British Columbia Boreal Caribou Health Research Program

Progress Report: Year 2 (February 1, 2015 – March 31, 2016)

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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 [e.g. 4-7].

Boreal caribou in Northeastern British Columbia (*Rangifer tarandus caribou*, population No.14, DU06 [8, 9]) are red-listed due to declines in abundance and distribution [10]. Since 2010, the *Implementation Plan for the Ongoing Management of Boreal Caribou (Rangifer tarandus caribou pop. 14) in British Columbia* (BCIP) has guided provincial efforts to manage and conserve this species [10]. As a component of the BCIP, 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 Northeastern BC (NE BC). In BCHRP Year 1, a comprehensive herd health assessment model was developed and applied across six boreal caribou herd ranges in the region [reviewed 11]. Biological samples collected from n=164 live-captured and n=12 dead 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, immunity, and nutrition. Evidence of notable and novel threats to caribou including the pathogenic bacterium *Erysipelothrix rhusiopathiae*, the protozoan parasite *Neospora caninum*, and severe winter tick (*Dermacentor albipictus*) infestations were identified. A preliminary investigation of selected health biomarkers that may show potential as simplified health assessment or monitoring tools was also performed [11].

Throughout 2014 and 2015 a continued radio-collar monitoring, maintenance, and replacement program in our study herds provided a unique opportunity for a short-term, longitudinal investigation of boreal caribou health in NE BC [reviewed 12]. 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 [reviewed 11]. An enhanced investigation of *E. rhusiopathiae* and winter tick was also initiated and tests for blood borne pathogens and to evaluate the trace nutrient status of caribou were added to the health assessment model. Results from BCHRP Year 1 and Year 2 were then combined to establish the first comprehensive herd health baselines for boreal caribou in NE BC.

Interpretation and analysis of our boreal caribou health dataset is ongoing. Overall, the occurrence of many pathogens and other health determinants appeared to vary across herd ranges and in some cases across study years. New findings in BCHRP Year 2 also confirmed that health and disease may have important implications for the long-term sustainability of boreal caribou in NE BC. Continued study of *E. rhusiopathiae* uncovered new evidence supporting the hypothesis that this pathogen may have played a role in the unusually high caribou mortality rate recorded in NE BC in 2013 including: the identification of a moribund caribou most likely clinically affected by the bacterium and a greater than 50% increase in exposure (seroprevalence) in two herd

ranges immediately after the high mortality period. Tick counts on hide samples collected from dead caribou and the development of a classification system for tick associated hair loss in live caribou reveled that moderate to severe infestations with *D. albipictus* are widespread in our study area. Winter tick may represent an emerging threat to caribou health in the region. Variation in bone marrow fat levels across study years and evidence of possible trace nutrient deficiencies in caribou were also identified.

In BCHRP Year 3 (April 1, 2016 -March 31, 2017) we will continue with our enhanced study of *E. rhusiopathiae* in free-ranging boreal caribou from NE BC. This work will be supported by an ongoing collaboration with caribou researchers in Alberta [Foothills Research Institute (fRI) Caribou Program, Hinton, AB] and a minimally invasive (vaccine based) experimental study in captive reindeer held at the University of Calgary's Faculty of Veterinary Medicine. The utility of selected stress and immune biomarkers as simplified health assessment and monitoring tools will also be tested and we will undertake a broader evaluation of temporal and spatial relationships between larger-scale (landscape level) factors and caribou health, reproduction, and survival. This program will provide a comprehensive picture of herd level health status that will inform boreal caribou management and conservation in NE BC. Investigative approaches and standardized protocols developed as part of the BCHRP will also benefit woodland caribou conservation initiatives elsewhere and provide a starting point for similar studies in other ungulates.

This report summarizes activities completed during BCHRP Year 2 and describes the baseline heard health status of boreal caribou in NE BC. An overview of *E. rhusiopathiae* results (available as of June 1, 2016), a novel classification system for winter tick related hair loss in caribou, and research efforts planned for BCHRP Year 3 are also presented.

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1.3 Consider BCHRP Year 1 and Year 2 findings to establish the first comprehensive herd health baselines for boreal caribou in NE BC and identify populations which may be "at risk" from compromised health.

1.4 Continue research into the potential role of the pathogenic bacterium *Erysipelothrix rhusiopathiae* as a factor in the relatively high number boreal caribou mortalities observed in NE BC between April, 2013 and September, 2013.

1.5 More thoroughly characterize the occurrence, distribution, and potential importance of *Neospora caninum*, blood borne pathogens, *Dermacentor albipictus* (winter tick) infestations, and the trace nutrient status of boreal caribou in NE BC.

1.6 Provide ongoing health related recommendations for boreal caribou management in NE BC.

2. Methods

2.1 Sample Collection and Storage

2.1.1 Sample Collection: Live-captured Caribou

Health based sampling in 2014 and 2015 was integrated into an ongoing monitoring program tracking adult caribou survival and juvenile caribou recruitment in NE BC [12]. Forty one adult female boreal caribou were live-captured and radio-collared in the Calendar (n=5), Chinchaga (n= 13), Maxhamish (n=11), Parker (n=1), Prophet (n=0) and Snake-Sahtaneh (n=11) herd ranges of NE BC (Fig. 1) between December 2013 and March 2014. Thirty three adult female caribou were live-captured and radio-collared in the Calendar (n=2), Chinchaga (n= 10), Maxhamish (n=9), Parker (n=2), Prophet (n=2) and Snake-Sahtaneh (n=8) herd ranges between December 2014 and April 2015. In 2014, n=1 caribou (first captured in 2013) was recaptured for radio-collar replacement. In 2015, n=13 caribou (first captured in 2013) were recaptured for radio collar replacement.

A standardized suite of basic biological samples including: hair, feces, whole blood, and serum were collected from every caribou captured in both 2013/2014 and 2014/2015. In 2014/ 2015, nasal swabs were also collected from all n=33 live-captured caribou and blood smears (minimum of two smears per individual) were made for n=27 of these animals. The occurrence

of ectoparasites (e.g. ticks, warbles), other health related anomalies, and any associated pathology were documented and samples (e.g. winter tick voucher specimens) were collected as encountered. Other biological data closely related to the health status of individual caribou (e.g. body condition, body size, age, lactation status, presence of a calf at heel) were also recorded at the time of capture. In 2014 and 2015 the pregnancy status of caribou was determined after capture by measuring serum progesterone concentration.

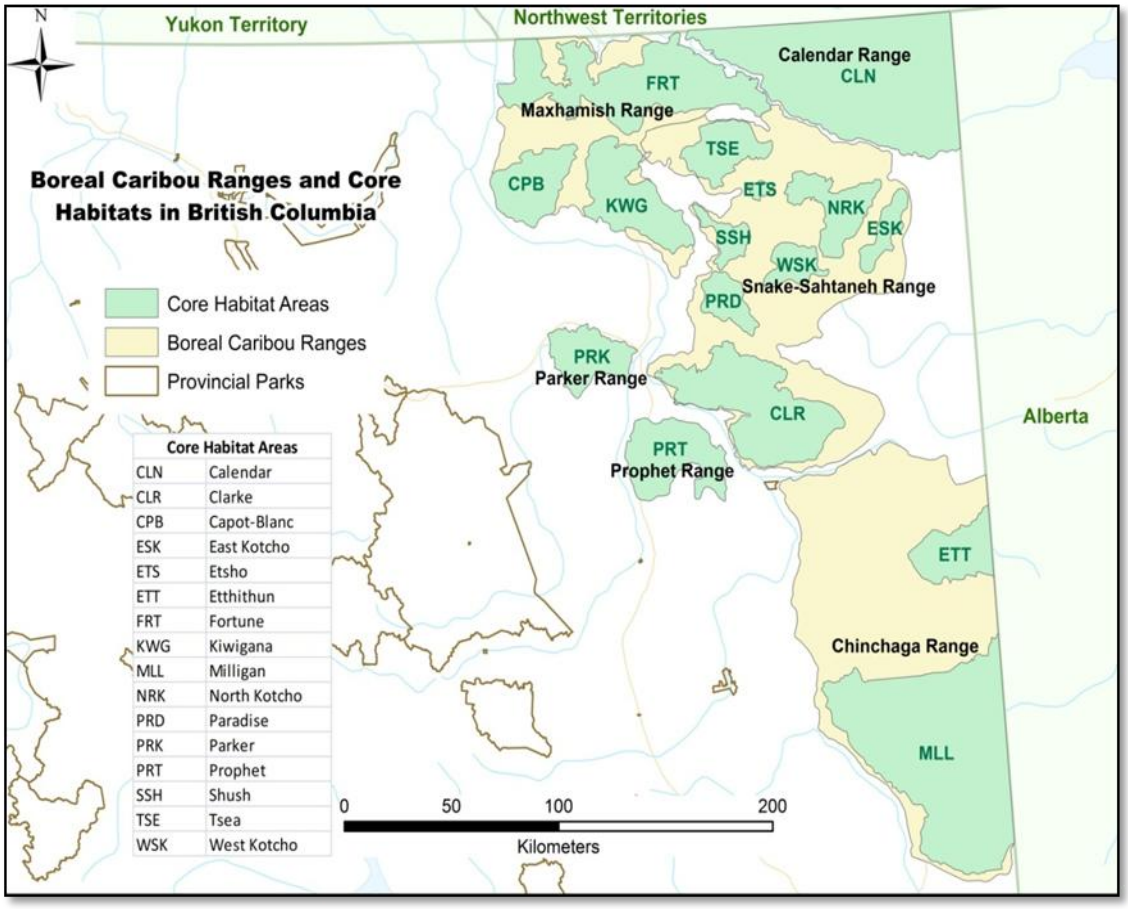

Figure 1. Boreal caribou herd ranges and core habitat areas in Northeastern British Columbia, Canada. Modified from: British Columbia Ministry of the Environment. (2010). Science update for the boreal caribou (*Rangifer tarandus caribou* pop. 14) in British Columbia. Victoria, BC. 54 pp.

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 [12]. In BCHRP Year 1, where available and when stage of decomposition permitted, biological samples (e.g. long bones, lower jaws, tissue samples, 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 2014 and 2015 were stored frozen (-20◦C) and then 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 Year 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 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 [11]. 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 [11] (available online: www.bcogris.ca). Based on preliminary results obtained in Year 1, the health assessment model was modified slightly in BCHRP Year 2 (Table 1). 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 nutrient status of boreal caribou in NE BC. Metagenomics [13] was also explored as a potential diagnostic technique to incorporate into future health assessment initiatives for caribou.

Table 1. Overview of pathogens, parasites, and non-infectious health determinants evaluated in Year 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 nutrient levels (serum)

2.3 Diagnostic Tests

The BCHRP's health testing efforts in Year 1 and Year 2 were based out of the University of Calgary's Faculty of Veterinary Medicine (UCVM) and the Canadian Wildlife Health Cooperative (CWHC) located in Calgary, AB, Canada. The BCHRP also 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 and specific diagnostic tests employed for pathogen and health screening in BCHRP Year 1 are described in detail in the BCHRP Year 1 Synthesis Report [11].

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 being deployed on caribou in the field. Detailed photographs from all animals captured in all years of the program (2013-2015) were used in BCHRP Year 2 to more thoroughly classify the occurrence and severity of winter tick infestations in boreal caribou in NE BC (see Section 3.4.7). In Year 2, trace nutrient levels (Mn, Fe, Co, Cu, Zn, Se, Mo) were also measured in serum from n= 211 caribou captured in winter 2012/2013 (n=137), 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 (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 also 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*). As in Year 1, all diagnostic tests in BCHRP Year 2 were performed by trained laboratory technicians, wildlife veterinarians, or board certified veterinary specialists.

