestations is the result of irritation and excessive grooming (92). Grooming behaviour may interrupt foraging and this response may lead to a decrease in body condition and a diminished probability of overwinter survival in affected individuals (92, 93). Heavy infestations may also cause anemia (due to blood loss) and epizootics of winter tick related mortality are known to occur in this species (93). Unlike moose, the effects of *D. albipictus* infestations on caribou are not well characterized and heavy burdens may occur in caribou with little or no hair loss. Emaciated caribou with heavy tick burdens (and no hair loss) have been observed in Alberta (65, 89). In addition to extensive hair loss, some heavily infested boreal caribou from NE BC (this study) were in poor body condition at the time of capture.

The number of boreal caribou from NE BC infested with *D. albipictus* along with the occurrence and severity of hair loss appears to have increased in approximately the last five years (94). This parasite appears to represent an emerging threat to caribou health in the region. Climate change leading to longer, drier, and warmer periods in autumn and earlier snowmelt in spring may improve conditions for winter ticks by increasing the survival of pregnant female ticks in the spring when they release from their host and the load of tick larvae in the fall. This may increase the risk of infestation and related effects in boreal caribou even further in NE BC in the near future (90). Likewise, recent landscape change may also enhance the risk of winter tick transmission to caribou due to an increase in the number of moose inhabiting caribou range. Ongoing surveillance and research into the occurrence and impact of winter tick infestations on caribou is warranted.

Winter ticks can carry and may transmit microorganisms (e.g. *Anaplasma* sp.) that have the potential to cause severe/fatal disease in cervids (95). We have collected and archived

winter ticks collected from caribou in NE BC to facilitate molecular investigation of tick borne pathogens in the future. Tick burdens and hair loss are not directly correlated in caribou and the validation and incorporation of alternate techniques for quantifying infestations on live-captured caribou (e.g., hair transect counts (96)) are recommended.

Hair loss in early and late winter across all caribou herds are shown in Figs. 25 and 26, respectively.

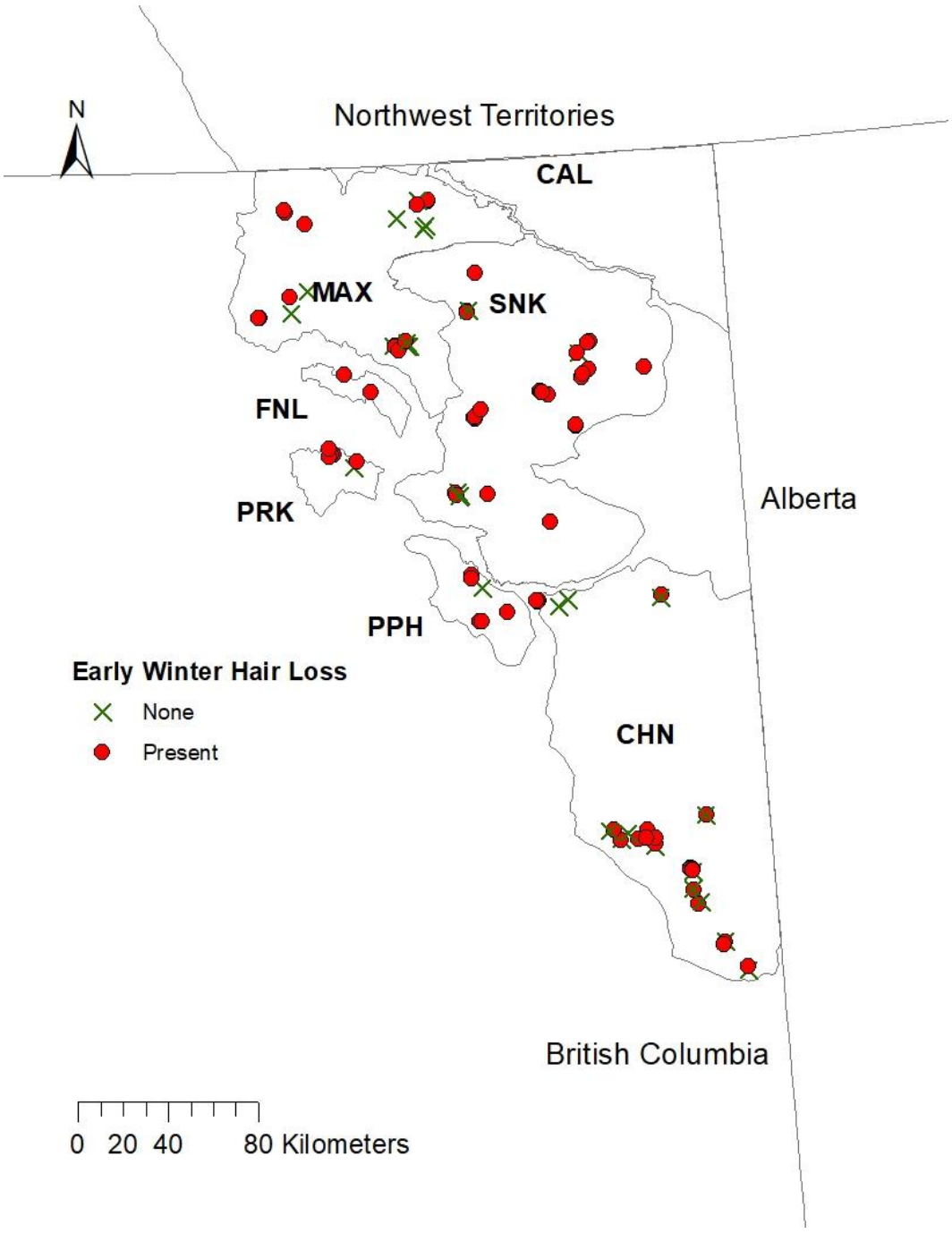

Figure 25. Spatial distribution of early winter (Dec. 1-Feb. 15) hair loss in adult female caribou in northeast B.C. in winters 2012/2013 to 2015/2016. Repeated captures are included on the map.

