

# **British Columbia Boreal Caribou Health Program**

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## **Progress Report: Year 1 (November 1, 2013 – December 31, 2014)**

**Prepared for:**

**The BC Science and Community Environmental Knowledge (SCEK) Funding Board  
Victoria, BC**

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## Executive Summary

Boreal caribou in Northeastern British Columbia (*Rangifer tarandus caribou*, population No.14, DU06<sup>1,2</sup>) are red-listed and believed to be declining<sup>1</sup>. 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<sup>3</sup>. Infectious diseases and other determinants of health are increasingly recognized as factors which may negatively impact caribou populations through direct and indirect effects on survival and reproduction<sup>e.g. 4-8</sup>. As a component of the BCIP, a three year Boreal Caribou Health Research Program (BCHRP) was created in the fall of 2013 to: 1) address knowledge gaps surrounding the current health status of boreal caribou in Northeastern British Columbia (NE BC), 2) determine if compromised health was a factor in the higher than expected mortality of caribou observed in the region between December 2012 and July 2013, and 3) provide health related recommendations for BC's ongoing boreal caribou management programs. The BCHRP represents the first large-scale study of boreal caribou health in British Columbia.

Research activities in BCHRP Year 1 focused on developing and employing a comprehensive health assessment program across six boreal caribou herd ranges in NE BC. Biological samples collected from n=164 live-captured and n=12 dead caribou during ongoing radio-telemetry based monitoring programs in 2012 and 2013 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, nutrition, and toxicology. Testing and data analysis are ongoing; however, preliminary findings indicate that health and disease may be important considerations for the long-term sustainability of boreal caribou in NE BC. Notably, the pathogenic bacterium *Erysipelothrix rhusiopathiae*, the protozoan parasite *Neospora caninum*, and the winter tick (*Dermacentor albipictus*) were identified in (or on) boreal caribou examined as part of BCHRP Year 1, and all may have distinct potential to adversely affect survival, reproduction, and ultimately population performance in this species. Preliminary findings also suggest that the prevalence of these (and other pathogens) may vary among different boreal caribou herds in NE BC.

In BCHRP Years 2 (January 1, 2015-December 31, 2015) and 3 (January 1, 2016-December 31, 2016), evaluation of additional biological samples collected in 2014 and 2015 will be combined with Year 1 results, and we will do an enhanced study of specific pathogens, and 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 novel and detailed picture of herd level health status and will inform boreal caribou management and conservation initiatives in NE BC. Strategies to evaluate, monitor, and protect the health of boreal caribou developed as part of the BCHRP will also benefit other woodland caribou conservation initiatives in BC and elsewhere and provide a starting point for similar studies in other species-at-risk.

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# Table of Contents

Executive Summary .....	i
Acknowledgements .....	ii
1. Introduction .....	1
2. Objectives .....	2
3. Methods.....	2
3.1 Sample Collection and Storage .....	2
3.2 Development of a Comprehensive Boreal Caribou Health Assessment Model .....	4
3.3 Pathogen and Health Testing .....	29
4. Preliminary Results, Discussion, and Recommendations.....	36
4.1 Considerations.....	36
4.2 Terminology.....	36
4.3 Preliminary Results, Discussion, and Recommendations (Pathogens).....	37
4.3.1 Viruses .....	37
4.3.2 Bacteria .....	38
4.3.3 Parasites .....	45
4.4 Preliminary Results, Discussion, and Recommendations (Other Health Indices).....	49
4.4.1 Serum Biochemistry.....	49
4.4.2 Chronic Stress .....	52
4.4.3 Immunity .....	52
4.4.4 Nutrition .....	53
4.4.5 Toxicology .....	55
5. General Recommendations .....	55

6. Future Directions: Research Objectives for BCHRP Year 2 and Year 3 .....	56
7. Literature Cited .....	58

## List of Tables

Table 1. Viral pathogens investigated as part of the Boreal Caribou Health Assessment Model in BCHRP Year 1.....	6
Table 2. Bacterial pathogens investigated as part of the Boreal Caribou Health Assessment Model in BCHRP Year 1 .....	8
Table 3. Parasites investigated as part of the Boreal Caribou Health Assessment Model in BCHRP Year 1.....	12
Table 4. Other health indices investigated as part of the Boreal Caribou Health Assessment Model in BCHRP Year 1 .....	24
Table 5. Diagnostic tests employed for pathogen and health screening in BCHRP Year 1 .....	30
Table 6. <i>Erysipelothrix rhusiopathiae</i> serology, PCR, and selective tissue culture results for n=12 boreal caribou ( <i>Rangifer tarandus caribou</i> ) from Northeastern BC, Canada that died in 2013 and were examined in BCHRP Year 1 .....	41
Table 7. Serum biochemical parameters for n=75 free-ranging, adult female, boreal caribou ( <i>Rangifer tarandus caribou</i> ) captured in 2102 and 2013 by net gun in Northeastern BC, Canada .....	51

## List of Figures

Figure 1. Boreal caribou ( <i>Rangifer tarandus caribou</i> ) herd ranges and core habitat areas in Northeastern British Columbia, Canada .....	3
Figure 2. Preliminary Case Report: <i>Erysipelothrix rhusiopathiae</i> in an un-collared yearling male boreal caribou ( <i>Rangifer tarandus caribou</i> ) found moribund in the Snake-Sahtaneh herd range of Northeastern BC in March, 2013 .....	43

## Appendices

Appendix 1: Data sheet and recommendations for the health-based evaluation and sampling of live-captured woodland caribou (*Rangifer tarandus caribou*).

Appendix 2: Data sheet and recommendations for the health-based evaluation and sampling of dead woodland caribou (*Rangifer tarandus caribou*).

# 1. Introduction

Wildlife health reflects the cumulative outcome of complex interactions between a species' evolutionary history and recent or past biotic and abiotic factors acting on individuals and populations<sup>9, 10</sup>. As such, "health" may be an indicator of an individual's and a population's capacity to cope with the combined effects of natural and anthropogenic challenges<sup>11, 12</sup>. Understanding and tracking the health status of free-ranging wildlife may, therefore, provide valuable information for the management and conservation of species-at-risk<sup>e.g. 13-17</sup>.

Health is increasingly recognized as an important factor that may contribute to diminished survival and reproduction in free-ranging caribou populations<sup>e.g. 4-8</sup>. Certain pathogens (e.g. West Nile Virus<sup>18</sup>) have the potential to kill caribou directly while others (e.g. gastrointestinal and other parasites<sup>e.g. 19-21</sup>) may have more subtle or chronic effects which may lead to morbidity and ultimately mortality caused by an enhanced risk of predation or a diminished ability to cope with other natural or anthropogenic stressors (e.g. random weather events, nutritional deficits, other infections, development, recreational land use etc.). Likewise, other pathogens (e.g. the protozoan parasites *Neospora caninum*<sup>22</sup> and *Toxoplasma gondii*<sup>23</sup>, the bacterium *Brucella suis* biovar 4<sup>24</sup>, alphaherpesvirus<sup>25</sup>) may reduce the reproductive success of caribou directly by causing abortions or indirectly by leading to calf morbidity and an enhanced risk of neonatal mortality due to predation, decreased resistance to environmental stressors, or failure to thrive. In turn, the effects of disease may eventually impact caribou populations as rates of survival, reproductive output, and overall abundance decline as the number of affected individuals grows<sup>14, 26</sup>. Interactions between, or the cumulative effects of, many factors influencing the health of individual caribou in a particular region, may also act as key drivers of caribou population dynamics with outcomes that are particularly relevant to caribou management and conservation. For example, the effects of compromised health and enhanced predation risk may be additive or synergistic<sup>27</sup> and among the most important interactions affecting adult caribou survival and abundance and juvenile caribou survival and recruitment. Similarly, poor health and prolonged periods of inclement weather (e.g. deep snow or ice crusting) may combine to reduce the foraging efficiency and body condition of female caribou below thresholds necessary to support pregnancy<sup>28, 29</sup>.