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 of the same species living in or on a single host.

3.1.2. Seropositive and Seronegative

An animal is considered to be seropositive when there is evidence that its immune system 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 specific antibodies against that pathogen (seroconvert). An increase in the number of seropositive animals is often recorded 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. No pathogen specific antibodies are found in seronegative animals indicating that they have not been exposed to the pathogen in question 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). Immunity may also have waned.

3.1.3 PCR and Culture Positive and Negative

A PCR 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. 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 that culture negative are not necessarily free of that pathogen.

3.2 Viruses

3.2.1 Alphaherpesvirus

Alphaherpesvirus is a pathogen causing both subclinical effects and overt disease in captive and free-ranging *Rangifer* including: keratoconjunctivitis, ulceration of the nasal, oral, and genital mucosa (+/- secondary bacterial infection), and severe respiratory disease, along with abortions, neonatal morbidity and mortality [14]. In BCHRP Year 1, the overall prevalence of exposure to alphaherpesvirus among adult female boreal caribou captured in NE BC in winter 2012/2013 was 62% (n=101/162). Prevalence of exposure also appeared to vary across herd ranges being highest (86%, n=6/7) in the Parker herd range and lowest (22%, n=2/9) in the Prophet herd range [11]. Notably, the prevalence of exposure to alphaherpesvirus recorded in boreal caribou from NE BC was also higher than that recorded in other woodland caribou herds from NWT (37.5%) [15], AB (52%) [16], and SK (55%) [17].

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. As such, 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* [18]. To confirm results from both assays were comparable we evaluated n=41 serum samples from BC boreal caribou collected in winter 2013/2014 and with both kits. Results agreed in 97% (n=40/41) of caribou tested.

In winter 2013/2014 and 2014/2015, the overall prevalence of exposure to alphaherpesvirus among adult female boreal caribou captured in NE BC in was 63% (n=25/40, n=1 recapture not included) and 63% (n=12/19, n=13 recaptures not included) respectively.

These findings are similar to results from Year 1 and appear to confirm that the overall prevalence of alphaherpesvirus in boreal caribou from NE BC may be higher than in woodland caribou from other areas. Some evidence that the prevalence of exposure may vary across herds and across years was also recorded in winter 2012/2013, 2013/2014, and 2014/2015 (Table 2). 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 [14]. The relatively high and somewhat variable prevalence of exposure to alphaherpesvirus may therefore indicate that factors influencing stress levels in boreal caribou from NE BC may be relatively prominent and may also vary as a function of landscape level features encountered by different caribou herds in the region.

The precise alphaherpesvirus found in boreal caribou is unknown at the present time. In winter 2014/2015 minimally invasive nasal swabs were collected from n=33 boreal caribou captured during ongoing radio-collaring initiatives. To identify the alphaherpesvirus in boreal caribou from NE BC, archived swabs will be subjected to molecular testing at the Norwegian School of Veterinary Sciences, Tromsø, Norway in BCHRP Year 3. In Year 3, alphaherpesvirus will also be incorporated into analyses designed to explore the broader relationships between: 1) specific pathogens and caribou survival and reproduction, 2) pathogens and other health indices, and 3) pathogens and larger scale (landscape level) factors (see Section 6).

Table 2. Prevalence of exposure to alphaherpesvirus recorded in live-captured, adult female boreal caribou from six herds in Northeastern British Columbia in winter 2012/2013, 2013/2014, and 2014/2015 [α].

Herd	**Alphaherpesvirus Prevalence and Sample Size**		
	2012/2013 Captures	**2013/2014 Captures**	**2014/2015 Captures**
Calendar	59% (n=16/27)	80% (n=4/5)	(-)
Chinchaga	69% (n=35/36)	69% (n=9/13)	43% (n=3/7)
Maxhamish	59% (n=16/27)	60% (n=6/10)	71% (n=5/7)
Parker	86 % (n=6/7)	0% (n=0/1)	(-)
Prophet	22% (n=2/9)	(-)	(-)
Snake-Sahtaneh	64% (n=36/56)	55% (n=6/11)	60% (n=3/5)

[α] Like other herpesviruses, alphaherpesvirus in boreal caribou is most likely a lifelong infection. As a result, alphaherpesvirus data from caribou originally captured in winter 2012/2013 and recaptured in winter 2013/2014 or 2014/2015 were not included in calculations. (-) designates herds where no previously un-collared individuals were captured in the sampling year.

3.2.2 Pestiviruses

In ruminants, pestiviruses are the causative agents of immunosuppression, respiratory and gastrointestinal disease as well as infertility, abortions, and neonatal morbidity and/or mortality [19-21]. In BCHRP Year 1, an ELISA (Synbiotics SERELISA BVD Kit**,** Synbiotics Corporation, Lyon, France) was used to test boreal caribou serum for exposure to pestiviruses. The prevalence of exposure to pestiviruses among adult female boreal caribou captured in NE BC in winter

2012/2013 was 0.6% (n=1/161) however, the serostatus of n=51/161 (32%) of caribou was unclear [11]. 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) may have adversely influenced antibody binding properties and the reliability of ELISA results obtained in Year 1 [11].

In BCHRP Year 2, a subset of serum samples tested in Year 1 using the ELISA were re-tested using a virus neutralization (VNT) assay (Prairie Diagnostic Services Inc., Saskatoon, SK). In this test, serial dilutions of caribou serum (possibly containing anti-pestivirus antibodies) were incubated with an infectious pestivirus of cattle (bovine viral diarrhea virus-BVDV). Susceptible cells 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=0/60) were identified using the VNT including all samples testing "negative" or "uncertain" using the ELISA in Year 1. Serum from the single seropositive caribou identified in Year 1 was also re-tested using the VNT and was negative. This could indicate that the Year 1 finding was a false positive. However, antigenic variability may also have influenced the reliability of VNT results. All considered, these findings appear to indicate that exposure to pestiviruses may be very uncommon in boreal caribou from NE BC. As a general consideration for evaluating *Rangifer* health, more research is needed to identify specific pestiviruses in caribou and reindeer and to identify and validate sensitive and specific diagnostic tests for this pathogen. Given the apparent low prevalence of exposure and the uncertainty surrounding test results, pestiviruses will not be examined further as part of the BCHRP.

3.3 Bacteria

3.3.1 *Brucella suis* biovar 4

Brucella suis biovar 4 is a bacterial pathogen of caribou and reindeer found in herds throughout Northern Canada and Alaska [e.g. 22-24.] In caribou, infection with *B. suis* biovar 4 may be 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 [25, 26].

In winter 2012/2013 there was no evidence that any adult female boreal caribou captured in NE BC had been previously exposed to *Brucella* sp. (n=162/162 were seronegative) [11]. All n= 74 samples collected from boreal caribou in winter 2013/2014 and 2014/2015 were also negative. In addition, there was no evidence that any of the n=14 caribou first captured in winter 2012/2013 and then recaptured in winter 2013/2014 or 2014/015 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 [28]. 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. Given the potential impact of *B. suis* biovar 4 for caribou (and other ungulates) 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.

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 [e.g. 29, 30]. Abortions caused by *E. rhusiopathiae* have also been recorded in some species [31]. *Erysipelothrix rhusiopathiae* may be 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 muskox in the Canadian Arctic [32]. This bacterium has also been recorded as the probable cause of severe disease or death in free-ranging deer and moose [33, 34] and may have been responsible for a historical (1930's) outbreak of severe/fatal disease in semi-domesticated Scandinavian and Russian reindeer [35]. *Erysipelothrix* sp. has also been recovered from the carcasses of free-ranging bison found dead in the Northwest Territories [36].

In BCHRP Year 1, *Erysipelothrix rhusiopathiae* was identified in the tissues of n=5 dead boreal caribou [11]. 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 and included n= 4 radio collared caribou found dead and an un-collared yearling male caribou found dying (Fig. 2) in NE BC between April and September 2013 [reviewed 11]. Using a proprietary ELISA under development at UCVM [101], evidence of exposure to *E. rhusiopathiae* was also identified in the serum of approximately 30% of boreal caribou captured in NE BC in winter 2012/2013 and 2013/2014 [11]. To our knowledge, these findings represented the first record 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 (Fig 2., also see Section 3.3.2.1.1) along with a concentration of unusual caribou deaths occurring in spring and summer (Fig. 3, Fig. 4) suggested that *Erysipelothrix rhusiopathiae* may have been a factor in the relatively high number of caribou mortalities recorded NE BC in 2013 [Annual finite survival rate for n=171 caribou during the 12 month period between May 1, 2013 and April 30, 2014, 0.72 (95% C.I. X-X)] [37].

Figure 2. An un-collared yearling male boreal caribou (*Rangifer tarandus caribou*) found moribund in the Snake-Sahtaneh herd range of Northeastern British Columbia in March, 2013. Photo credit: D. Culling and B. Culling, Diversified Environmental Services Inc., Fort St. John, BC.

3.3.2.1.1 Case Report: *Erysipelothrix rhusiopathiae* in an un-collared yearling male caribou from Northeastern 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. 2). 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 infectious disease.

Erysipelothrix rhusiopathiae was cultured from multiple organs (femur 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) [38, 39] (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 relatively poor condition (some internal fat reserves, 60.6 % femur marrow fat, mild hepatic atrophy). Liver levels of trace nutrients 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 in other ungulates infected with *E. rhusiopathiae*. The presentation of this case was also similar to muskox [32], moose [33], and reindeer mortalities [35] believed to have been caused by *E. rhusiopathiae* and with other caribou mortalities observed in NE BC in spring and summer, 2013 (Fig. 4). Together, these findings also suggest that *E. rhusiopathiae* may have been a factor in the relatively high number of caribou mortalities recorded NE BC in 2013.

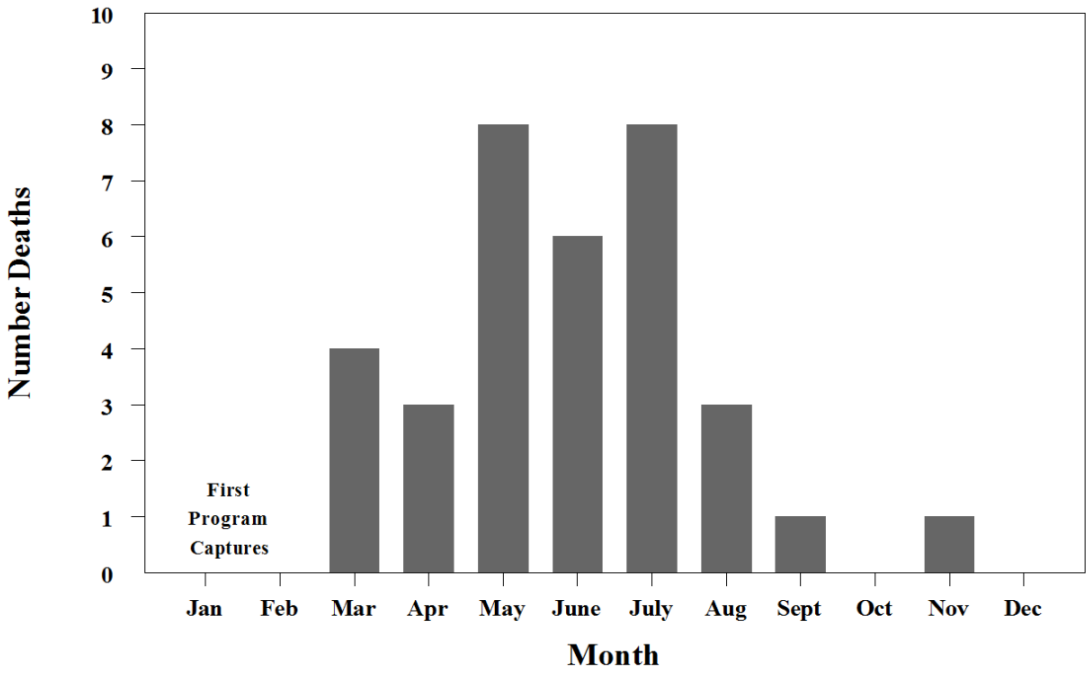

Figure 3. Distribution of boreal caribou mortalities recorded in Northeastern British Columbia between and March and December, 2013. The majority of boreal caribou deaths recorded in 2013 were concentrated in late spring and summer. Many mortalities occurring in this period were not caused by predation (Fig. 4).