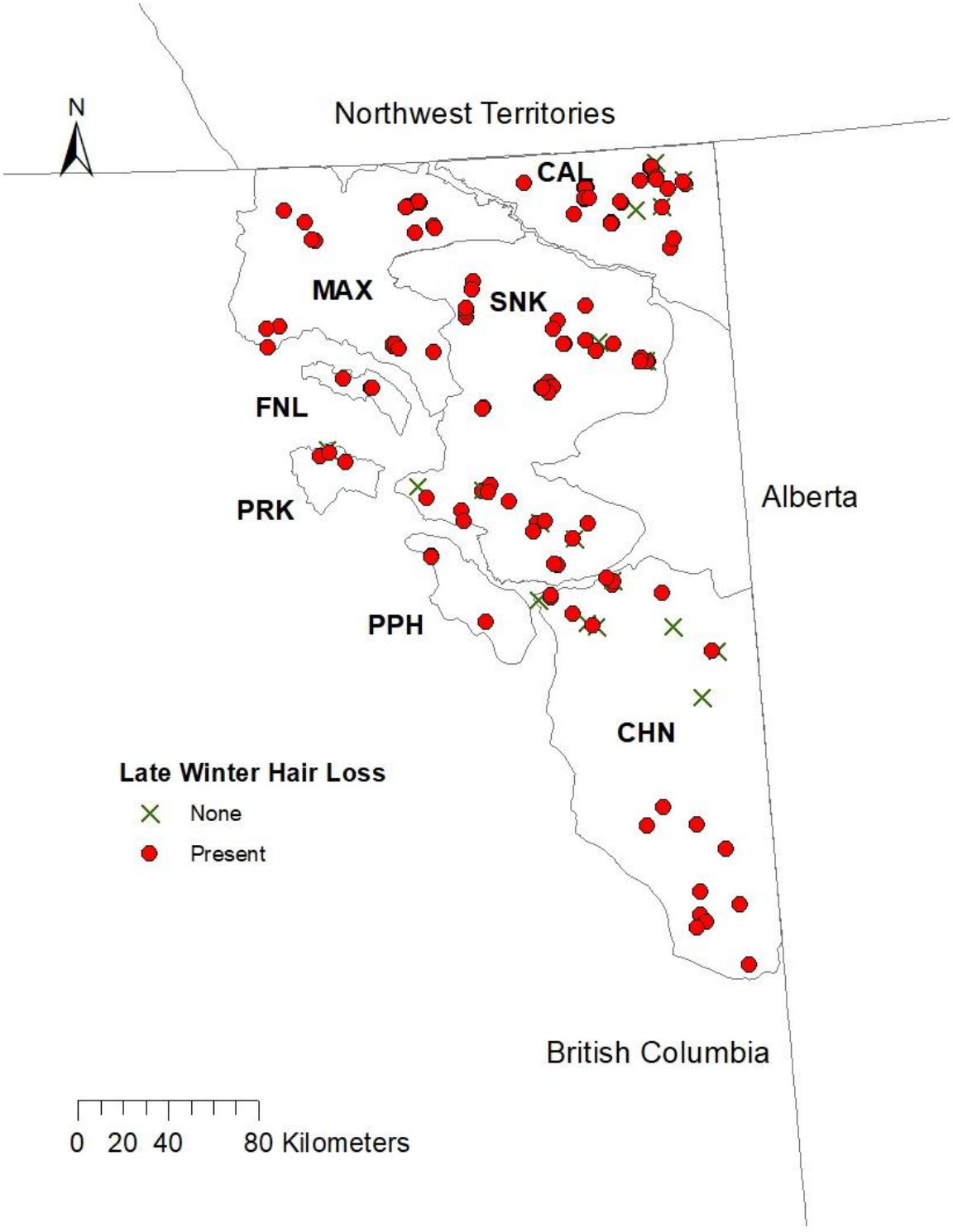

Figure 26. Spatial distribution of late winter (Feb. 16-Apr. 30) hair loss in adult female caribou in northeast B.C. in winters 2012/2013 to 2015/2016. Repeated captures are included on the map.

Warble fly (*Hypoderma tarandi*) larvae are considered one of the most important parasites of tundra *Rangifer* (97, 98). Heavy infections (1000+ larvae) have been reported and migrating and developing larvae may cause significant pathology in the skin and subcutaneous tissue (97) and a high energetic cost. In addition, avoidance behaviour may lead to a decrease in foraging efficiency and an increase in energy expended by caribou being harassed by adult flies. This may lead to a decrease in the body condition and reproductive success of adult caribou and diminished condition and overwinter survival in juvenile caribou (99, 100). Warbles were recorded on 3% (n=9/261) of caribou captured in NE BC from winter 2012/2013 to 2014/2015. No warbles were recorded in winter 2012/2013. However, warbles were detected in 4 caribou in winter 2013/2014 (2 caribou from each of Chinchaga and Snake-Sahtaneh), 4 caribou in winter 2014/2015 (3 caribou from Chinchaga and 1 from Prophet), and 1 caribou from Parker in winter 2015/2016. The distribution of warbles found on caribou in NE BC is shown in Fig. 27.

All warbles collected have been submitted to the CWHC for identification. Results are pending; however, *Hypoderma tarandi* is the species most likely to be identified. Warbles do not appear to be a limiting factor for boreal caribou in NE BC at the present time. To date, no other ectoparasites have been identified on any boreal caribou examined (live or dead) in this study.