The relative importance of health as a driver of population performance for woodland caribou in western Canada is anticipated to increase as the loss, degradation, and fragmentation of their habitat continues and the effects of climate warming [e.g. periods of thermal stress, forest fires, and the northward expansion of white-tailed deer (*Odocoileus virginianus*) and arthropod vectors of disease and other pathogens] occur with greater frequency<sup>e.g. 30-34</sup>. Moreover, most woodland caribou populations in western Canada are relatively small<sup>1, 34</sup> which greatly increases the risk that health related factors (which are currently unrecognized) could have serious effects at both the local (herd) and regional level<sup>35, 36</sup>. Despite these considerations, the lack of a broader understanding of relationships between environmental conditions, caribou health, and population performance has recently been highlighted as a knowledge gap in management, conservation, and recovery programs throughout the species' range<sup>e.g. 6-8, 37, 38</sup>.

In British Columbia the boreal ecotype of woodland caribou (*Rangifer tarandus caribou*, population No.14; DU06<sup>1, 2</sup>) is red-listed, designated as *Threatened* under the federal *Species at*

*Risk Act*<sup>39</sup>, and believed to be declining<sup>1</sup>. 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<sup>3</sup>. As part of the BCIP, a Boreal Caribou Health Research Program (BCHRP) was created in fall 2013 with a three year mandate (November 1, 2013–December 31, 2016) and the objectives of: 1) gathering comprehensive baseline data to determine the health status of live and dead boreal caribou in Northeastern British Columbia (NE BC), 2) assessing if health was a factor in the higher than expected mortality of boreal caribou observed in the region between December 2012 and July 2013, and 3) providing health related recommendations for BC's ongoing boreal caribou management programs. The BCHRP represents the first large-scale study of boreal caribou health in British Columbia. This report summarizes activities completed during BCHRP Year 1 and includes preliminary results of health and disease testing available as of December, 2014. An overview of research efforts planned for BCHRP Year 2 and Year 3 is also presented.

## **2. Boreal Caribou Health Program Objectives (Year 1)**

2.1 Develop and apply a comprehensive Boreal Caribou Health Assessment Model (testing plan) to evaluate biological samples (blood, feces, hair, tissue) collected from live-captured and dead boreal caribou across six herd ranges in NE BC in 2012 and 2013.

2.2 Determine the health status of live-captured and dead boreal caribou in NE BC using results obtained as part of 2.1 in conjunction with available ancillary biological data (e.g. pregnancy status, body condition, survival).

2.3 Determine health baselines for boreal caribou herds in NE BC and assess if poor health may have been a factor in the relatively high number boreal caribou mortalities observed in the region between December 2012 and July 2013.

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

2.5 Provide preliminary health related recommendations for boreal caribou management in NE BC.

## **3. Methods**

### **3.1 Sample Collection and Storage**

#### **3.1.1 Sample Collection: Live-captured Caribou**

As a component of the BCIP, 164 adult female boreal caribou were live-captured and radio-collared in the Calendar (n=27), Chinchaga (n= 37), Maxhamish (n=28), Parker (n=7), Prophet (n=9) and Snake-Sahtaneh (n=56) herd ranges of NE BC (Fig. 1) between December 2012 and March 2013 to support a large-scale monitoring program tracking adult caribou survival and juvenile caribou recruitment in the region. This program along with a detailed description of the study area, capture and handling protocols, boreal caribou habitat, and herd

ranges in NE BC, are reported in detail elsewhere<sup>40-42</sup>. Health based sampling was integrated into this ongoing program and hair, feces, whole blood, and serum were collected from every caribou captured<sup>42</sup>. The occurrence of ectoparasites, other health related anomalies, and any associated pathology were documented and samples 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<sup>42</sup> while pregnancy status was determined after capture by measuring serum progesterone and/or Pregnancy Specific Protein B (PSPB) concentrations<sup>42,43</sup>.

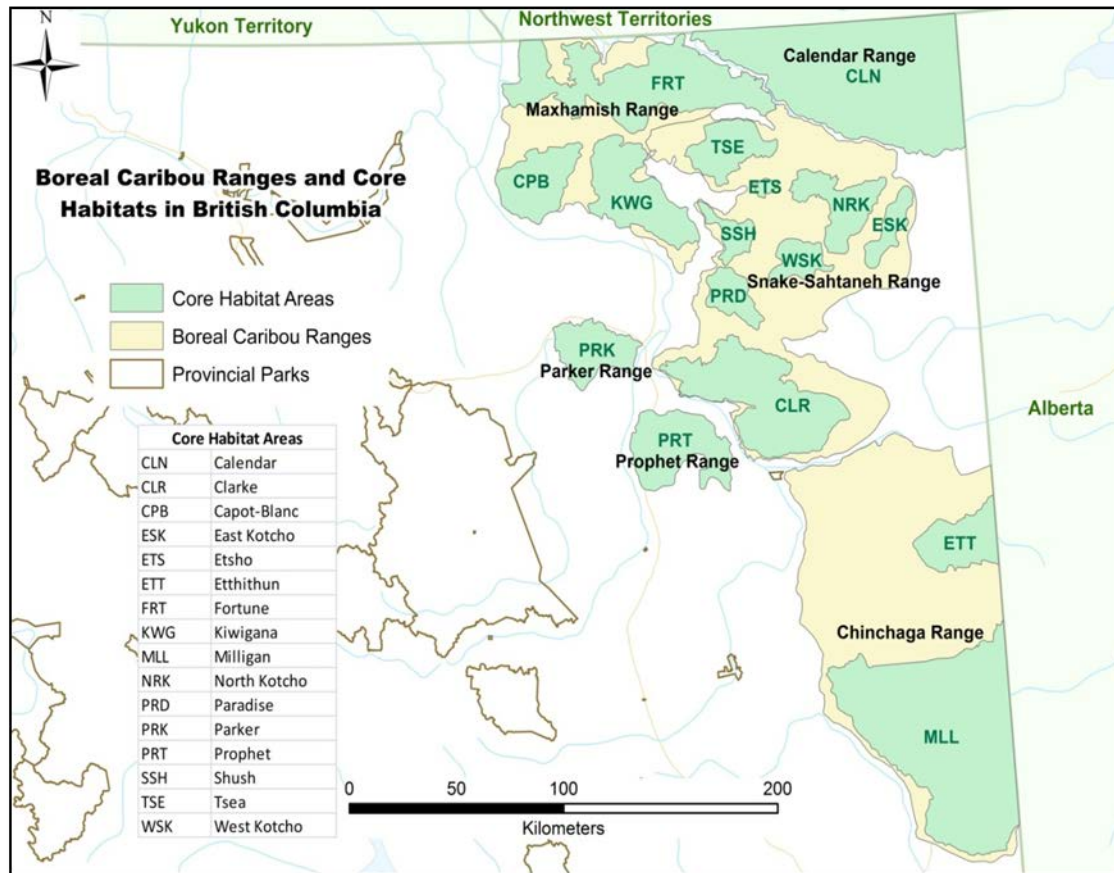


Figure 1. Boreal caribou herd ranges and core habitat areas in Northeastern British Columbia, Canada. Modified from: British Columbia Ministry of the Environment. (2010). Science update for the boreal caribou (*Rangifer tarandus caribou* pop. 14) in British Columbia. Victoria, BC. 54 pp.