Figure 4. Examples of unusual mortalities observed in boreal caribou from Northeastern British Columbia in spring and summer, 2013. 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. The majority of these unusual deaths occurred between April and September, 2013 and their presentation was similar to mortalities recorded in muskox, moose, and reindeer that may have been caused by *Erysipelothrix rhusiopathiae* [32, 33, 35] and also with the un-collared yearling male caribou identified in this study that was most likely clinically affected by this bacterium (Fig. 2). 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 Year 2

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 Year 2. 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 disease outbreaks in wildlife. In BCHRP Year 1, we employed a proprietary ELISA developed at UCVM to test and compare the prevalence of exposure to *E. rhusiopathiae* in boreal caribou captured in NE BC in 2012/2013 (n=161) and 2013/2014 (n=41). Preliminary results indicated that approximately 30% of caribou captured in both years may have been previously exposed to this pathogen (were seropositive, see Section 3.1.2). The overall prevalence of exposure to *Erysipelothrix* also appeared to vary across six boreal caribou ranges in NE BC [Calendar 31% (n=8/26), Chinchaga 35% (n=13/37), Maxhamish 30% (n=8/27), Parker 0% (n=0/7), Prophet 44% (n=4/9), and Snake-Sahtaneh 22% (n=12/55)] [11].

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. All samples from all boreal caribou collected in NE BC in winter 2012/2013, 2013/2014 and 2014/2015 were then tested a minimum of two times each. Cut-off values were refined based on this work and the concurrent analysis of a large number (n>200) of serum samples from other caribou populations being tested for exposure to *Erysipelothrix* as part of a separate research project at UCVM. For boreal caribou from NE BC, average results from all tests were then evaluated against refined cut-off values to more accurately determine the serostatus of individual animals.

In BCHRP Year 2, we found that the overall prevalence of exposure to *E. rhusiopathiae* across six boreal caribou herd ranges in NE BC in 2012/2013 was 21% (n=34/159). The prevalence of exposure also appeared to vary across herd ranges [Calendar 27% (n=7/26), Chinchaga 23% (n=8/35), Maxhamish 21% (n=5/24), Parker 0% (n=0/7), Prophet 13% (n=1/8), Snake-Sahtaneh 22% (n=12/54)]. Overall seroprevalence in caribou increased to 36% (n=14/39) in 2013/2014 and then declined to 15% (n=3/20) in 2014/2015 (Fig. 5). Differences in the pattern of change in seroprevalence from 2012/2013 to 2013/2014 were also recorded across four herds (Fig. 6). These findings may indicate that landscape level factors influence the ecology of this pathogen and subsequent rates exposure (and possibly infection) across boreal caribou herds in our study area.

Evidence of seroconversion was also recorded among caribou first captured in winter 2012/2013 and then recaptured in either winter 2013/2014 or 2014/2015 (Table 3). The serostatus of n=2 caribou testing positive at capture in winter 2012/2013 and then re-captured and tested again in winter 2014/2015 appeared to remain stable (Table 3). Seroprevalence and evidence of seroconversion often increases after an outbreak of infectious disease and these findings may offer further support for the potential role of *Erysipelothrix* in the caribou mortalities observed in NE BC in 2013. This hypothesis may also be supported by the changing pattern and presentation of caribou mortalities observed from 2013 through 2015. Fewer caribou mortalities were recorded in the spring or summer of 2014 and 2015 vs. 2013 (Fig. 7) and the number of unusual mortalities (intact carcasses, see Fig. 4.) observed declined from n=12+ in 2013 to n=1 in 2014 (health testing results pending) and n=0 in 2015.

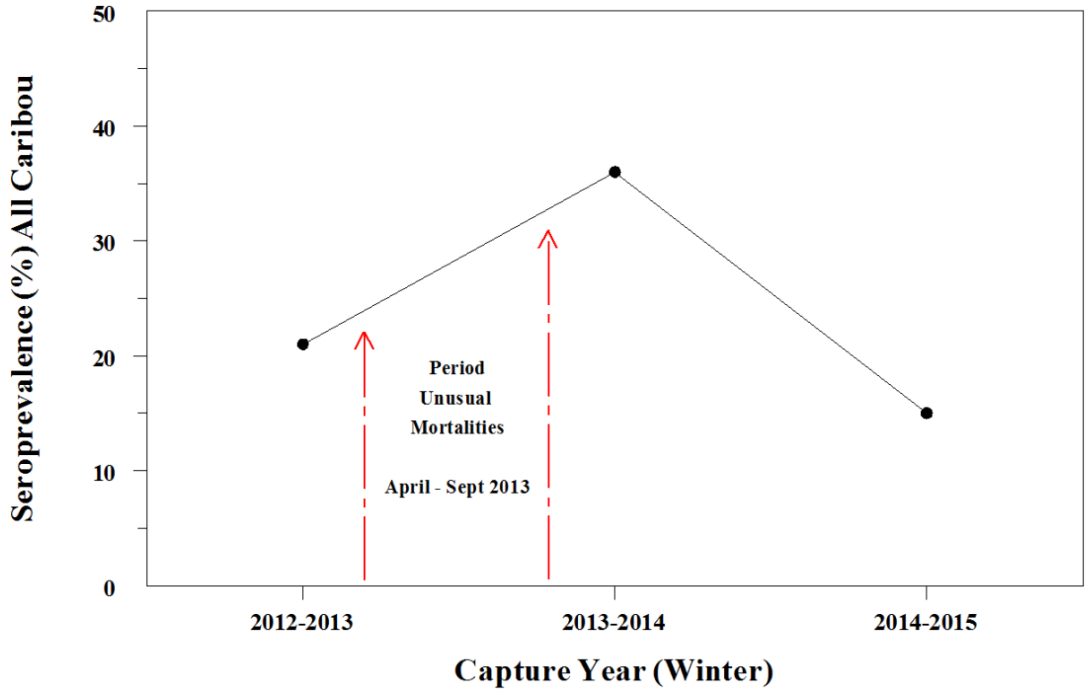

Figure 5. Prevalence of exposure to the bacterial pathogen *Erysipelothrix rhusiopathiae* recorded in adult female boreal caribou from Northeastern British Columbia in winter 2012/2103, 2013/2014 and 2014/2015. Seroprevalence increased from 2012/2103 to 2013/2014 following a period in which many unusual caribou mortalities (Fig.4) possibly caused by infectious disease were observed in the region. *Erysipelothrix* seroprevalence declined from 2013/2014 to 2014/2015 and only n=1 unusual caribou mortality was recorded from 2014 to 2015.

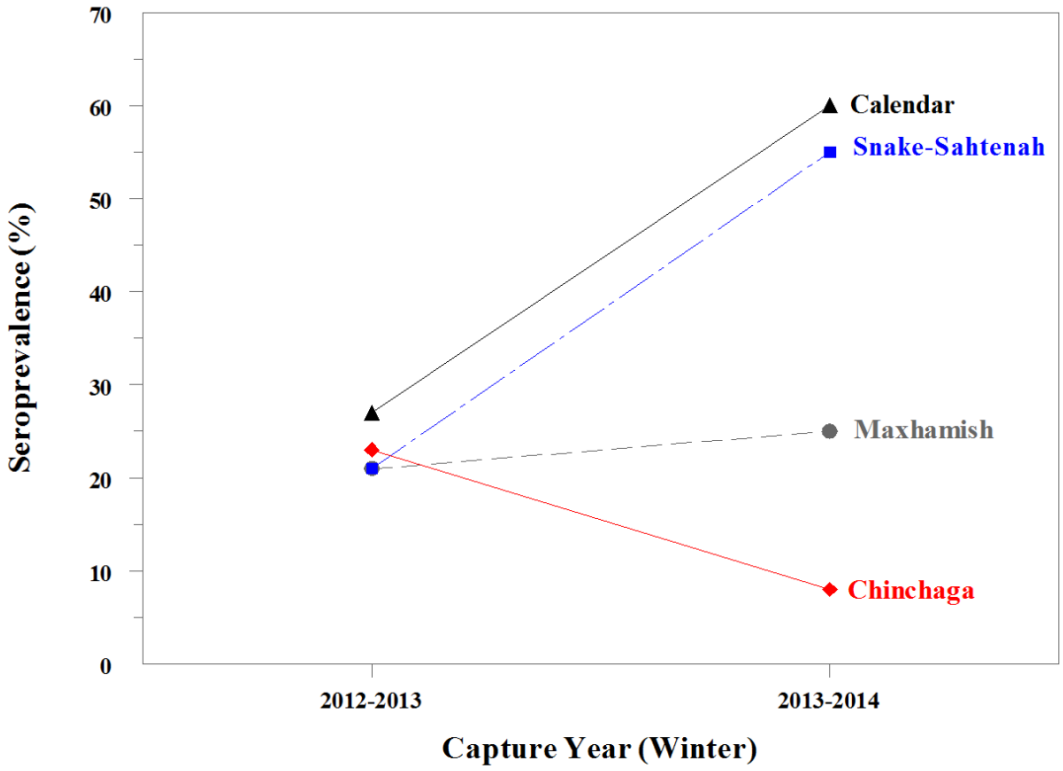

Figure 6. Prevalence of exposure to the bacterial pathogen *Erysipelothrix rhusiopathiae* recorded in adult female boreal caribou from four herd ranges in Northeastern British Columbia in winter 2012/2103 and 2013/2014. Seroprevalence increased dramatically in the Calendar and Snake-Sahtaneh herds from 2012/2013 to 2013/2014 while seroprevalence increased only slightly in the Maxhamish herd and declined in the Chinchaga herd in the same time period. These findings may indicate that local or landscape level factors influence the ecology of *Erysipelothrix rhusiopathiae* along with rates of exposure (and possibly infection) across boreal caribou herds in NE BC.