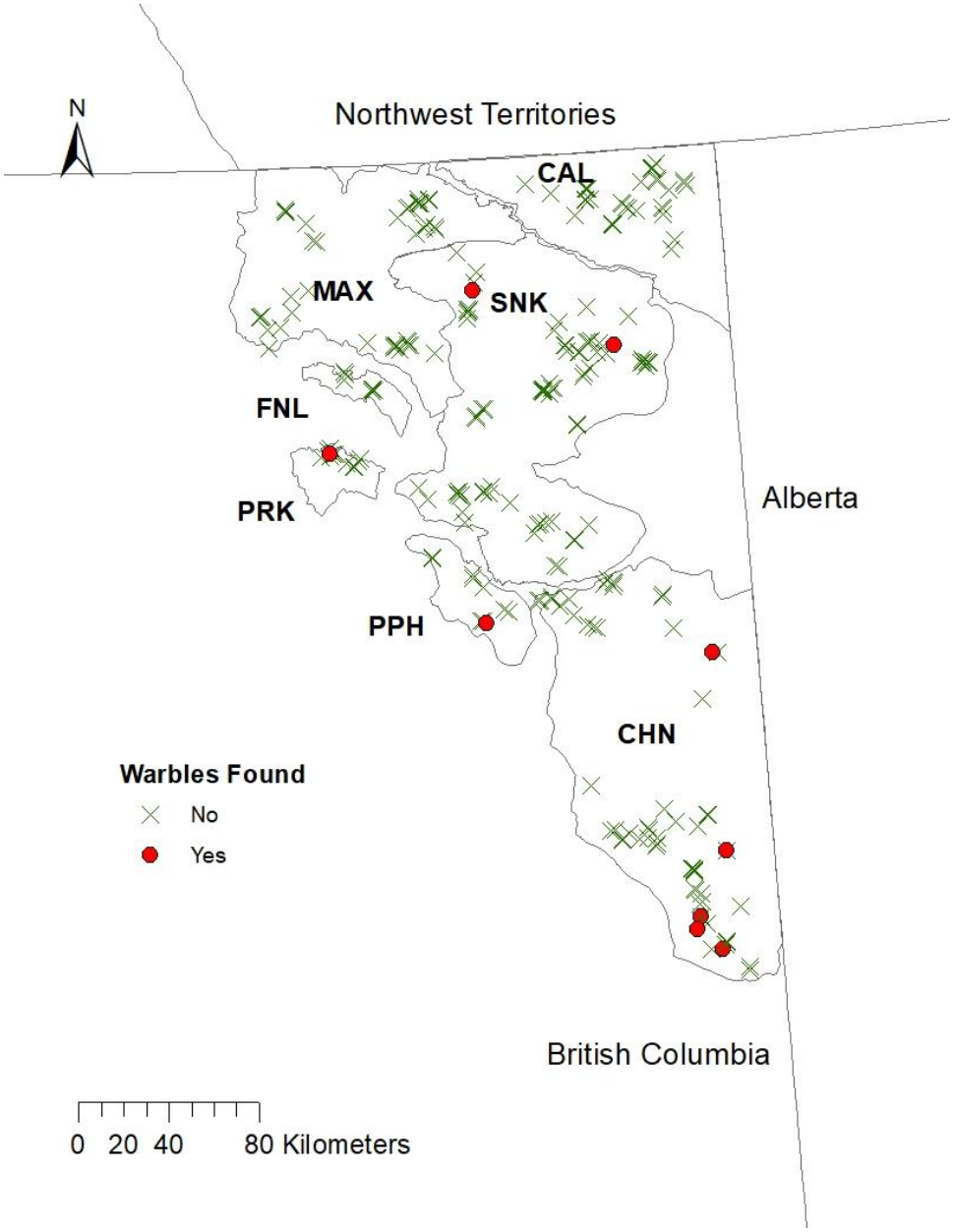

Figure 27. Spatial distribution of warbles in adult female caribou in northeast B.C. in winters 2012/2013 to 2015/2016. Repeated captures are included on the map. No warbles were found on caribou in the Fort Nelson, Maxhamish, and Calendar herds.

3.4.8 Arthropod Vectored Blood Borne Parasites (*Babesia, Anaplasma, Trypanosoma, Setaria, Onchocerca*)

In BCHRP Year 1, a caribou that died during the high mortality period in 2013 was found to be iron deficient (12). Blood borne pathogens and parasites such as *Babesia* and *Anaplasma* may be one of many causes of iron deficiency in ungulates. These organisms are also known or suspected to cause an array of subclinical and clinical disease syndromes which may adversely affect the survival and/or reproductive success of infected animals (100-102). Likewise, vector borne nematodes (*Setaria* sp.) have been implicated as the cause of severe disease in free-ranging and semidomesticated *Rangifer* (103) while new species of blood borne pathogens and extra-limital (or newly recognized) occurrences of known blood borne pathogens have been recently identified in Canadian cervids (including in BC) (104).

The occurrence, distribution, and impact of blood borne pathogens in free-ranging caribou are not currently known. In BCHRP Year 2, n=57 blood smears from n=27 caribou captured in 2015 (University of Guelph Animal Health Laboratory, Guelph Ontario) and molecular testing of blood from n=15 caribou that died in 2013 (PCR, Canadian Wildlife Health Cooperative, Calgary, AB) were employed to screen a subset of individuals for evidence of infection with blood borne pathogens or parasites (e.g. *Trypanosoma, Anaplasma, Babesia*) and/or vector borne nematodes (e.g. *Setaria, Onchocerca*). No evidence of blood borne pathogens was identified in any of the n=57 blood smears examined and PCR results for *Babesia* and *Anaplasma* were negative in all n=15 caribou tested. Microfilaria (larval Filarid nematodes) were identified in blood smears from 1% (n=3/32) caribou examined. Microfilaria counts were low (approximately 1 microfilaria /slide) in all cases. The microfilariae identified in boreal caribou from NE BC are most likely *Setaria* or *Onchocerca* sp. No evidence of *Trypanosoma* sp. was recorded in any blood smear collected from boreal caribou in NE BC. This finding was somewhat unexpected as *Trypanosoma* sp. appear to be very common (~ almost ubiquitous) in woodland caribou from AB and the NWT (28, 105); however, isolation of the buffy coat and/or blood culture, is often required in order to be able to visually detect this parasite. All considered, these findings may indicate that blood borne pathogens and parasites and vector borne nematodes are unlikely to be limiting factors for boreal caribou in NE BC at the present time. The occurrence of blood borne pathogens and parasites and vector borne nematodes in boreal caribou from NE BC is likely to increase as climate change supports an increase the number of arthropod vectors and/or the seasonal duration of arthropod activity in the region (103). Opportunistic and periodic surveillance for blood borne pathogens and parasites and vector borne nematodes in boreal caribou from NE BC is recommended

4. Results, Discussion, and Recommendations (Other Health Indices)

4.1 Serum Biochemistry

Patterns and levels of circulating enzymes, metabolites, and hormones measured in serum permit an evaluation of physiology and organ function which may reflect factors such as capture, stress, immunity, disease, and nutrition in individual caribou and caribou herds. In BCHRP Year 1, serum biochemistry was evaluated in n=75 adult female boreal caribou captured in NE BC in winter 2012/2013. In BCHRP Year 2, additional serum from caribou captured in winter 2012/2013 (n=7), 2013/2014 (n=40), and 2014/2015 (n=32) was tested (Table 31) to more thoroughly establish "normal" values and increase our understanding of potential variation around normal in individual caribou and across different caribou herds. Overall, serum biochemical parameters recorded in adult female boreal caribou from NE BC in 2012/2013, 2013/2014, and 2014/2015 were similar to those previously recorded in adult female boreal from the Northwest Territories (also captured by net-gun) [e.g.15].

4.2 Haematology

Complete blood counts (CBCs) measure the numbers, types and morphology or red blood cells, white blood cells, and platelets circulating in the blood stream and provide insight into processes such as inflammation, infection, anemia, and blood clotting which may reflect the general health status of caribou and/or the occurrence of specific pathogens (e.g. blood borne parasites) or disease processes (e.g. acute vs. chronic inflammation, parasitism) in caribou. Logistical considerations prevented the collection of samples (fresh blood preserved in EDTA) required for CBCs in BCHRP Year 1. IN BCHRP Year 2, n=57 blood smears were collected from n=27 caribou captured in winter 2014/2015 to support this analysis. Ambient conditions encountered in the field made the preparation of smears of sufficient quality for accurate red blood cell counts quite difficult and no samples collected were usable for this purpose. Nonetheless, no overt evidence of anemia was noted in any caribou. Data obtained from a subset of slides did provide useful baseline information regarding what may be "normal" total white blood cell counts and leukocyte differential profiles for adult female caribou captured by net-gun in NE BC in winter (Table 32). White blood cell counts and differentials obtained from free-ranging boreal caribou in NE BC fell within reference ranges for captive *Rangife*r (106) and were also similar to those recorded in generally healthy semi-domesticated reindeer (107). No evidence of severe infection was identified in any caribou examined.