### 3.1.2 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<sup>42,44</sup>. As part of these



investigations, 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<sup>42, 44</sup>.

### 3.1.3 Sample Storage

All biological samples from live-captured and dead caribou were stored frozen (-20°C) and shipped to the Provincial Wildlife Veterinarian in Nanaimo, BC where they were archived until forwarded to the University of Calgary, Faculty of Veterinary Medicine, Calgary, AB for in-house or referred health and diagnostic testing.

## 3.2 Development of a Comprehensive Boreal Caribou Health Assessment Model

Prior to the initiation of the BCHRP, the health status of boreal caribou in NE BC (as for the majority of woodland caribou populations in Canada) had not been thoroughly investigated at the population level and baseline data were lacking. Moreover, an unexpectedly high number of boreal caribou mortalities (including unusual spring and summertime deaths not related to predation) were observed in the region between December 2012 and July 2013 following an extended winter with high snow pack and significant crusting. Together these factors lead to the creation of the BCHRP in fall 2013.

The primary focus of BCHRP Year 1 was the development and application of a comprehensive Boreal Caribou Health Assessment Model (testing plan) to evaluate archived biological samples (whole blood, serum, feces, tissue, hair) collected from live-captured and dead boreal caribou in 2012 and 2013 (see Section 3.1). The primary goals of this health testing plan were: 1) to determine health baselines for boreal caribou herds in NE BC and 2) to evaluate health related considerations which may have been a factor in the relatively high number of boreal caribou mortalities observed in the region between December 2012 and July 2013. Other goals considered when developing the testing plan were to provide a foundation of knowledge to guide: 1) the short-term health monitoring program and in-depth (landscape level) analyses planned for BCHRP Years 2 and 3 (see Section 6), and 2) the development of health related recommendations to enhance boreal caribou management and conservation efforts in NE BC.

In this context, pathogens and other indices of caribou health were assessed as candidates for testing 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 (and other ungulates) in NE BC and elsewhere. Pathogens ultimately selected for study in BCHRP Year 1 focused on bacteria, viruses, and parasites deemed to represent a significant threat (directly or indirectly) to caribou survival and/or reproduction now or in the near future. In order to provide an enhanced understanding of both the general health of boreal caribou in NE BC and the biological mechanisms possibly influencing herd level health status other parameters were also considered including established and emerging indices of organ function, chronic stress, immunity, nutrition, and toxicology. Viruses, bacteria, and parasites evaluated as part of the Boreal Caribou Health Assessment Model in BCHRP Year 1 are presented in Table 1, Table 2, and Table 3. Other health related indices evaluated as part of the Boreal Caribou Health Assessment Model in BCHRP Year 1 are presented in Table 4.

It should be noted that testing efforts in BCHRP Year 1 were restricted by the availability, quality, quantity, and types of archived biological samples (frozen whole blood, serum, hair, feces, and the specific types of tissue) collected from individual boreal caribou in 2012 and 2013. As such, we were unable to assess all pathogens and all health indices in every animal. To address these considerations in BCHRP Years 2 and 3, recommendations were developed to enhance the quantity and scope of health related biological samples collected from live-captured (Appendix 1) and dead (Appendix 2) boreal caribou in 2014 and 2015. It is also important to note that both the health assessment model and field-based sampling recommendations are designed to be adaptive and may be modified if new health related considerations are identified or as the results of diagnostic tests are finalized. Although not considered directly in Year 1 (due to the lack of specific samples required for testing), the BCHRP will also support ongoing provincial surveillance programs for other potentially important pathogens [e.g. Chronic Wasting Disease (CWD), Tuberculosis (*Mycobacterium bovis*)] of boreal caribou (and other ungulates) when and if required samples are obtained as part of Year 2 and 3 research efforts.

**Table 1. Viral pathogens investigated as part of the Boreal Caribou Health Assessment Model in BCHRP Year 1.**

Pathogen	Brief Background and Potential Significance for Boreal Caribou in Northeastern British Columbia
<p><b>Alphaherpesvirus</b></p>	<p><b>1) Background:</b></p> <ul style="list-style-type: none"> <li>• A high prevalence of exposure to an alphaherpesvirus closely related to bovineherpesvirus (BoHV-1) has been recorded in woodland caribou herds from NT (37.5%) <sup>45</sup>, AB (52%) <sup>46</sup>, and SK (55%) <sup>47</sup>.</li> <li>• The specific alphaherpesvirus virus occurring in woodland caribou has not been identified but is most likely CvHV-2 (<i>Rangifer herpes virus</i>) <sup>25, 48</sup>.</li> </ul> <p><b>2) Potential Significance:</b></p> <ul style="list-style-type: none"> <li>• There is an emerging interest in alphaherpesvirus (CvHV-2) as a potentially important pathogen causing both subclinical effects and overt disease in captive and free-ranging <i>Rangifer</i> of all ages including: <ul style="list-style-type: none"> <li>• Mild to severe infectious keratoconjunctivitis <sup>49</sup></li> <li>• Erosion and ulceration of nasal, oral, and genital mucosa (+/- secondary bacterial infection) <sup>25, 50</sup></li> <li>• Respiratory disease complex including acute hemorrhagic, necrotizing bronchopneumonia <sup>51</sup></li> <li>• Abortion and neonatal morbidity or mortality <sup>25, 52</sup></li> </ul> </li> <li>• Herpesviruses are characterized by horizontal (animal to animal) and vertical (mother to calf) transmission as well as latency (non-clinical, persistent infections which can reactivate). Clinical disease and transmission are often triggered by stress in persistently infected animals <sup>25, 52</sup>.</li> <li>• The probability that an alphaherpesvirus occurs in boreal caribou from NE BC is very high. Chronic stress related to the cumulative effects of recent landscape and climatic change, increasing predation risk, nutrition, other diseases etc. may therefore have the potential to alter transmission dynamics and/or increase the probability that clinical disease or negative subclinical effects related to this pathogen may adversely impact the survival or reproductive success of boreal caribou in the region.</li> </ul>

<p><b>Pestiviruses</b></p>	<p><b>1) Background:</b></p> <ul style="list-style-type: none"> <li>• A high prevalence of exposure to a pestivirus closely related to Bovine Viral Diarrhea Virus (BVDV) has been recorded in caribou from the Western Arctic (19-56%)<sup>53, 54</sup> and Quebec (69%)<sup>55</sup>. To date, pestiviruses have not been reported in boreal or mountain caribou from NT, AB, SK, or YT<sup>45-47, 56</sup>.</li> <li>• It is probable that an uncharacterized <i>Rangifer</i> specific pestivirus exists which is closely related to BVDV or Border Disease Virus (BDV) of sheep<sup>57</sup>.</li> </ul> <p><b>2) Potential Significance:</b></p> <ul style="list-style-type: none"> <li>• In domestic ruminants pestiviruses are the causative agents of immunosuppression, mild to severe/ fatal respiratory and gastrointestinal disease as well as infertility, abortions, and neonatal morbidity and/or mortality<sup>58-62</sup>.</li> <li>• Immunosuppression related to infection with pestiviruses may also increase the susceptibility to or the severity of infections caused by other viruses, bacteria, and parasites<sup>59</sup>.</li> <li>• Pestiviruses are transmitted horizontally and vertically and persistent infections may occur in some animals exposed to the virus as fetuses at specific times during gestation<sup>59</sup>. Persistently infected animals are considered of particular importance for the maintenance and transmission of this pathogen in and among ungulate herds<sup>59, 60</sup>. Pestiviruses are also known to cross the species barrier (e.g. BVDV of cattle may infect and cause disease in wild cervids)<sup>58</sup>.</li> <li>• Experimental infections with BVDV have caused severe, fatal disease characterized by laminitis and diarrhea with blood and mucus in reindeer<sup>61</sup>.</li> <li>• A negative association between exposure to an unknown pestivirus and the occurrence of pregnancy has also been recently recorded in caribou from the George River Herd of Quebec, Newfoundland and Labrador<sup>63</sup>.</li> <li>• If identified in NE BC, pestiviruses may have the potential to enhance the impact of other pathogens and/or cause clinical or subclinical disease which may adversely affect the survival and reproductive success of boreal caribou in the region.</li> </ul>
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**Table 2. Bacterial pathogens investigated as part of the Boreal Caribou Health Assessment Model in BCHRP Year 1.**