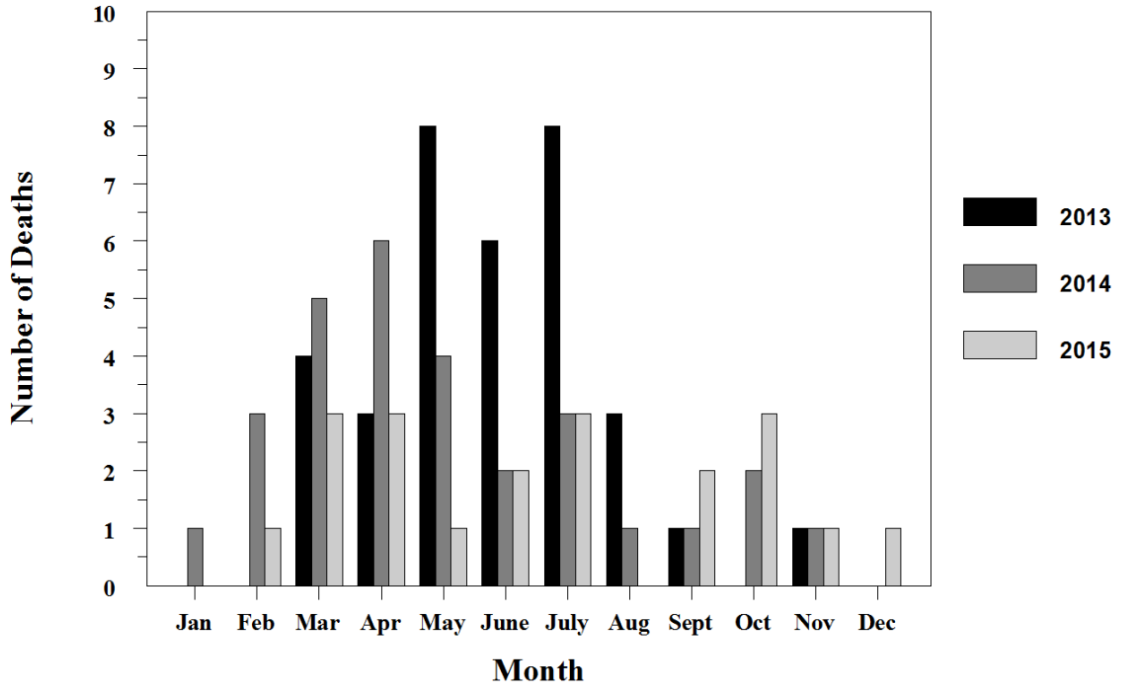

Figure 7. Distribution of mortalities recorded in radio-collared, adult female boreal caribou from Northeastern British Columbia between and March, 2013 and December, 2015. The number of caribou mortalities recorded in spring or summer was higher in 2013 compared to 2014 and 2015. N=12+ unusual caribou mortalities possibly related to infectious disease (Fig. 4) were recorded in 2013 vs. n=1 in 2014 and n=0 in 2015. The annual finite survival rate of boreal caribou increased from 0.72 (95% C.I. X-X, n=171) during the 12 month period between May, 2013 and April, 2014 to 0.86 (95% C.I. 0.81-0.91, n=181), during the twelve month period between May, 2014 and April, 2015 and 0.88 (95% C.I. 0.83-0.93, n=168), during the twelve month period between May 1, 2015 and April, 2016 [37]. 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* (Fig 5).

Table 3. *Erysipelothrix rhusiopathiae* serostatus of n= 14 adult female boreal caribou from Northeastern British Columbia first captured and tested in winter 2012/2013 and then recaptured and tested again in winter 2013/2014 (n=1) or 2014/2015 (n=13). Two caribou (highlighted in orange) that were seropositive at first capture in 2012/2013 were also seropositive at recapture in 2014/2015 indicating lasting antibody titres or re-exposure. Four caribou (highlighted in red) seroconverted sometime after their first capture in 2012/2013.

Caribou ID	Winter of First Capture	*Erysipelothrix* Serostatus at First Capture	Winter of Recapture	*Erysipelothrix* Serostatus at Recapture
SK005	2012/2013	Negative	2013/2014	Negative
SK007	2012/2013	Positive	2014/2015	Positive
SK009	2012/2013	Negative	2014/2015	Negative
SK014	2012/2013	Negative	2014/2015	Positive
SK016	2012/2013	Negative	2014/2015	Negative
SK020	2012/2013	Negative	2014/2015	Negative
SK033	2012/2013	Positive	2014/2015	Positive
SK036	2012/2013	Negative	2014/2015	Negative
SK079	2012/2013	Negative	2014/2015	Positive
SK097	2012/2013	Negative	2014/2015	Positive
SK110	2012/2013	Negative	2014/2015	Positive
SK126	2012/2013	Negative	2014/2015	Negative
SK136	2012/2013	Negative	2014/2015	Negative
SK161	2012/2013	Negative	2014/2015	Negative

In BCHRP Year 2, we also tested blood or "body cavity" fluids obtained from dead caribou for the presence of antibodies against *Erysipelothrix rhusiopathiae*. We identified one caribou (SK106, Calendar herd range) that was seronegative at capture in winter 2012/2013 but was "high seropositive" and culture/PCR positive at death in July, 2013 during the high mortality period. High seropositive caribou have evidence of antibody levels ≥ the maximal levels recorded 12 weeks after *Erysipelothrix rhusiopathiae* vaccination in experimental studies in muskoxen [40]. 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. 2), 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 [29, 30].

3.3.2.2.2 Molecular analysis 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 culture positive boreal caribou were determined using Illumina MiSeq platform for sequencing (Nextera XT sample preparation kit-generating 250 base pair paired-end reads) [38, 39] to explore the relationships between isolates from caribou and those obtained from other species. *E. rhusiopathiae* isolates from boreal caribou were found to be genetically different from those recorded in other domestic and free-ranging animals (both terrestrial and aquatic) [39]. Isolates from n=2 caribou found dead in the high mortality period in 2013 were identical [SCEK069 and the un-collared yearling (Fig. 2) 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 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 caribou found dying in 2013 (Fig. 2) [39]. Polyclonal infections have also been recently recorded in other ungulates [41] and it is important to note that every *E. rhusiopathiae* colony obtained from every caribou tissue sample was not sequenced in this study [39]. It is very possible that that a shared isolate (or isolates) common to all caribou may have been missed. Future research will employ sequencing of all suspected *E. rhusiopathiae* colonies obtained from culture of caribou tissues.

In some species, some animals carry *E. rhusiopathiae* in their tonsils, pharynx, bile, and muscles without developing clinical disease [29, 30]. 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 [29, 30]. *E. rhusiopathiae* infections are also known to be transmitted between different species [29, 30]. 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. The variation in seroprevalence recorded across study years and in different caribou herds could also indicate that other factors (e.g. predation risk, chronic stress, immune status, sympatric species, habitat use) potentially

influencing the risk of exposure to, infection with, and disease caused by *Erysipelothrix* may vary as a function of herd or landscape level features in NE BC.

Multiple serotypes (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) [30]. 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 currently no evidence that this pathogen can replicate outside a host [42, 43]. Rather, it is more likely that *Erysipelothrix* persists in nature primarily in carrier animals with a transient existence (of several weeks to months [44]) 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 [29]. 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 [29, 30]. Continued research is required to improve our understanding of the occurrence and significance of genetic variation in *E. rhusiopathiae* in boreal caribou.

In BCHRP Year 2, we explored metagenomics as a novel diagnostic technique for caribou health research. Metagenomics is an emerging discipline that enables the detailed study of uncultured microorganisms [13]. This technique employs sequencing and analysis of all genetic material recovered from a biological sample along with bioinformatics to identify ~ all microorganisms present in a tissue sample without the need for other diagnostics [13]. In addition, microorganisms do not have to be alive or present in large quantities to be detected. A metagenomics based approach may also permit the complete sequencing of genomes of microorganisms in a tissue sample. This information could then be used to identify/characterize both known and unknown pathogens, predict potentially relevant characteristics (e.g. virulence factors of specific pathogens), and to compare the microbiomes of "sick" vs. "healthy" animals [13]. Accordingly, metagenomics may be especially informative in wildlife mortality events where the microbiome of the affected species is poorly understood (as it is for all wildlife), where novel or poorly understood pathogens (e.g. *Erysipelothrix*) are believed to be acting, and if complex disease processes (e.g. co-infections) are involved.

3.3.2.2.3 *Erysipelothrix* in other caribou and 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 *Erysipelothrix rhusiopathiae* in the tissues of mountain

and boreal caribou from AB including a caribou found dead and intact (Fig. 8). A collaborative, interagency approach to evaluate the role of *Erysipelothrix* (and other health determinants) as drivers of woodland caribou population dynamics is strongly encouraged. We also recorded exposure to and infection with *Erysipelothrix* in serum and/or tissues collected from moose in both BC [45] and AB [46] and white-tailed deer in AB [46]. The investigation of *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 that could possibly transmit this bacterium to caribou. Serum samples from n=4 captive caribou located in Fort St. John, BC were also tested for exposure to *E. rhusiopathie* in BCHRP Year 2. All four captive caribou tested negative.

Figure 8. A radio-collared adult female mountain caribou (*Rangifer tarandus caribou*) 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 Northeastern British Columbia in the same year (Fig. 4). The bacterial pathogen *Erysipelothrix rhusiopathiae* was cultured from the tissues of this caribou. Photo credit: L. Finnegan, fRI Caribou Research Program, Hinton, AB.

3.3.2.3 Ongoing *Erysipelothrix rhusiopathiae* Research

Erysipelothrix rhusiopathiae will be a major focus of BCHRP Year 3. Research efforts will include analysis of serum collected from caribou live-captured in winter 2015/2016 as well as a retrospective analysis of serum collected from boreal caribou in the Snake-Sahtaneh herd range from 2002-2004. This work will provide further insight into longitudinal patterns of exposure to *E. rhusiopathiae* in caribou from NE BC. Tissue samples obtained from dead boreal caribou will continue to be evaluated using culture and molecular techniques and an experimental study to explore the timing and duration of the *E. rhusiopathiae* specific antibody response in *Rangifer* will occur (see Section 6). In depth analyses to evaluate potential relationships between *E. rhusiopathiae,* other pathogens, other health indices, and caribou survival/reproduction as well as temporal and spatial (landscape level) factors which may influence the transmission of *E. rhusiopathiae* and/or the occurrence of disease caused by this pathogen will be performed by a dedicated data analyst (see Section 6). We are also actively seeking to establish more research collaborations to explore the occurrence, distribution, and overall impact of this pathogen (and other health determinants) in woodland caribou herds across Canada.

3.4 Parasites

3.4.1 Gastrointestinal Parasites (Focus 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 [47]. 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 Trichostrongyle (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. Nonetheless, 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 long periods of freezing or freeze thaw cycles and sample storage prior to analysis may have influenced results obtained in Year 1.

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^{o}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 Strongylate 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 *Nematodirus*, *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) [47], 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* [47- 49]. 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 [47]. In BCHRP Year 1, dorsal-spined Protostrongylid larvae (DSLs) were found in 35% (n=55/157) of fecal samples collected from boreal caribou captured in NE BC in winter 2012/2013. The prevalence of DSLs appeared to vary somewhat across herd ranges [Calendar 48% (n=12/25), Chinchaga 51% (n=19/37), Maxhamish 35% (n=9/26), Parker 29% (n=2/7), Prophet 0% (n=0/8), and Snake-Sahtaneh 26% (n=14/54)]. In BCHRP Year 1, the average intensity of infection was 25 DSLs/gram feces (range < 1 - 191 DSLs/ gram feces) and was similar in all herd ranges (One-way ANOVA, P>0.05). All DSLs examined in BCHRP Year 1 were identified (using PCR) as the caribou muscle worm, *Parelaphostrongylus andersoni*.