Table 31. Serum biochemical parameters for n=154 free-ranging, adult female boreal caribou captured in winter 2102/2013, 2013/2014, and 2014/2015 by net gun in northeast British Columbia.

Parameter	Mean	Median	Range (95% C. I.)	S.D.
Calcium (mmol/l)	2.48	2.48	2.46 - 2.50	0.14
Phosphorus (mmol/l)	1.85	1.82	1.79 – 1.91	0.36
Ca:P Ratio*not normally distributed	1.41	1.35	1.35 - 1.47	0.39
Magnesium (mmol/l)*	1.07	1.10	1.05– 1.09	0.12
Sodium (mmol/l)*	141.29	145.00	138.55 – 144.04	17.37
Potassium (mmol/l)*	7.94	5.10	6.97-8.92	6.18
Chloride (mmol/l)	93.23	93.50	92.70-93.77	3.39
CO_2 (mmol/l)	7.35	7.00	6.83-7.88	3.34
Anion (mmol/l)	50.34	51.00	49.26-51.43	6.90
Na : K Ratio*	25.08	28.00	23.37-26.79	10.84
Total Protein (g/l)	70.21	70.00	69.37-71.06	5.35
Albumin (g/l)*	42.65	43.00	41.78-43.52	5.50
Globulin (g/l)*	27.05	26.00	26.13-27.96	5.78
Albumin : Globulin Ratio	1.67	1.67	1.61-1.72	0.36
Urea (mmol/l)*	1.66	1.40	1.49-1.82	1.06
Creatinine (mmol/l)*	211.89	207.00	203.84-219.94	50.95
Glucose (mmol/l)	6.99	6.90	6.70-7.29	1.86
Cholesterol (mmol/l)	1.17	1.14	1.14-1.21	0.20
Total Bilirubin (umol/l)*	1.68	2.00	1.56-1.79	0.74
Conjugated Bilirubin (umol/l)*	0.86	1.00	0.81-0.92	0.34
Free Bilirubin (umol/l)*	0.82	0.79	0.70-0.95	0.81
Alkaline phosphatase (ALP) (U/l)*	61.95	57.00	57.96-65.94	25.25
Gamma-glutamyltransferase (GGT) (U/l)*	19.75	17.00	17.53-21.97	14.06
Aspartate aminotransferase (AST) (U/l)	71.45	68.00	68.15-74.76	20.94
Creatine kinase (CK) (U/l)	260.30	247.00	239.76-280.84	130.05
Glutamate dehydrogenase (GLDH) (U/l)*	4.89	3.00	3.41-6.37	9.32
Betahydroxybutyrate (BHBA) (umol/L)	575.94	583.00	555.15-596-72	131.62
Non-Esterified Fatty Acids (NEFA) (mmol/L)	0.61	0.59	0.55-0.67	0.36

Table 32. Mean total white blood cell count and white blood cell differential for n=27 adult female boreal caribou captured in northeast British Columbia in winter 2014/2015.

Total White Blood Cell Count and White Blood Cell Differential (* 10^9/L + S.D.)					
Total WBC Count	Segmented Neutrophils	Lymphocytes	Monocytes	Eosinophils	Basophils
4.04±1.55	0.91±0.57	1.82±0.86	0.12±0.09	0.92±0.48	0.11±0.14

4.3 Hair Cortisol Concentration (Chronic Physiological Stress)

Chronic physiological stress is increasingly recognized as a factor that may contribute to diminished health in free-ranging wildlife and a mechanistic linkage between chronic stress and diminished growth, immunity, reproduction, and survival is recognized in many species (108)[reviewed]. The measurement of corticosteroids in hair is an emerging technique in wildlife health studies which has been previously evaluated in caribou/reindeer (108-110) and may represent the best integrated measure of chronic physiological stress currently available for this species. Hair was plucked from the shoulder of all caribou captured and was sent to the University of Saskatchewan, Western College of Veterinary Medicine for hair cortisol concentration testing using an Oxford EA–65 Cortisol Competitive EIA kit (Oxford Biomedical, Lansing, MI, USA).

In BCHRP Year 1, we measured hair cortisol concentration (HCC) in n=156 individual adult female boreal caribou captured in NE BC in winter 2013/2013 (mean 3.7 pg/mg, range 0.39-47.9). In BCHRP Year 2, we measured HCC in n=40 caribou and n=24 caribou captured in winter 2013/2014 (mean 5.4 pg/mg, range 0.70-42.4) and 2014/2015 (mean 7.0 pg/mg, range 1.0-47.4), respectively. We also measured HCC in n=13 radio-collared caribou that died between 2013 and 2015.

Hair cortisol concentration was significantly lower in winter 2012-2013 than in winter 2013-2014 and winter 2014-2015 (Kruskall-Wallis test; P = 0.003 and 0.045, respectively). There was no difference in HCC recorded in caribou captured in winter 2013/2014 and 2014/2015 (P > 0.999). Hair cortisol concentration in the individual was significantly and linearly associated with mortality within the next year (P = 0.028; Fig. 28).

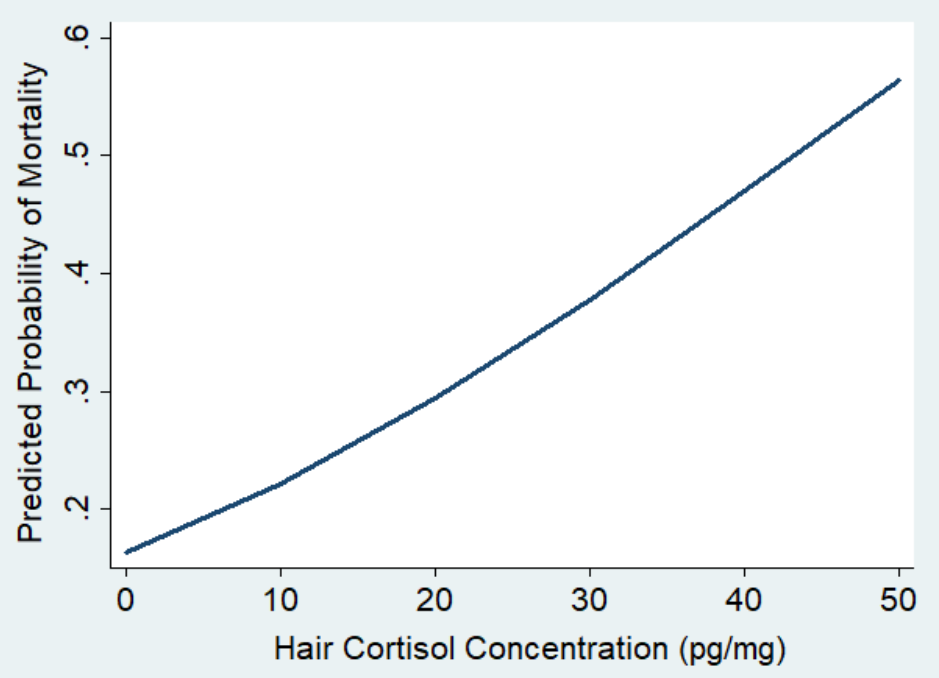

Figure 28. Association between hair cortisol concentration and the probability of an adult female caribou surviving to the next year in NE BC from winter 2012-2016.