Pathogen	Brief Background and Potential Significance for Boreal Caribou in Northeastern British Columbia
<b><i>Brucella suis</i> biovar 4</b>	<p><b>1) Background:</b></p> <ul style="list-style-type: none"> <li><i>Brucella suis</i> biovar 4 is a bacterial pathogen that is widely distributed in caribou and reindeer herds throughout Northern Canada and Alaska<sup>24, 53, 64, 65</sup>. Suspect lesions have also been recorded in boreal caribou from Eastern AB<sup>66</sup> but have not been recorded in boreal caribou from NT or YT<sup>45, 56</sup></li> </ul> <p><b>2) Potential Significance:</b></p> <ul style="list-style-type: none"> <li><i>Brucella suis</i> biovar 4 is often considered one of the most important infectious diseases affecting free-ranging caribou populations<sup>24, 65, 67-69</sup>.</li> <li>This pathogen is primarily transmitted among caribou by contact between uninfected animals and aborted fetuses or fetal membranes from infected animals<sup>70</sup>. Venereal transmission may also occur<sup>70</sup>.</li> <li>In caribou, infection with <i>B. suis</i> biovar 4 may be subclinical or may be associated with severe chronic disease including: <ul style="list-style-type: none"> <li>Chronic bursitis and/or arthritis (causing lameness)<sup>24</sup> and chronic nephritis<sup>65</sup></li> <li>A variety of reproductive disorders leading to reproductive failure and/or sterility in both female and male caribou including: abortion, retention of the placenta, metritis, mastitis, orchitis, and epididymitis<sup>69, 70</sup></li> <li>Neonatal morbidity and mortality<sup>70</sup></li> </ul> </li> <li>If identified in NE BC, <i>Brucella suis</i> biovar 4 may be an important factor contributing to the poor performance of boreal caribou populations in the region.</li> </ul>

<p><i>Mycobacterium avium</i> <i>ssp. paratuberculosis</i> (MAP)</p> <p><b>Paratuberculosis</b></p> <p><b>Johne's Disease</b></p>	<p><b>1) Background:</b></p> <ul style="list-style-type: none"> <li>• PCR-based testing has been used to identify <i>Mycobacterium avium ssp. paratuberculosis</i> (MAP) DNA in the feces of free-ranging caribou from Quebec (prevalence ~ 11.0%) <sup>71</sup> and boreal caribou from NT (prevalence 5.9%) <sup>45</sup>. Although PCR positive caribou are likely to be infected, only one case of a confirmed MAP infection (culture positive fecal sample) has been recorded in free-ranging caribou (from the Rivière-aux-Feuilles herd, Quebec) to date <sup>71</sup>.</li> </ul> <p><b>2) Potential Significance:</b></p> <ul style="list-style-type: none"> <li>• In domestic ruminants MAP causes chronic enteritis characterized by persistent diarrhea, progressive weight loss, morbidity and eventually death <sup>72</sup>.</li> <li>• Horizontal transmission of MAP may occur through contact between uninfected animals and the feces of an infected animal or through contaminated soil, forage, or water <sup>72</sup>. Vertical transmission between an infected female and her calf may also occur in utero or by ingestion of MAP in colostrum or milk after birth <sup>73</sup>.</li> <li>• In <i>Rangifer</i> MAP is known to cause a disease syndrome similar to that reported in domestic ruminants and characterized by poor body condition, subcutaneous edema, granulomatous ileitis, mesenteric lymphadenitis and hepatitis leading to progressive wasting, morbidity, and death <sup>71, 74</sup>. Unlike domestic species, diarrhea may be absent and chronic weight loss is a more typical sign of this disease in caribou <sup>71</sup>.</li> <li>• Lung lesions such as necrosis and mineralization similar to those more typically observed in <i>Mycobacterium bovis</i> (bovine tuberculosis) infections have also been recorded <sup>74</sup>.</li> <li>• In captive reindeer, MAP may be associated with an acute onset of illness and high mortality rate <sup>71, 74</sup> and clinical disease is often detected in young (9-10 months old) animals. Together these observations suggest that, unlike domestic ruminants, MAP may have a relatively rapid clinical course in caribou <sup>71</sup>.</li> <li>• In caribou, clinical disease associated with MAP may be precipitated by stress related to calving or other environmental factors <sup>71, 72</sup>.</li> <li>• If MAP occurs in boreal caribou from NE BC, it may directly and adversely affect the survival of both adults and juveniles. In addition, MAP may indirectly reduce the reproductive success of caribou through negative effects on maternal body condition. Chronic stress related to the cumulative effects of recent landscape and climatic change, increasing predation risk, nutrition, other diseases etc. may also</li> </ul>
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	increase the probability that clinical disease or other negative effects related to this pathogen could occur.
<b>Miscellaneous Bacterial Infections</b>	<p><b>1) Background:</b></p> <ul style="list-style-type: none"> <li>• A variety of bacterial pathogens have been reported in caribou and may be suspected as primary or secondary agents of morbidity and/or mortality in individual animals <sup>e.g. 75-86</sup>. However, the occurrence and importance of most bacterial pathogens have not been thoroughly investigated in free-ranging caribou populations.</li> <li>• Horizontal, vertical, and/or vector mediated transmission of bacterial pathogens may occur and the most common route(s) vary across different species of bacteria.</li> <li>• Selected bacteria of particular importance to free-ranging caribou in NE BC may include:</li> </ul> <p>a) <i>Erysipelothrix rhusiopathiae</i></p> <ul style="list-style-type: none"> <li>• <i>Erysipelothrix rhusiopathiae</i> is the causative agent of a spectrum of clinical conditions in domestic and free-ranging ungulates including: abortions, cutaneous lesions, chronic arthritis and endocarditis, pneumonia, and per acute, fatal septicemia <sup>79</sup>. Clinical disease related to <i>E. rhusiopathiae</i> often occurs secondary to stress or compromised immunity in both carrier and newly infected animals <sup>79</sup></li> <li>• The ecology of <i>E. rhusiopathiae</i> is poorly understood in wildlife. However, this bacterium may be emerging as an important pathogen of northern ungulates. <i>E. rhusiopathiae</i> has recently been implicated in the widespread mortality of muskoxen (<i>Ovibos moschatus</i>) on two islands in the Canadian Arctic archipelago <sup>87</sup> and as the cause of mortality in free-ranging Canadian moose (<i>Alces alces</i>) <sup>88</sup>. This bacterium has also been implicated as the likely cause of a historical (1930's) outbreak of fatal illness in semi-domestic reindeer (<i>Rangifer tarandus tarandus</i>) in Scandinavia and Russia <sup>89</sup>.</li> </ul> <p>b) <i>Pasturella multocida</i></p> <ul style="list-style-type: none"> <li>• <i>Pasturella multocida</i> has been recorded as the causative agent of fatal septicemia in free-ranging caribou with infections related to unsuccessful predation attempts by lynx (<i>Lynx canadensis</i>) which may carry the bacteria in their oral cavity <sup>78</sup>.</li> <li>• This pathogen may also be associated with fatal pneumonia in wild ungulates where it may be a primary</li> </ul>

agent of disease or occur secondary to other bacterial, viral, or parasitic infections<sup>80</sup>.