In BCHRP Year 2, DSLs were quantified in the feces of all caribou captured in winter 2013/2014 and 2014/2015. In 2013/2014, 37% (n=15/41) of all fecal samples contained DSLs. In 2014/2015, 32% (n=10/31) of all fecal samples contained DSLs. 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. In winter 2013/2014 the prevalence of DSLs varied slightly across herd ranges [Calendar 40% (n=2/5), Chinchaga 23% (n=3/13), Maxhamish 45% (n=5/11), Parker 0% (n=0/1), and Snake-Sahtaneh 45% (n=5/11)]. Some variation was also recorded in DSL counts across herd ranges in winter 2014/2015 [Calendar 100% (n=1/1), Chinchaga 30% (n=3/10), Maxhamish 44% (n=4/9), Parker 0% (n=0/2), Prophet 0% (n=0/2) and Snake-Sahtaneh 42% (n=3/7)]. The intensity of DSL infections was similar across all herd ranges in winter 2013/2014 and 2014/2015 (One-way ANOVA, P>0.05). Based on findings in BCHRP Year 1, it is most likely that DSLs identified in BCHRP Year 2 were *P. andersoni*, however all DSLs recovered from all fecal samples were archived should molecular identification be required in the future. The occurrence, prevalence, and intensity of DSLs will be evaluated in the context of health biomarkers, caribou survival and reproduction in BCHRP Year 3. Spatial and temporal patterns of infection will also be explored.

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 [50]. Although it causes significant liver pathology in caribou no reports of clinical disease due to *F.*

magna have been reported in this species [51, 52]. Infected caribou also shed *F. magna* eggs in their feces and may function as a definitive host for this parasite [51, 52]. 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 causes severe to fatal disease [53].

In BCHRP Year 1, no fluke eggs were identified in n=157/157 fecal samples collected from adult female boreal caribou in winter 2012/2013. In BCHRP Year 2, no fluke eggs were identified in n=41 and n=31 fecal samples collected in winter 2013/2014 and 2014/2015. To date we have evaluated n=229 fecal samples for evidence of giant liver fluke. 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. elk and deer) 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 [47, 54]. Pathology caused by *B. tarandi* is related to the presence of intracellular cysts containing bradyzoites which may occur in a wide variety of tissues [55, 56.] Clinical disease associated with this parasite most often manifests as hair loss and skin lesions [55, 56]. 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 [55-57]. Outbreaks of clinical disease may also occur [54, 57].

The overall prevalence of exposure to *B. tarandi* in adult female boreal caribou captured in NE BC in winter 2012/2013 was 60% (n=90/149). However, the prevalence of exposure varied across herd ranges [Calendar 40% (n=8/20), Chinchaga 85% (n=28/33), Maxhamish 59% (n=16/27), Parker 43% (n=3/7), Prophet 71% (n=5/7), and Snake-Sahtaneh 45% (n=25/55)] which may suggest that factors influencing *B. tarandi* transmission (e.g. infections in carnivores or insect vectors [11]) may be relatively prominent in NE BC and may also vary as a function of landscape level features encountered by different boreal caribou herds. The only serological test currently available for this parasite is an in house ELISA (with a posteriori Western Blot) at Complutense University, Madrid, Spain. In BCHRP Year 2, logistical considerations prevented the shipment of serum samples collected in 2013/2014 and 2014/2015 to Spain for *Besnoitia* testing. We are currently working to arrange testing in BCHRP Year 3.

3.4.5 *Toxoplasma gondii*

Toxoplasma gondii is a protozoan parasite with a felid definitive host (in caribou range most likely lynx) and a wide variety of intermediate hosts including wild ungulates such as caribou [58-60]. 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 [58].

In BCHRP Year 2, n= 229 serum samples collected from boreal caribou in NE BC in winter 2012/2013 (n=155), 2013/2014 (n=41), and 2014/2015 (n=33) were tested for exposure to *Toxoplasma*. No seropositive caribou were detected in any year and no evidence of seroconversion in recaptured caribou was recorded. 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* will not be evaluated further as part in the BCHRP.

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 [47, 61]. This parasite is suspected as a likely cause of abortions and unthrifty calves in free-ranging caribou [47]. 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 [62]. As such, infection with *N. caninum* may represent a form of apparent competition [63] that could adversely affect caribou as the number of alternate intermediate hosts for this parasite increase in caribou range due to landscape and climatic change.

In BCHRP Year 1, we found evidence that 2% (n=3/148) of adult female boreal caribou captured in winter 2012/2013 had been previously exposed to *N. caninum*. A single *N. caninum* positive animal was identified in each of the Parker, Calendar, and Chinchaga herd ranges. Positive caribou from the Parker and Chinchaga ranges were not pregnant at the time of capture (January 7 and 21, 2013 respectively) while the positive caribou from the Calendar range (captured February 25, 2013) was pregnant. None (n=0/3) of the *N. caninum* positive caribou had a calf at heel at the time of capture. In BCHRP 2, we found that n=1 caribou from the Chinchaga herd range had been previously exposed to *N. caninum*. This individual was pregnant and had a calf at heel at the time of capture (March 6, 2014). No seropositive caribou (n=0/33) were identified in winter 2014/2015. No evidence of seroconversion was recorded among n=14 caribou recaptured in 2013/2014 (n=1) or 2014/2015 (n=13).

The overall pregnancy rate for adult female boreal caribou captured in 2012/2013, 2013/2014, and 2014/2015 in NE BC was 86% (n=204/237). The relative risk of "not being pregnant" was 4.2 times greater (Z=2.708, 95% C.I. 1.5-11.8 times greater, P=0.006) in *N. caninum* positive caribou than in *N. caninum* negative caribou. At ~2% (n=4/222), the overall prevalence of *N. caninum* in boreal caribou from NE BC appears to be low and to fall within the range previously recorded in other free-ranging caribou herds [e.g. 64, 65]. Nonetheless, *N. caninum* may represent an emerging threat to the reproductive success of boreal caribou in NE BC. This may be especially true in the Parker herd range (overall prevalence 13%, n=1/8) which currently has few caribou and in the Chinchaga herd range (overall prevalence 4%, n=2/57) where 50% of

seropositive animals were identified. Continued monitoring of this parasite in caribou as well as in other ungulate intermediate hosts and canid definitive hosts in NE BC is recommended.

3.4.7 Ectoparasites

In BCHRP Year 1, winter ticks (*Dermacentor albipictus*) and/or tick associated hair loss/breakage (ranging from mild to severe) were recorded as incidental observations in 16% (n=26/163) of adult female boreal caribou captured in NE BC in winter 2012/2013. In BCHRP Year 2, we used photographs taken of all n=238 individual caribou captured in NE BC in winter 2012/2013, 2013/2014, and 2014/2015 to develop a classification system for tick associated hair loss in live caribou (Fig. 9). 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. Across all years, the overall prevalence of winter tick associated hair loss in boreal caribou from NE BC was 76% (n=182/238). Moderate, severe, or extreme hair loss was recorded in 26%, 11%, and 2.5% of caribou examined over all years. The prevalence of winter tick infestations also appeared to vary across the six boreal herd ranges in NE BC and across study years (Table 4).

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) [66] and burdens typically recorded in moose (~1-2 ticks/cm^2) [67].

Figure 9. Recommended classification score for *Dermacentor albipictus* (winter tick) related hair loss in boreal caribou from Northeastern British Columbia. 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.

Table 4. Prevalence of hair loss related to *Dermacentor albipictus* (winter tick) infestations on adult female boreal caribou live-captured in Northeastern British Columbia in winter 2012/2013, 2013/2014, and 2014/2015*.

Herd Range	Prevalence of Winter Tick Associated Hair Loss (%) and Sample Size		
	Winter 2012/2013	Winter 2013/2014	Winter 2014/2015
All herds	75% (124/164)	85% (n=35/41)	69% (n=23/33)
Calendar	85% (n=23/27)	100% (n=5/5)	100% (n=2/2)
Chinchaga	59% (22/37)	61% (n=8/13)	70% (n=7/10)
Maxhamish	75% (n=23/28)	100% (n=11/11)	44% (n=4/9)
Parker	85% (n=6/7)	100% (n=1/1)	100% (n=2/2)
Prophet	88% (n=8/9)	No sample	50% (n=1/2)
Snake-Sahtaneh	78% (n=44/56)	91% (n=10/11)	88% (n=7/8)

* NB: The majority of BCHRP caribou captures occurred in late winter (late January to early April) however, some caribou in some years were captured in December when winter ticks may be relatively difficult to locate on infested animals. In addition, caribou with high tick burdens often do not exhibit any hair loss. Accordingly, prevalence estimates based on hair loss should be viewed as a crude index of infestation at the herd or population level only.

In moose, hair loss associated with *Dermacentor albipictus* infestations is the result of irritation and excessive grooming [68]. 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 [68, 69]. Heavy infestations may also cause anemia (due to blood loss) and epidemics of winter tick related mortality are known to occur in this species [69]. 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 [46, 66]. 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 [37]. This parasite may 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 be improving conditions for winter ticks and may increase the risk of infestation and related disease in boreal caribou even further in NE BC in the near future [67]. Likewise, recent landscape change may also enhance the risk of winter tick transmission to caribou due to an increase in the number of moose (or elk) inhabiting caribou range. Further research into the occurrence and impact of winter tick infestations on caribou is warranted. In BCHRP Year 3 we will investigate the occurrence and distribution of winter tick as part of in depth analyses looking at spatial and temporal relationships between caribou health determinants and caribou fitness.

Winter ticks can carry and likely transmit microorganisms (e.g. *Anaplasma* sp.) that have the potential to cause severe/fatal disease in cervids[70]. We have also 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 [71]) are recommended.

Warble fly (*Hypoderma tarandi*) larvae are considered one of the most important parasites of tundra *Rangifer* [72, 73]. Heavy infections (1000+ larvae) have been reported and migrating and developing larvae may cause significant pathology in the skin and subcutaneous tissue [72]. 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 that may lead to a decrease in the body condition and reproductive success of adult caribou and diminished condition and overwinter survival in juvenile caribou [74, 75]. Warbles were recorded on 0.4% (n=1/238) of caribou captured in NE BC in winter 2012/2013, 2013/2014, and 2014/2015. The single infected caribou was captured in winter 2014/2015 in the Chinchaga herd range and had a low warble burden (n=2 warbles identified). These warbles 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) for this study.

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 [11]. 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 [e.g. 75-77]. Likewise, vector borne nematodes (*Setaria* sp.) have been implicated as the cause of severe disease in free-ranging and semidomesticated *Rangifer* [78] 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) [79].

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 [15, 80]. 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 [78]. 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 5) 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]. In BCHRP Year 3, serum biochemical panels will be evaluated in the context of individual caribou and caribou herd health.

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 6). White blood cell counts and differentials obtained from free-ranging boreal caribou in NE BC fell within reference ranges for captive *Rangifer* [81] and were also similar to those recorded in generally healthy semi-domesticated reindeer [82]. No evidence of severe infection was identified in any caribou examined. In BCHRP Year 3, blood counts will be more closely evaluated in the context of individual caribou and caribou herd health.

Table 5. 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 Northeastern 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 6. Mean total white blood cell count and white blood cell differential for n=27 adult female boreal caribou captured in Northeastern 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 [reviewed 83]. The measurement of corticosteroids in hair is an emerging technique in wildlife health studies which has been previously evaluated in caribou/reindeer [83-85] and may represent the best integrated measure of chronic physiological stress currently available for this species.

In BCHRP Year 1, we measured hair cortisol concentration (HCC) in n=163 adult female boreal caribou captured in NE BC in winter 2013/2013. In BCHRP Year 2, we measured HCC in n=41 caribou and n=32 caribou captured in winter 2013/2014 and 2014/2015 respectively. We also measured HCC in n=13 radio-collared caribou that died between 2013 and 2015. Across all years, a wide range of HCC values were identified in live-captured animals (mean 4.36 pg/mg, range 0.16 - 47.94 pg/mg, n=236). Overall, HCC recorded in boreal caribou from NE BC was higher (Unpaired t-test, $t_{231}=3.281$, $P = 0.001$) than previously determined (using the same assay) in n=24 captive reindeer and caribou from Alaska and n=97 free-ranging caribou from Greenland [83]. Across all years, HCC was also lower in caribou captured in winter 2012/2013 than in winter 2013/2014 or 2014/2015 (One-way ANOVA, $F_{2,234} = 6.905$, $P=0.001$, Tukey-Kramer, $P<0.05$). There was no difference in HCC recorded in caribou captured in winter 2013/2014 and 2014/2015. 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). Multivariate analyses remain necessary to gain meaningful insight into all factors that may explain hair cortisol concentrations measured in boreal caribou and will occur in BCHRP Year 3.