HCC measured in caribou captured in winter reflects Hypothalamic-Pituitary-Adrenal (HPA) axis activity that occurred in the preceding spring through late summer (i.e. the period of active hair growth). Accordingly, these findings may indicate that stress levels experienced by boreal caribou in NE BC in the spring and summer of 2013 (during the high mortality period) and 2014 (after the high mortality period) were higher than stress levels experienced by caribou in spring and summer of 2012 (before the high mortality period).

Interestingly, HCC levels recorded in dead radio-collared caribou were higher than levels initially recorded in the same animal when it was captured (Paired t-test, t_{12}=4.766, P= 0.0005, n=13). In addition, HCC levels recorded in boreal caribou from NE BC (mean 4.36 pg/mg, range 0.16 - 47.94 pg/mg, n=236) were higher overall (Unpaired t-test, t_{231}=3.281, P = 0.001) than HCC levels previously determined (using the same assay) in n=24 captive reindeer and caribou from Alaska and n=97 free-ranging caribou from Greenland (108).

Multivariate analyses remain necessary to gain meaningful insight into all factors that may explain hair cortisol concentrations measured in boreal caribou. However, based on the relationship with apparent survival, there is supporting evidence that HCC may be a useful management/research tool in caribou.

4.4 Acute Phase Proteins

Acute phase proteins such as haptoglobin and serum amyloid A (SAA) are believed to play critical roles in combating the deleterious effects of infectious agents and inflammatory processes by removing cellular debris, neutralizing enzymes, and modulating the specific (B and T cell mediated) immune response (111). Circulating concentrations of APPs increase in response to inflammatory stimuli and haptoglobin and SAA levels are known to rise during the course of both acute and chronic bacterial or viral infections in caribou and other ruminants (112-116). As such, haptoglobin and SAA may be useful indicators of both the occurrence and severity of pathological conditions in these species.

4.4.1 Haptoglobin

A range of haptoglobin levels were recorded in n=152 adult female boreal caribou captured in NE BC in winter 2012/2013 (mean 0.15 g/L; range 0.11-0.39 g/L). In 2012/2013, the mean haptoglobin levels (mean 0.17 g/L; range 0.11-0.87 g/L) of caribou in NE BC fell within the test range (0.00-0.50 g/L) considered to be normal in domestic ruminants (117). In BCHRP Year 2, we measured haptoglobin levels in n=40 caribou captured in winter 2013/2014 (mean 0.40 g/L, range 0.27-0.90 g/L) and n=25 caribou captured in 2014/2015 (mean 0.56 g/L, range 0.38-1.16 g/L). Haptoglobin levels measured in boreal caribou in winter 2013/2014 and winter 2014/2015 were higher than winter 2012/2013 (Kruskall Wallis test; All $P < 0.001$). Haptoglobin levels did not differ between winter 2014/2015 and 2013/2014 (Kruskall Wallis test; $P = 0.716$).

To provide further context for results obtained from free-ranging boreal caribou in NE BC, we also evaluated haptoglobin levels in n=4 captive barren ground caribou bulls with known clinical histories in BCHRP Year 2. These captive caribou were in excellent nutritional condition and had high total body fat. However, upon euthanasia of three of the caribou, evidence of parasitic infections (moderate larval migrans of the liver and the thorax) and chronic inflammation negative for *Mycobacterium avium* ssp. *paratuberculosis* (Tb), and are likely to have been (severe multifocal pulmonary granulomas) were found. The granulomas tested caused by chronic parasitic infections.

In these four captive caribou, haptoglobin levels (mean 0.64 g/L, range 0.49-0.88 g/L) measured in n=4 captive caribou with moderate pathology (determined at post-mortem) due to parasite infections were higher (One-way ANOVA, $F_{3,225}$ =100.00, P<0.0001, Tukey Kramer P<0.05) than haptoglobin levels determined in free-ranging boreal caribou from NE BC in winter 2012/2013 and 2013/2014 but similar (Tukey Kramer P>0.05) to haptoglobin levels determined in free-ranging caribou in winter 2014/2015. Haptoglobin levels measured in both captive and free-ranging caribou were also lower than the level measured in n=1 free-ranging mountain caribou with an extensive, chronic, severe bacterial infection (5.70 g/L).

4.4.2 Serum Amyloid A

In BCHRP Year 1, a wide range of SAA levels were recorded in n=156 adult female boreal caribou captured in NE BC in winter 2012/2013 (mean 84.37 ug/ml, range 0.00 - 1060.00) ug/ml). In BCHRP Year 2, SAA levels were measured in n= 41 caribou captured in winter 2013/2014 (mean 50.53 ug/ml, range 0.00 - 204.10 ug/ml) and n=25 caribou captured in 2014/2015 (mean 159.7 ug/ml, range 7.4 - 1609.00 ug/ml).

Serum Amyloid A levels measured in the caribou from NE BC did not significantly differ among years (Kruskall Wallis test, P = 0.28), but did differ among herds. In winter 2012-2013, SAA levels in the Calendar herd (median 94.7, intraquartile range (IQR) 75-125) were significantly higher than those in the Chinchaga (median 26.7, IQR 19.9-44.9), Maxhamish (median 45.7, IQR 20.2-96.0), and Snake-Sahtaneh herds (median 54.3, IQR 26.4-81.8) (Kruskall Wallis; All P < 0.05).

Serum Amyloid A levels in the caribou from NE BC appeared to be higher than SAA levels from two euthanized adult caribou cows (SAA = 32.9 and 31.5 ug/ml) that had no evidence of parasitic or other infections upon necropsy. In addition, SAA levels in the caribou from NE BC were similar (One-way ANOVA, P>0.05) to SAA levels determined in n= 4 captive caribou bulls with moderate pathology due to parasite infections (mean 200.2 ug/ml, range 192.5-206.6 ug/ml). Likewise, SAA levels in the Calendar herd were uniformly high and similar to those of the reindeer exposed to endotoxin in a challenge trial by Orro et al. (115), suggesting an inflammatory process.

However, SAA levels in most boreal caribou from NE BC were lower than those reported for captive reindeer administered bacterial endotoxins in experimental studies or affected by severe bacterial infections (116). For example, SAA levels measured in free-ranging boreal caribou from NE BC were lower than the level measured in n=1 free-ranging mountain caribou with an extensive, chronic, severe bacterial infection (>10,000 ug/ml).