c) *Fusobacterium necrophorum* and *Tuperella* (*Archanobacterium*) *pyogenes*

- *Fusobacterium necrophorum* and *Tuperella* (*Archanobacterium*) *pyogenes* have been recorded as the cause of recent outbreaks of digital necrobacillosis (footrot) in wild reindeer from Norway<sup>75</sup> and caribou from Northern Canada<sup>90, 91</sup>
- Outbreaks have occurred following extended periods of usually warm and wet weather<sup>75, 90</sup>.
- Severe pathology (including: severe chronic periostitis and osteomyelitis and necrotizing deforming arthritis), lameness, and poor body condition have been reported in affected animals<sup>75, 90</sup>.

d) Other bacteria of potential significance for caribou may include:

- *Leptospira interrogans*<sup>54, 81</sup>, *Helicobacter*<sup>82</sup>, *Yersinia pseudotuberculosis*, *Y. enterocolitica*<sup>83</sup>, *Chlamydophila* sp., *Nocardia asteroides*<sup>76</sup>, *Listeria monocytogenes*<sup>77</sup>, *Clostridium* sp.<sup>85, 86</sup>, *Salmonella* sp.<sup>84</sup>, and *Escherichia coli*<sup>83</sup>.

**2) Potential Significance:**

- The significance of miscellaneous bacterial infections for boreal caribou in NE BC is likely to be pathogen dependent. Some organisms have the potential to negatively affect boreal caribou survival or reproduction by causing chronic disease leading to morbidity which may ultimately enhance the risk of predation, decrease resistance to other stressors, or lead to diminished body condition. Other bacterial pathogens may cause abortions or outbreaks of acute fatal illness.
- Factors which may lead to compromised immunity (e.g. chronic stress, toxins, other diseases, poor nutrition etc.) may be of particular importance to the occurrence and overall significance of disease caused by miscellaneous bacterial infections in boreal caribou from NE BC.
- Increasing potential for vector mediated transmission of some bacterial pathogens may also be relevant as climate warming alters the distribution, numbers and seasonal activity of arthropod vectors in the region<sup>32</sup>.



**Table 3. Parasites investigated as part of the Boreal Caribou Health Assessment Model in BCHRP Year 1.**

Pathogen	Brief Background and Potential Significance for Boreal Caribou in Northeastern British Columbia
<b>Protostrongylid Nematodes</b>	<p><b>1) Background:</b></p> <ul style="list-style-type: none"> <li>• Protostrongylids are nematode parasites found in the lungs, muscle, and nervous system of ungulates.</li> <li>• Species reported in <i>Rangifer</i> from North America include: <i>Parelaphostrongylus odocoilei</i>, <i>P. andersoni</i>, <i>P. tenuis</i>, <i>Elaphostrongylus rangiferi</i> (introduced in Newfoundland only), and <i>Varestrongylus eleguneniensis</i> <sup>19, 92-96</sup>.</li> <li>• Members of this group have characteristic dorsal-spined larvae (DSL) that may be found in the feces of infected animals <sup>19</sup>.</li> <li>• Protostrongylids have a complex lifecycles where an infected ungulate (definitive host) excretes DSLs in feces which then penetrate the foot of gastropods (intermediate hosts). In gastropods, DSLs develop to a third stage (L3) larvae that may infect a new definitive (ungulate) host when gastropods, or emerged L3, are passively ingested while foraging <sup>19</sup>.</li> <li>• The types of Protostrongylids and prevalence of infection found in caribou vary across the species distributional range <sup>19</sup>.</li> </ul> <p><b>2) Potential Significance:</b></p> <ul style="list-style-type: none"> <li>• For caribou, the consequences of infection with Protostrongylids may range from subclinical/mild to highly pathogenic /fatal depending on the intensity of infection, the age of host, and the species of parasite <sup>19, 92-96</sup>.</li> <li>• Subclinical effects on mobility and body condition as well as the cumulative effects of co-infections with other pathogens (or interactions with other factors) may be especially important <sup>19</sup>.</li> <li>• The species of Protostrongylids where adults inhabit the muscle or nervous system may be of particular importance for the health of boreal caribou in NE BC and include:</li> </ul>

a) *Parelaphostrongylus andersoni*

- Adult *P. andersoni* are found in the muscle of caribou and this parasite has been reported throughout species' North American range<sup>19, 92, 93</sup>.
- *Parelaphostrongylus andersoni* is often considered to cause subclinical to mild disease. However, in high intensity infections adults in skeletal muscle may cause severe pathology and larvae and eggs in the lungs may be cause severe pneumonia<sup>19, 92, 93</sup>.
- Subclinical effects on mobility and body condition may be particularly important<sup>19, 92</sup> and the cumulative effects of infection with *P. andersoni* and other pathogens (or interactions with other factors) may adversely affect the general health status of boreal caribou in NE BC.

b) *Parelaphostrongylus odocoilei*

- Adult *P. odocoilei* are typically found in the muscle of mule deer (*Odocoileus hemionus*) but have also been reported in caribou from westcentral AB<sup>19, 97</sup>.
- The effects of *P. odocoilei* on woodland caribou are not well characterized however this parasite is known to cause severe/fatal muscle and lung pathology in ungulates other than mule deer<sup>19, 97</sup>.
- It is probable that, like *P. andersoni*, clinical disease may occur in heavily infected animals while subclinical or cumulative effects on mobility, body condition, and general health may also be important<sup>19</sup>.

c) *Parelaphostrongylus tenuis*

- *Parelaphostrongylus tenuis* is the meningeal worm of white-tailed deer causing no ill effects in this species but often leading to fatal neurological disease in caribou and other cervids<sup>19, 94</sup>.
- Although unlikely, if *P. tenuis* occurs in white-tailed deer in NE BC it may be a direct (and indirect) cause of caribou mortalities in the region<sup>19, 94</sup>.
- Until recently *P. tenuis* was not believed to occur much further west than the MB/SK border<sup>98</sup>. However, *P. tenuis* was recently identified in a clinically ill moose from Northwestern, SK<sup>99</sup> which reflects a significant westward range expansion and indicates that this parasite may have the potential to become an important limiting factor for woodland caribou<sup>100</sup> in western Canada in the near future.