4.4 Haptoglobin and Serum Amyloid A (SAA)

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 [86]. 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 [e.g. 87-91]. As such, haptoglobin and SAA may be useful indicators of both the occurrence and severity of pathological conditions in these species.

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, all haptoglobin levels in all caribou examined fell within the test range (0.00-0.50 g/L) considered to

be normal in domestic ruminants [92]. 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=32 caribou captured in 2014/2015 (mean 0.57 g/L, range 0.37-1.16 g/L). Haptoglobin levels measured in boreal caribou increased from winter 2012/2013 through winter 2014/2015 (One-way ANOVA, $F_{2, 221}$=132.36, P<0.0001, n=223, Tukey-Kramer, P<0.05). To provide further context for results obtained from free-ranging boreal caribou in NE BC, we also evaluated haptoglobin levels in n=6 captive caribou with known clinical histories in BCHRP Year 2. Haptoglobin levels (mean 0.64 g/L, range 0.49-0.88 g/L) measured in n=5 captive caribou with moderate pathology (determined at post-mortem) due to parasite infections or bacterial disease 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).

In BCHRP Year 1, a wide range of SAA levels were recorded in n=162 adult female boreal caribou captured in NE BC in winter 2012/2013 (mean 84.19 ug/ml, range 0.00 - 1016.00) 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 - 204.00 ug/ml) and n=32 caribou captured in 2014/2015 (mean 145.33 ug/ml, range 7.4 - 1609.00 ug/ml). Across all years, SAA levels measured in free-ranging caribou were similar (One-way ANOVA, P>0.05) to SAA levels determined in n= 5 captive caribou with moderate pathology due to parasite infections or bacterial disease and to levels reported in other ungulates harbouring chronic viral or bacterial infections [87, 88]. Overall, 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 [90, 91]. Likewise, 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). SAA levels measured in free-ranging boreal caribou in 2012/2013 were similar (Tukey Kramer, P>0.05) to levels measured in 2013/2014 and 2014/2015 while levels measured in 2013/2014 were marginally lower (Tukey Kramer, P<0.05) than those measured in 2014/2015 (One -way ANOVA, $F_{2, 232}$=3.218, P=0.04). No differences (One-way ANOVA, P>0.05) in SAA levels were apparent from 2012/2013-2014/2015 in the Calendar, Chinchaga, Maxhamish or Snake Sahtaneh herd ranges. In BCHRP Year 3, haptoglobin and SAA levels will be evaluated in the context of individual caribou and herd health. Spatial and temporal relationships between haptoglobin and SAA levels and landscape level features will also be explored.

4.5 Bone Marrow Fat Content

The nutritional status of free-ranging ungulates is closely related to their health, fitness, and population performance [e.g. 93, 94]. In BCHRP Year 2, we determined the % marrow fat in bone samples collected from boreal caribou mortality sites in NE BC in 2013, 2014 and 2015 (Table 7). Overall, marrow fat levels appeared to be lower in caribou that died in 2013 vs. 2014 or 2015. All caribou that died in 2013 appeared to be nutritionally stressed (marrow fat <85%) [95] to some degree and two caribou that died in summer 2013 (high mortality period) were likely

starving (marrow fat <12%) [95]. 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 7). 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 7. 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 Northeastern 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 [β]	April, 2014	Negative
SK154	jaw	69.8	April, 2014	Negative
SK165	jaw	82.1 [γ]	April, 2014	Pending
SK094	jaw	87.4 [γ]	October, 2014	Pending
SK044	jaw	88.0 [γ]	Nov, 2014	Pending
SK207	femur	84.0	Feb, 2015	Pending
SK052	jaw	80.8 [γ]	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 Nutrients

In domestic and free-ranging ungulates, trace nutrient levels are critically important determinants of immunity, health, growth, reproductive output, and survival e.g. [96-98]. 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 2.) and SK075 an adult female found dead (and intact) in July, 2013 during the high mortality period]. Liver levels of all trace nutrients measured in the un-collared yearling were within normal limits while iron deficiency and marginal copper levels were identified in the adult female caribou.

Liver samples are the preferred tissue in which to examine the trace nutrient 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 nutrients measured in serum can be an informative substitute and are commonly evaluated in livestock herd health assessment programs [99]. 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 8). The goals of this analysis were: to broadly evaluate caribou herds for evidence of potential trace nutrient deficiencies and to establish trace nutrient reference ranges (serum) for free-ranging boreal caribou in NE BC.

Preliminary findings suggest that trace nutrient deficiencies may occur in boreal caribou from NE BC. In particular, Cu and Se levels recorded in many boreal caribou fell below established laboratory reference ranges for captive *Rangife*r (Table 8). 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 [100]. Mn, Fe, Cu, Se, and Mo levels in boreal caribou were similar in winter 2012/2013, 2013/2014, and 2014/2015 (One-way ANOVA, $P>0.05$) while Co and Zn 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 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 which were similar (Tukey Kramer, $P>0.05$). Overall, these findings represent important baseline information and suggest that diet quality (nutrition) may have implications for the health status of caribou in NE BC. The significance of trace nutrient levels measured in boreal caribou will be evaluated in the context of the overall health status of individual caribou and caribou herds in BCHRP Year 3.

Table 8. Trace nutrient levels measured in serum collected from n=211 adult female boreal caribou captured by net-gun in Northeastern British Columbia in winter 2012/2013 (n=137), 2013/2014 (n=41) and 2014/2015 (n=33).

Trace Nutrient	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 captive *Rangifer* - University of Guelph Animal Health Laboratory
[β] 85% (n=180/211) samples below detection limits for Mo
NE: serum reference ranges not currently established in *Rangifer*

5. General Recommendations

In BCHRP Years 1 and 2, we established the first comprehensive herd health baselines for boreal caribou in NE BC. Our findings to date indicate that the health status of caribou may have important implications for the management and conservation of this threatened species. Interpretation and analysis of our boreal caribou health dataset is ongoing however the BCHRP working group currently recommends that, at a minimum, a longitudinal health monitoring program is continued for *Erysipelothrix*, *Neospora caninum*, and winter tick in at least some caribou herds in the current study area. We also recommend that biological samples continue to be collected and 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.

In BCHRP Year 2, we initiated a health research collaboration with the Foothills Research (fRI) 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 nutrient deficiencies in mountain and boreal caribou from west-central AB [46]. These findings clearly indicate that health may also have important implications for caribou populations outside of NE BC. 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 (Table 9) 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 and disease as a potential cause of death or a contributing factor. Detailed photographs of all mortality sites should be taken and all efforts should be made to collect any and all tissue remaining at mortality sites for review by a wildlife health specialist. 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). 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.

Table 9. Recommended minimum set of biological samples that should be collected from any and all live-captured caribou. 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. These samples can also 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 [37]. Detailed photographs should also be taken of each individual animal and of any abnormalities observed.

Sample	Sample Quantity	Sample Collection	Sample Processing and Storage
Serum	• n=3, 10 ml serum separator (SST) tubes	• Jugular or cephalic vein • 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- • 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

6. Research Objectives, BCHRP Year 3

6.1 Continue to enhance our understanding of the significance of exposure to and infection with *Erysipelothrix rhusiopathiae* in boreal caribou.

To better understand the importance of *Erysipelothrix rhusiopathiae* in free-ranging boreal caribou, a minimally invasive experimental trial will be performed at the University of Calgary in which vaccination of captive *Rangifer* will be used to evaluate the timing and duration of the antibody response when exposed to this pathogen. This trial was originally scheduled for BCHRP Year 2 however, management priorities (scheduled breeding and subsequent calving) in the captive herd dictated that the experiment was rescheduled to BCHRP Year 3. In BCHRP Year 3 we will also analyze serum collected from caribou live-captured in winter 2015/2016 as well as serum collected from boreal caribou in the Snake-Sahtaneh herd range from 2002-2004. This work will provide further insight into longitudinal patterns of exposure to *E. rhusiopathiae* in caribou from NE BC. In addition, we will continue to use tissue culture and molecular techniques to more thoroughly characterize *E. rhusiopathiae* in caribou.

6.2 Evaluate selected health biomarkers as simplified tools for monitoring boreal caribou health and evaluate relationships between boreal caribou health and larger scale (landscape level) features in NE BC.

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 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.

A data analyst with expertise in wildlife health and epidemiology joined the BCHRP research team in Spring, 2016. In BCHRP Year 3, this analyst will use the comprehensive herd health baselines established in Years 1 and 2 along with available ancillary data relating to the body condition, pregnancy status, and survival of individual caribou to validate selected health biomarkers (hair cortisol concentration, haptoglobin, SAA, serum biochemical parameters) in order to determine if they accurately reflect individual animal health and if they may ultimately have potential as simplified and relatively cost effective health monitoring tools.

The analyst will also use data gathered from current research partners and through new collaborations to evaluate selected temporal and spatial relationships between caribou health and larger-scale (landscape level) features (e.g. weather, habitat characteristics, predation risk, sympatric species, levels of natural or anthropogenic disturbance). This will allow us to determine if the health status of boreal caribou is linked with environmental conditions in NE BC. Landscape level analyses will also increase our understanding of the cumulative effects of environmental change on boreal caribou and the relative importance of health and disease as potential drivers of boreal caribou population performance in the region. This information will be used to develop recommendations to monitor and maintain healthy caribou populations in NE BC that may also benefit woodland caribou conservation initiatives elsewhere.

7.0 Literature Cited

1. Stephen, C. (2014). Toward a modernized definition of wildlife health. *Journal of Wildlife Diseases*, 50(3):427–430.

2. Busch, D. S. and Hayward, L.S. (2009). Stress in a conservation context: a discussion of glucocorticoid actions and how levels change with conservation-relevant variables. *Biological Conservation*, 142:2844-2853.

3. Linklater, W.L. (2010). Distress-an underutilised concept in conservation and missing from Busch and Hayward (2009). *Biological Conservation*, 143:1037-1038.

4. Patyk, K.A., Duncan, C., Nol, P., Sonne, C., Laidre, K., Obbard, M., Wigg, O., Aars, J., Regehr, E., Gustafson, L.L., and Atwood, T. (2015). Establishing a definition of polar bear (*Ursus maritimus*) health: a guide to research and management activities. *Science of the Total Environment*, 514:371-378.

5. Ellis, R.D., McWhorter, T.J., and Maron, M. (2012). Integrating landscape ecology and conservation physiology. *Landscape Ecology in Review*, 27:1-12.

6. Pedersen, A. B. and Babayan, S.A. (2011). Wild immunology. *Molecular Ecology*, 20:872–880.

7. MacPhee, R.D.E. and Greenwood, A.D. (2013). Infectious disease, endangerment, and extinction. *International Journal of Evolutionary Biology*, Volume 2013, Article ID 571939, 9 pp.

8. British Columbia Ministry of the Environment. (2010). Science update for the boreal caribou (*Rangifer tarandus caribou* pop. 14) in British Columbia. Victoria, BC. 54 pp.