Hair cortisol concentrations, and haptoglobin and serum amyloid A levels for the recaptured caribou in NE BC are shown in Table 33.

Table 33. Hair cortisol concentration (HCC), Haptoglobin (HAP), and Serum Amyloid A levels (SAA) of n=18 recaptured adult female boreal caribou from northeast British Columbia.

Animal ID	Herd	Age[a]	2012/2013	2013/2014	2014/2015	2015/2016
SK005	Maxhamish	OA	SAA – 45.7	SAA – 41.0		
SK007	Maxhamish	YA; MA	HCC – 1.94 HAP – 0.14 SAA – 23.0		HCC – 1.41 HAP – 0.48 SAA – 106.9	
SK009	Fort Nelson	MA; OA	HCC – 0.69 HAP – 0.19 SAA – 17.8		HCC – 0.81 HAP – 0.81 SAA – 29.8	
SK014	Parker	MA	HCC – 3.99 HAP – 0.15 SAA – 15.2		HCC – 41.8 HAP – 0.85 SAA – 26.2	
SK016	Parker	YA; MA	HCC – 1.71 HAP – 0.12 SAA – 0.00		HCC – 1.44 HAP – 0.52 SAA – 18.6	
SK020	Snake-Sahtaneh	MA	HCC – 0.87 HAP – 54.3 SAA – 44.0		HCC – 0.37 HAP – 120.2 SAA – 43.0	
SK026	Chinchaga	MA	HCC – 2.08		HCC – 1.14	
SK033	Chinchaga	MA	HCC – 1.20 HAP – 0.51 SAA – 7.5		HCC – 4.20 HAP – 0.37 SAA – 79.7	
SK036	Chinchaga	MA	HCC – 0.53 HAP – 0.41 SAA – 12.4		HCC – 1.37 HAP – 0.49 SAA – 33.1	
SK066	Maxhamish	JUV; YA	HAP – 0.12 SAA – 218.3			HAP – 0.45 SAA – 39.8
SK079	Snake-Sahtaneh	YA; MA	HCC – 1.14 HAP – 0.36 SAA – 32.1		HCC – 8.15 HAP – 0.48 SAA – 136.5	
SK097	Snake-Sahtaneh	YA; MA	HCC – 1.22 HAP – 0.34 SAA – 26.3		HCC – 7.37 HAP – 0.70 SAA – 20.6	
SK100	Snake-Sahtaneh	YA; MA	HCC – 0.91 HAP – 0.32 SAA – 99.9		HCC – 5.29 HAP – 0.38 SAA – 266	
SK110	Snake-Sahtaneh	YA; MA	HAP – 0.19		HAP – 0.51	
SK126	Calendar	YA; MA	HCC – 2.16 HAP – 0.26 SAA – 125.2		HCC – 1.63 HAP – 0.69 SAA – 114.6	
SK 136	Calendar	YA; MA	HCC – 1.89 HAP – 0.14		HCC – 14.23 HAP – 0.81	

			SAA – 267.6		SAA – 365.1	
SK146	Calendar	MA; OA	HCC – 1.04 HAP – 0.85 SAA – 50.0		HCC – 3.32 HAP – 0.53 SAA – 56.1	
SK 161	Prophet	MA; OA	HCC – 17.5 HAP – 0.17 SAA – 49.8		HCC – 5.75 HAP – 0.57 SAA – 70.3	

[a] YA – young adult; MA – mature adult; OA – old adult.

4.5 Bone Marrow Fat Content

The nutritional status of free-ranging ungulates is closely related to their health, fitness, and population performance (118, 119). In BCHRP Year 2, we determined the % marrow fat in large bone samples collected from boreal caribou mortality sites in NE BC in 2013, 2014 and 2015 (Table 34). Overall, marrow fat levels appeared to be lower in caribou that died in 2013 vs. 2014 or 2015. All caribou that died in 2013 had marrow fat <85% (120), and and two caribou that died in summer 2013 (high mortality period) were likely starving (marrow fat <12%) (120). Evidence of nutritional stress was also identified in some caribou that died in 2014 and 2015. Preliminary results suggest that the number of *Erysipelothrix* culture/PCR positive mortalities may have decreased as caribou condition increased (Table 34). This could suggest that nutritional stress experienced by caribou in the harsh winter of 2012/2013 may have contributed to the occurrence of disease caused by *Erysipelothrix* in the following spring and summer.

Table 34. Marrow fat (%) measured in bones (femur, radius/ulna, jaw) collected from n=16 dead radio-collared adult female and n=1 un-collared yearling male boreal caribou in northeast British Columbia in 2013, 2014, and 2015.

Caribou ID	Bone	% Marrow Fat [α]	Month of Death	*Erysipelothrix* Status (Culture/PCR)
Uncoll545M	femur	60.6	April, 2013	Positive
SK069	femur	79.8	May, 2013	Positive
SK075	femur	9.6	July, 2013	Negative*
SK106	femur	8.8	July, 2013	Positive
SK067	femur	82.9	Feb, 2014	Negative
BC1015	femur	86.9	Feb, 2014	Positive
SK018	femur	84.0	April, 2014	Negative
SK037	radius/ulna	89.0 [β]	April, 2014	Negative
SK104	jaw	72.9	April, 2014	Pending
SK130	radius/ulna	88.0$_{\beta}$	April, 2014	Negative
SK154	jaw	69.8	April, 2014	Negative
SK165	jaw	82.1$_{\gamma}$	April, 2014	Pending
SK094	jaw	87.4$_{\gamma}$	October, 2014	Pending
SK044	jaw	88.0$_{\gamma}$	Nov, 2014	Pending
SK207	femur	84.0	Feb, 2015	Pending
SK052	jaw	80.8$_{\gamma}$	April, 2015	Pending
SK210	jaw	69.9	June, 2015	Pending

[α] Determined using marrow fat drying and assessment protocol of the Canadian Wildlife Health Cooperative (CWHC). Only marrow from intact bones (not cracked) was evaluated. [β] Correlation between total body fat and fat levels measured in radius/ulna marrow not known in caribou. Caution re: interpretation is warranted.

[γ] Probable overestimate, marrow in relatively poor condition (dry). * Suspect positive case currently being re-tested.