	<p>d) <i>Elaphostrongylus rangiferi</i></p> <ul style="list-style-type: none"> <li>• <i>Elaphostrongylus rangiferi</i> is an introduced Protostrongylid of European reindeer now endemic in caribou herds from Newfoundland<sup>19, 101</sup>.</li> <li>• This parasite inhabits central nervous system and is known to cause sporadic cases or epizootics of severe to fatal disease characterized by hind limb paresis in free-ranging caribou<sup>19, 101</sup>.</li> <li>• Although very unlikely, if <i>E. rangiferi</i> occurs in NE BC it may be a direct (and indirect) factor in caribou mortalities observed in the region<sup>19</sup>.</li> <li>• Importantly, the geographic distribution, prevalence, and intensity of Protostrongylid infections in boreal caribou from NE BC may increase as climate change supports alterations in the life cycle patterns of parasites and/or the incursion of other definitive hosts (e.g. white-tailed deer) into caribou habitat<sup>102-104</sup>.</li> </ul>
<p><b>Gastrointestinal Parasites</b></p> <p>↓</p> <p><b>Focus</b></p> <p><b>Abomasal Nematodes</b></p>	<p><b>1) Background:</b></p> <ul style="list-style-type: none"> <li>• A wide variety of nematode and cestode parasites have been recovered from gastrointestinal tracts or feces of Canadian caribou<sup>e.g. 45, 105</sup></li> <li>• <i>Ostertagia gruehneri</i> is the most common abomasal nematode in caribou and the most well studied gastrointestinal parasite in this species<sup>e.g. 5, 45, 105, 106</sup>.</li> <li>• <i>Marshallagia marshalli</i> is the second most abundant abomasal nematode in caribou<sup>5, 107</sup>.</li> <li>• These nematodes have a direct life cycle and caribou are passively infected when they consume forage contaminated with L3<sup>5, 106, 107</sup>.</li> </ul> <p><b>2) Potential Significance:</b></p> <ul style="list-style-type: none"> <li>• Infection with abomasal nematodes may adversely affect boreal caribou survival and reproduction in NE BC. These parasites have been associated with diminished food intake, weight loss, and reduced pregnancy rates in free-ranging caribou herds<sup>20, 107-109</sup>. Infection with abomasal nematodes is also known to alter caribou grazing behaviour and may even contribute to caribou population cycles<sup>110, 111</sup>.</li> <li>• The cumulative effects of (or interactions between) infections with abomasal nematodes and other parasites, nutrition etc. may also be important.</li> </ul>

	<ul style="list-style-type: none"> <li>As for Protostrongylids, the prevalence, intensity, and relative importance of abomasal nematode infections for boreal caribou in NE BC may increase if climate change supports alterations in the life cycle patterns of these parasites that enhance transmission to caribou <sup>e.g. 106</sup>.</li> </ul>
<p style="text-align: center;"> <b>Trematodes</b>  ↓  <b>Focus</b>  <i>Fascioloides magna</i> </p>	<p><b>1) Background:</b></p> <ul style="list-style-type: none"> <li><i>Fascioloides magna</i> (the giant liver fluke) is a large trematode parasite which may be found in the liver and bile ducts of ungulates including white-tailed deer, elk (<i>Cervus elaphus</i>), moose and caribou <sup>112-114</sup>.</li> <li><i>Fascioloides magna</i> has a complex lifecycle where adults encysted in the liver of infected ungulate (definitive) hosts release eggs which are ultimately excreted in the feces <sup>114</sup>. In the environment, the eggs hatch and develop into a free swimming larval stage (miracidium) that penetrates a snail intermediate host <sup>114</sup>. In the snail, further development and asexual reproduction occurs culminating in the release of many motile larvae (cercariae) that leave the snail and encyst (as metacercaria) on vegetation. Ungulates are infected when they passively ingest metacercaria while foraging <sup>114</sup>.</li> <li>To date <i>F. magna</i> has been identified in caribou from Northern Quebec (prevalence 78%) <sup>113</sup>, and mountain caribou from AB <sup>115</sup>. Enzootic foci of infection are known to occur in elk and white-tailed deer (considered the typical definitive hosts for this parasite) in the Rocky Mountains and foothills of southwestern AB and southeastern BC <sup>116, 117</sup>.</li> </ul> <p><b>2) Potential Significance:</b></p> <ul style="list-style-type: none"> <li>Although it causes significant liver pathology in caribou no reports of morbidity or mortality due to <i>F. magna</i> have been reported in this species <sup>113, 118</sup>. Infected caribou shed <i>F. magna</i> eggs in their feces and function as a definitive host for this parasite <sup>118</sup>. Nonetheless, subclinical effects of <i>F. magna</i> infections have been reported in other cervid definitive hosts <sup>e.g. 119</sup> and it is probable <i>F. magna</i> causes similar problems in caribou.</li> <li>The risk of boreal caribou becoming infected with <i>F. magna</i> is greatest when their habitat overlaps with elk and/or white-tailed deer range <sup>114</sup> and may be increasing in NE BC as climate and landscape change facilitate an increase in the number of these species in caribou habitat.</li> <li>Like other parasites, the cumulative effects of (or interactions between) <i>F. magna</i> infections, other diseases, nutrition etc. may have the potential to adversely impact survival or reproduction in boreal caribou.</li> </ul>

	<ul style="list-style-type: none"> <li>• In moose, infection with <i>F. magna</i> may cause severe or fatal disease and the occurrence of this parasite in boreal caribou (or elk and deer) in NE BC could also adversely affect moose in the region <sup>120</sup>.</li> </ul>
<i>Besnoitia tarandi</i>	<p><b>1) Background:</b></p> <ul style="list-style-type: none"> <li>• <i>Besnoitia tarandi</i> is a protozoan parasite found in caribou throughout their distributional range (although much more common in barren-ground vs. woodland caribou) <sup>121, 122</sup>.</li> <li>• <i>Besnoitia tarandi</i> has a poorly characterized life cycle. Carnivores are believed to be the definitive hosts while caribou are thought to act as intermediate hosts and are infected when they passively ingest oocysts in soil, forage, or water contaminated by the feces of infected carnivores. Biting arthropods may also play an important role in transmission between caribou <sup>121</sup>.</li> <li>• The prevalence of <i>B. tarandi</i> varies widely across the distributional range of caribou. In BC, this parasite was identified in 23% (overall prevalence) of 320 caribou leg pairs examined in 1980s <sup>123</sup>. The prevalence of infection varied (0-63%) among caribou from different regions with the highest prevalence recorded in the North and Northwestern portions of the province <sup>123</sup>.</li> </ul> <p><b>2) Potential Significance:</b></p> <ul style="list-style-type: none"> <li>• Pathology caused by <i>B. tarandi</i> is related to the presence of intracellular cysts containing bradyzoites which may occur in a wide variety of tissues <sup>124</sup>.</li> <li>• The intensity of <i>B. tarandi</i> infections may be cumulative <sup>121</sup> and clinical disease is most often characterized by progressive thickening of dermis, hyperkeratosis, severe skin lesions, subcutaneous edema and hair loss. Bone lesions and severe orchitis have also been reported and diminished mobility, poor body condition, morbidity, and mortality have all been attributed to this parasite in free-ranging caribou <sup>121, 124, 125</sup>. Sterility may also be an important consequence of heavy infections in caribou bulls <sup>121</sup>.</li> <li>• Subclinical effects on growth and reproduction are also probable but have not been thoroughly investigated <sup>121</sup>.</li> <li>• Severe disease associated with <i>B. tarandi</i> has traditionally been considered to be a sporadic phenomenon <sup>121, 125</sup>. However, outbreaks of severe/fatal disease have been reported in captive <i>Rangifer</i> <sup>126</sup>. Reports of widespread and severe disease also appear to be increasing in free-ranging caribou populations <sup>121, 122</sup>. For example, <i>Besnoitia</i> emerged in the George and Leaf River caribou herds in the</li> </ul>