9. COSEWIC. (2011). Designatable units for caribou (*Rangifer tarandus*) in Canada. Committee on the Status of Endangered Wildlife in Canada. Ottawa, ON. 88 pp.

10. British Columbia Ministry of the Environment. (2011). Implementation plan for the ongoing management of boreal caribou (*Rangifer tarandus caribou* pop. 14) in British Columbia. Victoria, BC. 17pp.

11. Schwantje, H., Macbeth, B., Kutz, S., and Elkin, B. (2014). British Columbia Boreal Caribou Health Program: Year 1 (November 1, 2013- December 31, 2014) Synthesis Report. Available from: www.bcogris.ca.

12. British Columbia Oil and Gas Research and Innovation Society (OGRIS) Active Projects: Boreal Caribou (2016). Available from: www.bcogris.ca

13. Mill, R.R., Montoya, M.V., Gardy, J.L., and Tang, P. (2013) Metagenomics for pathogen detection in public health. *Genome Medicine*, 5:81, DOI:10,1186/gm485.

14. das Neves, C.G., Roth, S., Rimstad, E., Thiry, E., and Tryland, M. (2010). Cervid herpesvirus 2 infection in reindeer: a review. *Veterinary Microbiology*, 143:70-80.

15. Johnson, D., Harms, N.J., Larter, N.C., Elkin, B.T., Tabel, H., and Guojian, W. (2010). Serum biochemistry, serology, and parasitology of boreal caribou (*Rangifer tarandus caribou*) in the Northwest Territories, Canada. *Journal of Wildlife Diseases*, 46:1096-1107.

16. Tessaro, S.V., Deregt, D., Dzus, E., Rohner, C., Smith, K., and Gadboury, T. (2005). Herpesvirus infection in woodland caribou in Alberta, Canada. *Journal of Wildlife Diseases*, 41:803-805.

17. Jordan, L., Rettie, W., and Tessaro, S. (2003). Evidence of herpesvirus infection in woodland caribou in Saskatchewan. *Journal of Wildlife Diseases*, 39:216-220.

18. Das Neves, C.G., Roger, M., Yoccoz, N.G., Rimstad, E. and Tryland, M. (2009). Evaluation of three commercial bovine ELISA kits for detection of antibodies against alphaherpesvirus in reindeer (*Rangifer tarandus tarandus*). *Acta Veterinaria Scandinavia*, 51:9-19.

19. Van Campen, H., Frölich, K. and Hofmann, M. (2001). Pestivirus infections, In: *Infectious Diseases of Wild Mammals, Third Edition*. Williams, E.S. and Barker, I.K. (Eds.). Iowa State University Press, Ames, Iowa, USA. doi: 10.1002/9780470344880.ch12.

20. Kelling, C.L. and Topliff, C.L. (2013). Bovine maternal, fetal and neonatal responses to bovine viral diarrhea virus infections. *Biologicals*, 41:20-25.

21. Morton, J.K.M., Evermann, J.F., and Dieterich, R.A. (1990). Experimental infection of reindeer with bovine viral diarrhea virus. *Rangifer*, 10:75-77.

22. Ferguson, M.D. (1997). Rangiferine brucellosis on Baffin Island. *Journal of Wildlife Diseases*, 33:536-543.

23. Curry, P. (2012). Blood on filter paper for monitoring caribou health: efficacy, community-based collection, and disease ecology in circumpolar herds. PhD Dissertation, Department of Faculty of Veterinary Medicine, University of Calgary, Calgary, AB, Canada. 309 pp

24. Tessaro, S.V., and Forbes, L.B. (1986). *Brucella suis* biotype 4: a case of granulomatous nephritis in a barren-ground caribou (*Rangifer tarandus groenlandicus*) with a review of the distribution of Rangiferine brucellosis in Canada. *Journal of Wildlife Diseases*, 22:479-483.

25. Rausch, R.L. and Huntley, B.E. (1978). Brucellosis in reindeer inoculated experimentally with *Brucella suis* type 4. *Canadian Journal of Microbiology*, 24:129-135.

26. Neiland, K.A., King, B., Huntley, E., and Skoog, R.O. (1968). The diseases and parasites of Alaskan wildlife populations: Part 1. Some observations on brucellosis in caribou. *Bulletin of the Wildlife Disease Association* 4:27-36.

27. Nymo, I.H. Godfroid, J., Åsbakk, K., Larsen, A.K., das Neves, C.G., Rødven, R. and Tryland, M. (2013). A protein A/G indirect enzyme-linked immunosorbent assay for the detection of anti-*Brucella* antibodies in Arctic wildlife. *Journal of Veterinary Diagnostic Investigation*, DOI: 10.1177/1040638713485073.

28. Personal communication (2016), M. Watters, Ecosystem Biologist, British Columbia Ministry of Forests, Lands and Natural Resource Operations, North Area-Northeast.

29. Leighton, F.A. (2001). Miscellaneous bacterial infections: *Erysipelothrix* infection. In: *Infectious Diseases of Wild Mammals*. Williams, E.S. and Barker, I.K. (eds.). Iowa State University Press, Iowa. pp. 491-493.

30. Wang, Q., Chang, B.J., and Riley, T. (2010). *Erysipelothrix rhusiopathiae*. *Veterinary Microbiology*, 140:405–417.

31. Atyabi, N., Youssefi, R., Javdani, G., Tavasoli, A., Vojgani, M., and Gharegozloo, F. (2012). Isolation of *Erysipelothrix rhusiopathiae* from aborted lambs in Iran: a case report *Iranian Journal of Veterinary Medicine*, 6(2):129-132.

32. Kutz, S., Bollinger, T., Branigan, M., Checkley, S., Davison, T. et al. (2015). *Erysipelothrix rhusiopathiae* associated with recent widespread muskox mortalities in the Canadian Arctic. *Canadian Veterinary Journal*, 56:561-563.

33. Campbell, G.D., Addison, E.M., Barker, I.K., and Rosendal, S. (1994). *Erysipelothrix rhusiopathiae*, Serotype 17, septicemia in moose (*Alces alces*) from Algonquin Park, Ontario. *Journal of Wildlife Diseases*, 30:436-438.

34. Bruner J.A., Griffith R.W., Greva J.H., and Wood, R.L. (1984), *Erysipelothrix rhusiopathiae* serotype 5 isolated from a white-tailed deer in Iowa *Journal of Wildlife Diseases* 20:235-236.

35. Stepaikin, P.P. (1939). Occurrence of *Erysipelothrix rhusiopathiae* in reindeer. *Sovetskaia Veterinariia*, 16(5):52-53 (In Russian).

36. Langford, E.V., and Dorward, W.J. (1977). *Erysipelothrix insidiosa* recovered from sylvatic mammals in northwestern Canada during examinations for rabies and anthrax. *Canadian Veterinary Journal*, 18:101-104.

37. Personal communication (2016). D. Culling, Biologist, Diversified Environmental Services Inc., Fort St. John, BC.

38. Forde, T., R. Biek, R. Zadoks, M. L. Workentine, J. De Buck, S. Kutz, T. Opriessnig, H. Trewby, F. van der Meer, and Orsel, K. (2016). Genomic analysis of the multi-host pathogen *Erysipelothrix rhusiopathiae* reveals extensive recombination as well as the existence of three generalist clades with wide geographic distribution. *BMC Genomics* (in press).

39. Forde, T., Orsel, K., Zadoks, R.N., Biek, R., Adams, L.G., Checkley, S., L., Davison, T., De Buck, J., Dumond, M., Elkin, B.T., Finnegan, L., Macbeth, B., Nelson, C., Niptanatiak, A., Sather, S., Schwantje, H., van der Meer, F., and Kutz, S.J. (2016). Bacterial genomics reveal the complex epidemiology of an emerging pathogen in arctic and boreal ungulates. Submitted: *Molecular Ecology* (under review).

40. Personal communication (2015). W. Hutchins, University of Calgary, Faculty of Veterinary Medicine.

41. Coutinho, T.A., Moreno, A.M., Imada, Y., Lopez, R.P.G., and Neto, J.S.F. (2012) Characterization of *Erysipelothrix rhusiopathiae* isolated from Brazilian *Tayassu pecari*. *Tropical Animal Health and Production*, 44:689-692.

42. Wood, R.L. (1973). Survival of *Erysipelothrix rhusiopathiae* in soil under various environmental conditions. *The Cornell Veterinarian*, 63:390-410.

43. Chandler, D.S., and Craven, J.A. (1980). Persistence and distribution of *Erysipelothrix rhusiopathiae* and bacterial indicator organisms on land used for disposal of piggery effluent. *Journal of Applied Bacteriology*, 48:367-375.

44. Mitscherlich, E. and Marth, E. (Eds). (1984). *Erysipelothrix ehusiopathiae*. In: *Microbial Survival in the Environment*. Springer-Verlag, Berlin. pp 162-165.

45. Unpublished data. (2015, 2016). H. Schwantje, Wildlife Veterinarian, British Columbia Ministry of Forests, Lands and Natural Resource Operation.

46. Unpublished data (2015, 2016). L. Finnegan, Program Lead, Foothills Research Institute (fRI) Caribou Program, Hinton, AB.

47. Kutz, S., Ducrocq, J., Verocai, G., Hoar, B., Colwell, D., Beckmen, K., Polley, L., Elkin, B., and Hoberg, E. (2012). Parasites in ungulates of Arctic North America and Greenland: a view of contemporary diversity, ecology, and impact in a world under change. In: *Advances in Parasitology*. Rollinson, D., and Hay, S. (eds.). Academic Press, London.

48. Verocai, G.G., Lejeune, M., Finstad, G.L., and Kutz, S.J. (2013). A Nearctic parasite in a Palearctic host: *Parelaphostrongylus andersoni* infecting semi-domesticated reindeer in Alaska. *International Journal for Parasitology: Parasites and Wildlife*, 2:119-123.

49. Lankester, M.W. (2001). Extrapulmonary lung worms of cervids. In: *Parasitic Diseases of Wild Mammals Second Edition*. Samuel, W.M., Pybus, M.J., and Kocan, A.A. (eds.). Iowa State University Press, Ames, Iowa. pp. 228-278.

50. Pybus, M. J. (2001). Liver flukes. In: *Parasitic Diseases of Wild Mammals Third Edition*. W. M. Samuel, M. J. Pybus, and A. A. Kocan (eds.). Iowa State University Press, Ames, Iowa. pp. 121-149.

51. Lankester, M.W. and S. Luttich. (1988). *Fascioloides magna* (Trematoda) in woodland caribou (*Rangifer tarandus caribou*) of the George River Herd, Labrador. *Canadian Journal of Zoology*, 66:475-79.

52. Polluck, B., Penashue, B., McBurney, S. et al. (2009). Liver parasites and body condition in relation to environmental contaminants in caribou (*Rangifer tarandus*) from Labrador, Canada. *Arctic*, 60:1-12.

53. Murray, D. L., Cox, E. W., Ballard, W. B., Whitlaw, H. A., Lenarz, M. S., Custer, T. W., Barnett, T., and Fuller, T. K. (2006). Pathogens, nutritional deficiency, and climate influences on a declining moose population: *Wildlife Monographs*, 166:1-29.

54. Ducrocq, J., Beauchamp, G., Kutz, S. et al. (2013). Variables associated with *Besnoitia tarandi* prevalence and cyst density in barren-ground caribou (*Rangifer tarandus*) populations. *Journal of Wildlife Diseases*, 49:29-38.

55. Ayroud, M., Leighton, F.A., and Tessaro, S.V. (1995). The morphology and pathology of *Besnoitia* sp. in reindeer (*Rangifer tarandus tarandus*). *Journal of Wildlife Diseases*, 31:319-326.