4.6 Trace Minerals

In domestic and free-ranging ungulates, trace mineral levels are critically important determinants of immunity, health, growth, reproductive output, and survival (121-123). In BCHRP Year 1, levels of vitamin A, vitamin E, beryllium (Be), magnesium (Mg), vanadium (V), chromium (Cr), manganese (Mn), iron (Fe), cobalt (Co), nickel (Ni), copper (Cu), zinc (Zn), arsenic (As), selenium (Se), strontium (Sr), molybdenum (Mo), cadmium (Cd), tin (Sn), antimony (Sb), barium (Ba), thallium (Tl), and bismuth (Bi) were measured in liver samples obtained from n=2 boreal caribou found dead in the Snake-Sahtaneh range in NE BC in 2013 [the un-collared yearling male found dying in April, 2013 (Fig 5.) and SK075 an adult female found dead (and intact) in July, 2013 during the high mortality period]. Based on the normal limits of trace minerals for 5 captive *Rangifer*, liver levels of all trace minerals measured in the un-collared yearling were within normal limits while iron deficiency and marginal copper levels were identified in the adult female caribou (120). Liver samples from some of the dead caribou were recently submitted for trace mineral status and were found to be deficient in copper and selenium based on trace mineral reference ranges in *Rangifer* published by Puls (124).

Liver samples are the preferred tissue to examine the trace mineral status of individual animals. Unfortunately, it is not usually practical to obtain liver biopsies from free-ranging, live-captured wildlife and liver samples are rarely collected during caribou mortality site investigations because they are usually consumed by predators or scavengers. At a herd level, trace minerals measured in serum can be an informative substitute and are commonly evaluated in livestock herd health assessment programs (125). In BCHRP Year 2, we evaluated serum levels of Manganese (Mn), Iron (Fe), Cobalt (Co), Copper (Cu), Zinc (Zn), Selenium (Se), and Molybdenum (Mo) in n=211 boreal caribou from NE BC that were captured in winter 2012/2013 (n=137), 2013/2014 (n=41), and 2014/2015 (n=33) (Table 35). The goals of this analysis were: to broadly evaluate caribou herds for evidence of potential trace mineral deficiencies and to establish trace mineral reference ranges (serum) for free-ranging boreal caribou in NE BC.

In winter 2012-2013, Cu and Se levels for 98% and 34 % of the individuals from all herds, respectively, were below the minimum range reported by Puls (124) for 100 reindeer (*Rangifer tarandus*) and caribou. Differences were found between herds for Cu, Co, Mn, and Zn levels in early winter and for Co, Fe, and Mn in late winter. Molybdenum levels were not analyzed by herd because 85 % of the samples tested were below the detection limit of the test, which was 0.0009 ppm. The results of these analyses are presented further in the manuscript on boreal caribou health that was recently submitted for publication (13).

Co, Cu and Se values recorded in boreal caribou were also significantly lower (Unpaired t-tests, all comparisons, $P<0.0001$) than levels measured in n=6 captive caribou from NE BC (maintained on a combination of natural and pelleted ration) while Mn, Fe, Zn, and Mo levels were similar (Unpaired t-tests, all comparisons, $P>0.05$). Serum Cu levels in boreal caribou from NE BC also appeared to be lower than Cu levels (1.2 ± 0.3 ppm) reported in other captive *Rangifer* herds (126).

Mn, Fe, Cu, Se, and Mo levels in boreal caribou from 2012-2015 did not significantly differ by year (One-way ANOVA, $P>0.05$). However, Co levels recorded in 2012/2013 were lower (Co: One-way ANOVA, $F_{2,191} = 44.87$, $P<0.0001$, Tukey Kramer, $P<0.05$) and Zn levels were higher (Zn:One -way ANOVA, $F_{2,191} = 24.855$, $P<0.0001$, Tukey Kramer, $P<0.05$) than levels recorded in 2013/2014 and 2014/2015.

Overall, our trace mineral findings represent important baseline information and suggest that diet quality (nutrition) may have implications for the health status of caribou in NE BC.

Table 35. Trace mineral levels measured in serum collected from n=211 adult female boreal caribou captured by net-gun in northeast British Columbia in winter 2012/2013 (n=137), 2013/2014 (n=41) and 2014/2015 (n=33).

Trace Mineral	Mean and Range of Values Free-ranging Boreal Caribou	Laboratory Reference Range [α]
Manganese (Mn)	0.027 ppm (0.001-4.80) ppm	NE
Iron (Fe)	5.61 ppm (1.70-140.00 ppm)	NE
Cobalt (Co)	0.65 ppb (0.27-1.70 ppb)	NE
Copper (Cu)	0.43 ppm (0.11-0.74 ppm)	0.70-1.80 ppm
Zinc (Zn)	1.04 ppm (0.59-3.00 ppm)	1.10-2.50 ppm
Selenium (Se)	0.054 ppm (0.030-0.51 ppm)	0.050-0.140 ppm
Molybdenum (Mo)	0.034 ppm (0.0009-1.00 ppm) [β]	NE

[α] Reference ranges for caribou and reindeer- Puls (1994)
[β] 85% (n=180/211) samples below detection limits for Mo
NE: serum reference ranges not currently established in *Rangifer*

5. General Findings and Recommendations

In BCHRP Years 1 and 2, we established a standardized model of health sampling and testing of and developed the first comprehensive herd health baselines for boreal caribou in NE BC. With this information we explored the cause of death of animals dying in 2013. Our findings to date indicate new data that the health status of caribou may have important implications for the management and conservation of this threatened species. We determined that *E. rhusiopathiae* may have played a role in the high mortalities observed in winter 2012-2013 and that hair cortisol concentration was positively associated with mortality within the next year. We also found hair loss in more than 70% of all caribou in early and late winter and identified potential trace mineral deficiencies for copper and selenium in this population of boreal caribou. We identified that exposure to *Besnoitia tarandi* in caribou was associated with anthropogenic landscape features and that caribou exposed to *Neospora caninum* were less likely to be pregnant. These findings provide a comprehensive and standardized approach to health evaluation and provide critical new information on several health indices for boreal caribou and can be used to monitor population health in this population of boreal caribou, anticipate trends, and detect and suggest mitigation of new and emerging threats.

Although infection with or exposure to some bacterial and protozoan pathogens (e.g., *Brucella* spp., *Mycobacterium avium* ssp. *paratuberculosis, Babesia*, and *Toxoplasma gondi)*, ectoparasites and helminths (e.g., H. *tarandi* and *F. magna)* were not detected in all of the herds, these pathogens may still be present but below the detection/sensitivity limit of our existing tests. However, they may represent future emerging threats to caribou in this or other regions.

In BCHRP Year 3, we identified some current diagnostic methodologies (e.g., pestivirus tests and others developed for domestic livestock) that require further exploration in *Rangifer*. It must be noted that some of our interpretations are limited because 'normals' were not available for most serum trace minerals and acute phase proteins, the pathophysiology and epidemiology of many diseases are not well described for *Rangifer* and inferences had to be made from the domestic animal literature. However, our results greatly expand the health knowledge for free-ranging boreal caribou and help identify priorities for future surveillance and research in the herds in NE BC and elswhere. The standardized approach we used provides a model for sampling and analyses to determine health status in caribou and in other free-ranging ungulate species. Such sampling (and testing) should be considered an essential component in any collaring program, providing critical data on infectious disease, immune, and trace mineral status, as well as several physical health indicators. Capturing caribou for studies on survival and reproduction provides an excellent opportunity to collect much needed baseline health data that is lacking in most ungulate populations. Baseline caribou health data is essential in order to identify current or emerging health threats to caribou survival and reproduction and can also be used to aid conservation and management strategies of this species.