	<p>mid 2000s where it caused widespread disease and some mortalities<sup>121, 122, 127</sup>. Prior to this outbreak, <i>B. tarandi</i> had not been recorded in these caribou populations which may indicate a recent introduction, a higher susceptibility of the woodland ecotype, or both.</p> <ul style="list-style-type: none"> <li>• Recent studies suggest that caribou of the genetic lineage including the North American boreal ecotype may be naive and particularly susceptible to clinical disease caused by this parasite<sup>128, 129</sup>.</li> <li>• If <i>B. tarandi</i> occurs in NE BC it may adversely affect the survival and reproductive success of female caribou by leading to diminished condition, an enhanced risk of predation, or decreased overwinter survival<sup>121</sup>. The fitness of male caribou may also be reduced by these factors as well as sterility directly associated with cyst mediated damage to gonadal tissue<sup>121</sup>.</li> <li>• It is also possible that the risk of transmission (and the intensity of cumulative infections) may increase as climate change alters the number of insects or the patterns of insect activity in the region<sup>121</sup>.</li> </ul>
<i>Toxoplasma gondii</i>	<p><b>1) Background:</b></p> <ul style="list-style-type: none"> <li>• <i>Toxoplasma gondii</i> is a protozoan parasite with a felid definitive host (in caribou range most likely lynx<sup>130</sup>) and a wide variety of intermediate hosts including wild ungulates such as caribou<sup>131</sup>.</li> <li>• Intermediate hosts become infected with <i>T. gondii</i> after ingesting soil, water, or forage contaminated with oocysts shed in the feces of infected cats<sup>131</sup>. When ingested, oocysts develop into motile tachyzoites which migrate through the body of the intermediate host and ultimately localize in neural and muscle tissue where they develop into persistent tissue cysts containing bradyzoites<sup>131</sup>. Felids are most commonly infected after consuming intermediate hosts harboring tissue cysts<sup>131</sup>.</li> <li>• In the intermediate host, vertical transmission from mother to offspring may also occur in utero<sup>131</sup>.</li> <li>• The prevalence of exposure to <i>T. gondii</i> has been recorded ranging from 0 to 37% in barren-ground caribou from NT, NU, and Alaska<sup>22, 53, 132, 133</sup> and ~0.5% to 3% in boreal caribou from NT<sup>45</sup> and Quebec<sup>53</sup>. No evidence of exposure to <i>Toxoplasma gondii</i> was recorded in a serosurvey of 111 mountain caribou from BC in 2001<sup>117</sup>.</li> </ul> <p><b>2) Potential Significance:</b></p> <ul style="list-style-type: none"> <li>• <i>Toxoplasma gondii</i> causes a spectrum of diseases in intermediate hosts ranging from mild/sub clinical to severe/fatal and may include anemia, pneumonia, enteritis, hepatitis, and encephalitis<sup>131</sup>.</li> <li>• This parasite is also the causative agent of congenital defects, abortions, still births, and weak neonates</li> </ul>

	<p>in many species including caribou<sup>131</sup></p> <ul style="list-style-type: none"> <li>• To date, clinical disease associated with <i>T. gondii</i> has not been recorded in free ranging caribou<sup>22, 45, 53, 132, 133</sup>, however, acute, severe, fatal hemorrhagic enteritis and abortions have been recorded from experimental infections in this species<sup>23, 134</sup>.</li> <li>• Although the ecology and impact of <i>T. gondii</i> in free-ranging caribou are poorly characterized, this parasite may have distinct potential to cause both subclinical and severe/ fatal illness and/or reproductive failure if occurs in boreal caribou from NE BC.</li> </ul>
<i>Neospora caninum</i>	<p><b>1) Background</b></p> <ul style="list-style-type: none"> <li>• <i>Neospora caninum</i> is a protozoan parasite with a canid definitive host [in caribou range most likely wolf (<i>Canis lupis</i>), coyote (<i>Canis latrans</i>), or fox (<i>Vulpes vulpes</i>)] and a ruminant intermediate host<sup>135, 136</sup>.</li> <li>• Horizontal and vertical transmissions both play important roles in the infection and persistence of <i>N. caninum</i> in ungulate herds<sup>137</sup>.</li> <li>• Horizontal transmission occurs when an intermediate host ingests soil, water or forage contaminated with infective <i>N. caninum</i> oocysts shed in the feces of infected canids<sup>137</sup>. When ingested, oocysts develop into motile tachyzoites which migrate through the body of the intermediate host and ultimately localize in vascular, muscle, hepatic, uterine, or neural tissue where they develop into persistent tissue cysts containing bradyzoites<sup>137</sup>. Intermediate hosts may remain infected with <i>N. caninum</i> for life. Canids are most commonly infected after consuming intermediate hosts or host tissues (e.g. expelled placentas) harboring cysts<sup>137</sup>.</li> <li>• Vertical transmission of <i>N. caninum</i> may occur in two ways. If an ungulate is pregnant when it is infected by <i>N. caninum</i> for the first time trans-placental transmission of oocyte derived tachyzoites may also infect the fetus<sup>137</sup>. Alternately, the recrudescence of maternal persistent infections may occur during gestation. Importantly, infected females may transmit the infection to their offspring over several consecutive pregnancies or intermittently<sup>137</sup>.</li> <li>• Depending on the age of the fetus, both types of trans-placental infections may cause abortions (epidemic or endemic) <u>or</u> may result in the birth of un-infected calves, infected calves which are infected and unthrifty, or clinically normal but persistently infected calves<sup>137, 138</sup>. By propagating the infection to successive generations, the later route is believed to be the primary pathway contributing to the persistence of <i>N. caninum</i> infections in ungulate herds<sup>137, 138</sup>. However, horizontal transmission</li> </ul>

	<p>(from canid definitive hosts) is also believed to be necessary to spread the infection and maintain infection levels in ungulate herds<sup>137</sup>.</p> <ul style="list-style-type: none"> <li>• The prevalence of exposure to <i>Neospora caninum</i> has been recorded between 1 and 11% in free-ranging caribou<sup>22, 53, 139, 140</sup> including an overall prevalence of 6.2% (range 0-33% depending on conservation area) in mountain caribou from BC<sup>117</sup>. Antibodies against <i>N. caninum</i> were recorded in 13.6% of woodland caribou examined in the NT<sup>141</sup>. An exceptionally high prevalence of exposure (73%) has also been reported in captive reindeer from AB<sup>53</sup>.</li> </ul> <p><b>2) Potential Significance:</b></p> <ul style="list-style-type: none"> <li>• Although the ecology and significance of <i>N. caninum</i> in free-ranging caribou is poorly characterized, this parasite is suspected as a likely cause of abortions and unthrifty calves in free-ranging caribou<sup>22, 53, 140, 142</sup>.</li> <li>• If <i>N. caninum</i> is found in boreal caribou from NE BC, epizootic or endemic abortions and the production of unthrifty calves may adversely affect the reproductive success and recruitment of boreal caribou in the region.</li> <li>• Importantly, the persistent and trans-generational nature of <i>N. caninum</i> infections in ungulates also suggests that this parasite may limit the recovery of caribou populations even if it occurs at low levels.</li> <li>• White-tailed deer may be particularly important in the maintenance of this parasite and its transmission to definitive hosts such as wolves, coyotes, and foxes<sup>138</sup>. As such, the risk infection with <i>N. caninum</i> may be increasing as recent landscape and climatic change in NE BC facilitate an increase in the number of white-tailed deer and wolves in boreal caribou habitat. Changes in the number of other potential intermediate hosts (e.g. elk, moose) in NE BC may also have a similar (but currently unrecognized) effect.</li> </ul>
<p><b>Ectoparasites</b></p> <p>↓</p> <p><b>Focus</b></p> <p><b>Ticks and Flies</b></p>	<p><b>1) Background and Potential Significance:</b></p> <p>A variety of ticks and fly species may infest or infect caribou. The primary species which may be of importance for boreal caribou in NE BC are:</p> <p>a) Winter ticks (<i>Dermacentor albipictus</i>)</p>