56. Wobeser, G. (1976). Besnoitiosis in a woodland caribou. *Journal of Wildlife Diseases*, 12:566-571.

57. Glover, G.J., Swendrowski, M., and Cawthorn, R.J. (1990). An epizootic of besnoitiosis in captive caribou (*Rangifer tarandus caribou*), reindeer (*Rangifer tarandus tarandus*) and mule deer (*Odocoileus hemionus hemionus*). *Journal of Wildlife Diseases*, 26:186-195.

58. Dubey, J.P. and Odening, K. (2001). Tissue inhibiting protozoans: Toxoplasmosis and related infections. In: *Parasitic Diseases of Wild Mammals Second Edition*. Samuel,W.M., Pybus, M.J., and Kocan, A.A. (eds.). Iowa State University Press, Ames, Iowa. pp 478-520.

59. Kutz, S.J., Elkin, B.T., Panayi, D., and Dubey, J.P. (2007). Prevalence of *Toxoplasma gondii* antibodies in barren-ground caribou (*Rangifer tarandus groenlandicus*) from the Canadian Arctic. *Journal of Parasitology*, 87:439-442.

60. Zarnke, R.L., Dubey, J.P., Kwok, O.C.H., and Ver Hoeff, J. (2000). Serologic survey for *Toxoplasma gondii* in selected wildlife species from Alaska. *Journal of Wildlife Diseases*, 36:219-224.

61. Dubey, J.P. (2003). Review of *Neospora caninum* and neosporosis in animals. *The Korean Journal of Parasitology*, 41:1-16.

62. Dubey, J.P., Jenkins, M.C., Kwok, O.C.H., Ferreira, L.R. et al. (2013). Congenital transmission of *Neospora caninum* in white-tailed deer (*Odocoileus virginianus*). *Veterinary Parasitology*, 196:519-522.

63. Tompkins, D.M., Draycott, R.A.H., and Hudson, P.J. (2000), Field evidence for apparent competition mediated via the shared parasites of two gamebird species. *Ecology Letters*, 3: 10–14. doi:10.1046/j.1461-0248.2000.00117.x

64. Stieve, E., Beckmen, K., Kania, S.A., Widner, A., and Patton, S. (2010). *Neospora caninum* and *Toxoplasma gondii* antibody prevalence in Alaskan wildlife. *Journal of Wildlife Diseases*, 46:348-355.

65. Curry, P. (2012). Blood on filter paper for monitoring caribou health: efficacy, community-based collection, and disease ecology in circumpolar herds. PhD Dissertation, Department of Faculty of Veterinary Medicine, University of Calgary, Calgary, AB, Canada. 309 pp.

66. Welch, D.A., Samuel, W.M., and Wilke, C.J. (1990). *Dermacentor albipictus* on captive reindeer and free-ranging woodland caribou. *Journal of Wildlife Diseases*, 26:410-411.

67. Leighton, F.A. (2000). Winter tick in moose and other ungulates. Available from: <http://www.ccwhc.ca/wildlife_health_topics/winter_tick/wintertick.php>Accessed June 1, 2016

68. Samuel, W.M. (1990). Grooming by moose (*Alces alces*) infested with the winter tick, *Dermacentor albipictus*: a mechanism for premature loss of winter hair. *Canadian Journal of Zoology*, 69:1255-1260.

69. Pybus, M.J. (2000). Moose and ticks in Alberta: a die-off in 1998-99. Occasional Paper No. 20. Government of Alberta, Fisheries and Wildlife Management Division, Environment and Sustainable Resource Development. 18 pp.

70. Baldridge, G. D., Scoles, G.A., Burkhardt, N.Y., Schloeder, B., Kurtti, T.J., and Munderloh, U.G. (2009). Transovarial transmission of *Francisella*-like endosymbionts and *Anaplasma phagocytophilum* variants in *Dermacentor albipictus* (Acari: Ixodidae). *Journal of Medical Entomology*, 46(3):625–632.

71. Sine, M., Morris, K., and Knupp, D. (2009). Assessment of a line transect field method to determine winter tick abundance on moose. *Alces*, 45:143-146.

72. Colwell, D. D. (2001) Bot Flies and Warble Flies (Order Diptera: Family Oestridae), In: *Parasitic Diseases of Wild Mammals,Second Edition*. W. M. Samuel, M. J. Pybus and A. A. Kocan (eds.). Iowa State University Press, Ames, Iowa, USA. doi: 10.1002/9780470377000.ch3

73. Thomas, D.C. and Kiliaan, H.P.L. (1990). Warble infestations in some Canadian caribou and their significance. *Rangifer*, Special Issue 3:409-417.

74. Cuyler, C., White, R.R., Lewis, K., Soulliere, C., Gunn, A., Russell, D.E., and Daniel, C. (2012). Are warbles and bots related to reproductive status in West Greenland caribou? *Rangifer*, 32(2):243-257.

75. Pattullo, K., Wobeser, G., Lockerbie, B.P., and Burgess, H.J. (2013). *Babesia odocoilei* infection in a Saskatchewan elk (*Cervus elaphus canadensis*) herd. *Journal of Veterinary Diagnostic Investigation*, 25:535–540.

76. Ameri, M., Anderson, W., Holman, P.J., and Palmer, G.W. (2012). *Babesia odocoilei* infection in a North American elk (*Cervus elaphus canadensis*). *Comparative Clinical Pathology*, 21:363-365.

77. Petrini, K.R., Holman, P.J., Rhyan, J.C., Jenkins, S.J., and Wagner, G.G. (1995). Fatal babesiosis in an American woodland caribou (*Rangifer tarandus caribou). Journal of Zoo and Wildlife Medicine*, 26:298-305.

78. Laaksonen, S. (2010). *Setaria tundra*, an emerging parasite of reindeer, and an outbreak it caused in Finland in 2003-2006. Ph.D. Dissertation. Faculty of Veterinary Medicine, University of Helsinki, Finland. 80 pp.

79. Lobanov, V.A., Gajadhar, A.A., Al-Adhami, A., and Schwantje, H.M. (2012). Molecular Study of free-ranging mule deer and white-tailed deer from British Columbia, Canada, for evidence of *Anaplasma* spp. and *Ehrlichia* spp. *Transboundary and Emerging Diseases*, 59: 233–243.

80. Lefebvre, M.F., Semalulu, S.S., Oatway, A.E., and Nolan, J.W. (1997). Trypanosomiasis in woodland caribou of Northern Alberta. *Journal of Wildlife Diseases*, 33:271-277.

81. Western College of Veterinary Medicine Specialized Livestock Program, Saskatoon, SK. (2016). Normal hematology and serum chemistry values for captive reindeer (*Rangifer tarandus*). Available from: < http://www.usask.ca/wcvm/herdmed/specialstock/deer/reindeer_values.html> accessed June 1, 2016.

82. Nieminen, M. and Timisjarvi, J. (1981). Blood composition of reindeer 1: hematology. *Rangifer*, 1:10-26.

83. Macbeth, B.J. (2013). An evaluation of hair cortisol concentration as a potential biomarker of long-term stress in free-ranging grizzly bears (*Ursus arctos*), polar bears (*Ursus maritimus*), and caribou (*Rangifer tarandus* sp.). PhD Dissertation, Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, SK. 298 pp.

84. Ashley, N.T., Barboza, P.S., Macbeth, B.J., Janz, D.M., Cattet, M.R.L., Booth, R.K., and Wasser, S.K. (2011). Glucocorticosteroid concentrations in feces and hair of captive caribou and reindeer following adrenocorticotropic challenge. *General and Comparative Endocrinology*, 172:382-391.

85. Renaud, L.A. (2012). Stress-inducing landscape disturbances: linking habitat selection and physiology in woodland caribou. *In*: Impacts de l'aménagement forestier et des infrastructures humaines sur les niveaux de stress du caribou forestier. MSc Thesis, Universite du Quebec a Rimouski. 97 pp.

86. Gruyse, E., Toussaint, M.J.M., Niewold, T.A., and Koopmans, S.J. (2005). Acute phase reaction and acute phase proteins: a review. *Journal of Zhekiang University Science*, 6B:1045-1056.

87. Sharifiyazida, H., Nazifi, S., Nikseresht, K., and Shahirari, R. (2012). Evaluation of serum Amyloid A and haptoglobin in dairy cows naturally infected with brucellosis. *Journal of Bacteriology and Parasitology*, 3:9.

88. Ulutas, B., Tan, T., Ulutas, P.A., and Bayramli, G. (2011). Haptoglobin and serum amyloid A responses in cattle persistently infected with bovine viral diarrhea Virus. *Acta Scientiae Veterinariae,* 39:973.

89. Alaska Department of Fish and Game. (2009). Caribou management report of survey inventories activities 1 July 2006-30 June 2008. P. Harper, ed. Project 3.0. Juneau, Alaska.

90. Orro, T. (2008). Acute phase proteins in dairy calves and reindeer: changes afterbirth and in respiratory infections. Ph.D. Dissertation. Faculty of Veterinary Medicine, University of Helsinki, Finland. 80 pp.

91. Orro, T. Sankaria, S., Pudsab, T., Oksanen, A., and Soveria, T. (2003). Acute phase response in reindeer after challenge with *Escherichia coli* endotoxin. *Comparative Immunology, Microbiology and Infectious Diseases,* 27:413–422.

92. University of Guelph Animal Health Laboratory, Clinical Pathology Services, Guelph, ON (2015). Reference range for serum haptoglobin levels in domestic ruminants.

93. National Council for Air and Stream Improvement, Inc. (NCASI). (2007). A review of ungulate nutrition and the role of top-down and bottom-up forces in woodland caribou population dynamics. Brown G. and Mallory, F. eds. Technical Bulletin No. 934. Research Triangle Park, N.C.: National Council for Air and Stream Improvement, Inc. 108 pp.

94. Cook, J., and R. Cook. 2014. Nutritional condition of caribou in northern British Columbia, 2012-2014. Annual Progress Report, 30 May 2014. National Council for Air and Stream Improvement, La Grande, OR. 14pp.

95. Personal communication (2016). R. Cook, Research Scientist, National Council for Air and Stream Improvement, Inc. (NCASI), LA Grande, OR, USA.

96. O'Hara, T.M., Carroll, G., Barboza, P., Mueller, K., Blake, J., Woshner, V., and Willetto, C. (2001). Mineral and heavy metal status as related to a mortality event and poor recruitment in a moose population in Alaska. *Journal of Wildlife Diseases,* 37(3):509–522.

97. Handeland, K., Bernhoft, A., and Aartun, M.S. (2008). Copper deficiency and effects of copper supplementation in a herd of red deer (*Cervus elaphus*) *Acta Veterinaria Scandinavica*, 50:8 doi:10.1186/1751-0147-50-8

98. Gogan, J.P., Jessup, D.A., and Akeson, M. (1989).Copper deficiency in tule elk at Point Reyes, California. *Journal of Range Management*, 42(3):233-238.

99. Herdt, T.H. and Hoff, B. (2011). The use of blood analysis to evaluate trace mineral status in ruminant livestock. *Veterinary Clinics of North America: Food Animal Practice*, 27:255–283.

100. Barboza, P.S., and Blake, J.E. (2001). Ceruloplasmin as an indicator of copper reserves in wild ruminants at high latitudes. *Journal of Wildlife Diseases*, 37:324-31.

101. Kutz, S., Checkley, S., Hutchins, W., and Leclerc, L.M. (2014). Final report to the Nunavut Harvesters Association on the Development of a Serologic Test for *Erysipelothrix rhusiopathiae* in Muskoxen.