The BCHRP Working Group recommends that, at a minimum, a longitudinal health monitoring program be continued to monitor for *Erysipelothrix*, *Neospora caninum*, winter tick, and trace minerals in at least some caribou herds in the current study area. We also recommend that biological samples continue to be collected, tested and/or archived from any and all caribou captured or found dead in the region. An evaluation of key caribou pathogens (e.g., *Erysipelothrix*) in sympatric species (e.g., moose) is also recommended and the establishment of community based programs to gather biological samples from commonly harvested species is encouraged.

Metagenomics, an emerging discipline that enables the detailed study of uncultured microorganisms from biological samples, should be considered for future health assessments in live and dead animals since it is not subject to or may be less affected by the same issues as traditional veterinary diagnostics (e.g., microorganisms don't have to be alive or present in large quantities to be detected and the complete sequencing of genomes of microorganisms in a tissue sample is possible).

In BCHRP Year 2, we initiated a caribou health research collaboration with the Foothills Research Caribou Program in Hinton, AB. Although this study is ongoing, we have already identified *Erysipelothrix* and winter tick associated mortalities along with evidence of possible trace mineral deficiencies in mountain and boreal caribou from west-central AB (65). These findings clearly indicate that health has important implications for caribou populations outside of NE BC and that provincial and territorial borders do not matter. More comparative health assessments and the broader integration of health into other woodland caribou research and management programs are recommended. Based on BCHRP findings to date, the directed study of *Erysipelothrix* in archived serum or tissue samples collected across different woodland caribou populations may provide a starting point. Moving forward, the collection of a standardized set of biological samples from all live-captured caribou (Appendix 1) is strongly recommended for any research or management program in which caribou are captured or handled. In addition to predation, all caribou mortality site investigations should also consider health in a broad sense and infectious disease as potential causes of death or contributing factors. A standardized protocol (as an example, that developed by BCHRP) with detailed photographs of all mortality sites and all efforts should be made to collect any and all tissue remaining at mortality sites for review by a wildlife health specialist/veterinary pathologist.

We strongly recommend tissues of special importance (Appendix 2) for health based analyses in dead caribou to be collected as soon as possible and submitted immediately to a veterinary diagnostic laboratory. Where caribou are harvested, community based health monitoring and sample collection programs should also be initiated to support health research.

Full health assessments in wild species are logistically challenging and expensive. Practical, reliable, validated, and preferably minimally invasive biomarkers of health that consistently predict the health status of caribou or other wildlife are desirable as population level management and monitoring tools. Integrated indices of health which may reflect both the health status of individual caribou and biological mechanisms that may drive health related impacts on caribou populations may be especially informative. This information in this report were to develop recommendations to monitor and maintain healthy caribou populations in NE BC but will also benefit woodland caribou and other wild ungulate conservation initiatives elsewhere.

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7.0 APPENDICES

Appendix 1: Recommended minimum set of biological samples to be collected from live-captured caribou[a, b].

Sample	Sample Quantity	Sample Collection	Sample Processing and Storage
Serum	• Minimum pf 35 ml in serum separator (SST) tubes, royal blue top and lavender top tubes	• Jugular or cephalic vein • 18 or 19G, 1.5 inch needle and 35 cc syringe	• Spin SST tubes in centrifuge • Decant serum into cryogenic vials (1 cc serum/vial) • Store frozen (minimum -20^{o}C)
Whole Blood	• n=1, 10 ml EDTA tube	• Jugular or cephalic vein- • 18 or 19G, 1.5 inch needle and 35 cc syringe	• Slowly rotate tubes immediately after collection to mix blood and EDTA anticoagulant • Prepare n=4 blood smears as soon as possible after collection. Air dry (do not fix) smears and store at room temperature in a slide box protected from light, heat, and moisture • Store remaining whole blood frozen in original EDTA tube (minimum-20^{o}C)
Feces	• Approximately a "palm full" of pellets	• Collect per rectum or off snow at capture	• Transfer to Whirlpack ® (or similar) and remove as much air as possible without crushing pellets • Store frozen (minimum -20^{o}C)
Hair	• Minimum 100 mg (~ coin envelope stuffed full)	• Pluck from the top of the shoulder	• Air dry and store at room temperature in paper envelope protected from heat, light and moisture
Skin biopsy	• n=1-2, 6mm biopsy punches from ear	• Pre-punch hole for each ear tag(s) and collect skin plug(s)	• Air dry and store at room temperature in paper envelope protected from heat, light and moisture

[a] These recommendations provide basic biological samples of the proper type and of sufficient quantity to thoroughly evaluate the health status (and genetics) of free-ranging caribou. Some samples can be archived for future analysis. Experienced personnel can generally collect this set of samples in the time required for a radio-collar to be deployed on caribou

captured by net-gun in winter. To date, this protocol has been successfully applied in over n= 450 caribou live-captured in BC. Detailed photographs should also be taken of each individual animal and of any abnormalities observed.

[b] A data form and complete protocol for health and assessment and sampling of live-captured caribou can be obtained by contacting the Government of BC Wildlife Health Program.

Appendix 2

Tissues of special importance for health based analyses in dead caribou include: the head, pluck (heart and lungs), heart blood, liver, spleen, fetuses/placenta, hide (10x10cm^2 sections from rump, shoulder, neck), and intact long bones (e.g. femur, metatarsal and metacarpal bones). If intact carcasses (non-predation mortalities) are encountered all efforts should be made to retrieve the whole carcass as soon after death as possible and submit it immediately to a veterinary diagnostic laboratory. If this is not possible, the carcass can be frozen [whole (best) or disarticulated] until transport can be arranged. If a carcass cannot be removed from the field, an enhanced field necropsy should be performed in which extensive photo documentation occurs and where many samples from all organ systems are collected. A wildlife health specialist should be engaged to help develop field necropsy protocols that adequately address requirements for health testing. Tissue samples collected from caribou mortality site investigations can be stored (at minimum -20^{o}C) until submission to a diagnostic laboratory can be arranged. The collection of tissue samples fixed in 10% neutral buffered formalin from all major organ systems (and any lesions) may also be informative and is recommended if the carcass is relatively fresh (<24hrs since death or found in cool weather/winter). Where caribou are harvested, community based health monitoring and sample collection programs should also be initiated to support health research. A standardized mortality site assessment data form and sample collection protocol for caribou can be obtained by contacting the Government of British Columbia Wildlife Health Program.