- *Dermacentor albipictus* is an Ixodid tick typically associated with moose but which is also known to infect elk, white-tailed deer, mule deer, and woodland caribou <sup>143</sup>.
  - The winter tick is a one-host tick which completes its life cycle on a single ungulate host <sup>143</sup>. Larvae in the environment quest for and attach to ungulates beginning in fall (September to November). Development (larvae to nymphs to adult), repeated feedings (blood meals), and mating occur over winter and early spring<sup>143</sup>. Adult ticks drop off the ungulate host between late March and early May and female ticks lay eggs on the ground in early summer<sup>143</sup>. Larva hatch in summer but remain inactive until decreasing temperatures and photoperiod stimulate the search for a host thus beginning the life cycle again <sup>143</sup>.
  - *Dermacentor albipictus* infestations are irritating and excessive grooming may lead to significant hair loss in heavily infected ungulates <sup>144</sup>. Grooming behaviour may also interrupt foraging and together these responses may lead to a decrease in body condition and a diminished probability of overwinter survival in affected individuals <sup>144, 145</sup>. Heavy infestations may also cause anemia (due to blood loss) and winter ticks can carry and likely transmit microorganisms (e.g. *Anaplasma* sp.) that have the potential to cause severe/fatal disease in cervids <sup>145, 146</sup>. Epidemics of winter tick related disease and mortality are also known to occur in moose <sup>145</sup>.
  - Although the effects of *D. albipictus* infestations on caribou are not well characterized, emaciated caribou with extensive hair loss and heavy tick burdens have been observed in AB <sup>147</sup>.
  - There are also increasing reports of winter ticks and associated hair loss affecting boreal caribou from NE BC <sup>148</sup>.
  - Climate change leading to longer, drier, and warmer periods in autumn and earlier snowmelt in spring is predicted to improve conditions for winter ticks and may increase the risk of infestation and related disease in boreal caribou in NE BC in the near future <sup>143</sup>. Likewise, recent landscape change may also enhance the risk of winter tick transmission to caribou due to an increase in the number of moose inhabiting caribou range.
- b) Rocky mountain tick (*Dermacentor andersoni*)
- *Dermacentor andersoni* is a three-host Ixodid tick, where larvae, nymphs, and adults take a single blood meal from a different mammalian host <sup>149</sup>.
  - *Dermacentor andersoni* is a known vector of many wildlife pathogens and salivary neurotoxins produced by this tick species may cause tick paralysis in infested animals <sup>149</sup>.
  - Tick paralysis was recently recorded in a translocated woodland caribou that dispersed from southern

	<p>BC into the northern US<sup>150</sup>.</p> <ul style="list-style-type: none"> <li>• In BC, climate warming may facilitate a northward range expansion of this tick species<sup>149</sup> and an enhanced risk of disease transmission to, or tick paralysis in, boreal caribou.</li> </ul> <p>c) Warbles (<i>Hypoderma tarandi</i>)</p> <ul style="list-style-type: none"> <li>• <i>Hypoderma tarandi</i> is an Oestrid fly commonly reported in tundra caribou across their distributional range<sup>e.g. 21, 151, 152</sup>. This parasite also occurs in woodland caribou but may be less common.</li> <li>• In mid-summer, adult female <i>H. tarandi</i> lay eggs on the skin of the legs and lower body of caribou<sup>151</sup>. When the egg hatches, larvae penetrate the skin and burrow into the subcutaneous tissue where they grow and feed<sup>151</sup>. By late winter to early spring larvae are concentrated along the dorsal surface (spine and rump) of the infected host where they open a breathing hole in the skin<sup>151</sup>. In early summer fully developed larvae emerge from the host, fall to the ground, pupate and develop into adults beginning the life cycle anew<sup>151</sup>.</li> <li>• <i>Hypoderma tarandi</i> is considered one of the most important parasites of tundra <i>Rangifer</i><sup>21, 151-154</sup>. Heavy infections (1000+ larvae) have been reported in caribou<sup>21, 152</sup> and migrating and developing larvae may cause a local immune reaction, inflammation, and significant pathology in the skin and subcutaneous tissue<sup>151, 152</sup>.</li> <li>• 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<sup>153, 154</sup>.</li> <li>• Together avoidance behaviour and pathology may ultimately lead to a decrease in the body condition and reproductive success of adult caribou and diminished condition and overwinter survival in juvenile caribou<sup>21, 151-154</sup>.</li> <li>• <i>Hypoderma tarandi</i> larvae have been observed in live-captured boreal caribou from NE BC,<sup>42</sup> however the prevalence and intensity of infection have not been thoroughly investigated to date.</li> </ul> <p>d) Nasal Bots (<i>Cephenemyia trompe</i> = <i>C. nasalis</i> = <i>Oestrus trompe</i>)</p> <ul style="list-style-type: none"> <li>• <i>Cephenemyia trompe</i> is an Oestrid fly which is commonly reported in caribou across their range<sup>151, 155</sup></li> <li>• In fall, female <i>C. trompe</i> lay larvae on the face of caribou around the nostrils. Larvae then migrate into the mouth, nasal sinuses and throat where they grow throughout the winter<sup>151, 155</sup>. In the spring, larvae emerge from the host, fall to the ground, pupate, and develop into adults, beginning the life cycle anew<sup>151, 155</sup>.</li> </ul>
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	<ul style="list-style-type: none"> <li>• Infections with <i>C. trompe</i> are generally considered subclinical. However, heavy infections (&gt;50 larvae) can occur and may result in suffocation, local inflammation, and pneumonia which may ultimately lead to morbidity or mortality <sup>151, 155</sup>. As for, <i>H. tarandi</i>, avoidance of adult flies and an associated decrease in foraging efficiency may also contribute to reduced foraging efficiency and diminished body condition in caribou <sup>151, 155</sup>. Traditional Ecological Knowledge (TEK) indicates that this parasite often causes visible clinical signs (sneezing) and discomfort which may disrupt the resting patterns of migrating caribou <sup>115</sup>. Harassment by adults and/or the effects of larvae in the nasopharynx may also be associated with chronic physiological stress in this species <sup>156</sup>.</li> <li>• It is likely that <i>C. trompe</i> occurs in boreal caribou from NE BC. However the prevalence, intensity, and overall significance of infections have not been evaluated to date.</li> </ul>
<p><b>Arthropod Vectedored Blood Borne Pathogens</b></p> <p>↓</p> <p><b>Miscellaneous Blood Borne Pathogens</b> e.g. <i>Trypanosoma</i>, <i>Anaplasma</i>, <i>Babesia</i>, <i>Bartonella</i>, <i>Ehrlichia</i>, and <b>Vector Borne Nematodes</b> e.g. <i>Setaria</i>, <i>Onchocerca</i></p>	<p><b>1) Background and Potential Significance:</b></p> <p>a) Miscellaneous Blood Borne Protozoa and Bacteria</p> <ul style="list-style-type: none"> <li>• Arthropod vectored blood borne pathogens of the genera <i>Trypanosoma</i>, <i>Babesia</i>, <i>Anaplasma</i>, <i>Ehrlichia</i>, and <i>Bartonella</i> have been recorded in both captive and free-ranging cervids in North America <sup>157-160</sup>.</li> <li>• Many of these organisms are known or suspected to cause both subclinical and clinical disease syndromes which may adversely affect the survival and/or reproductive success of infected animals <sup>160-163</sup>.</li> <li>• New species of blood borne pathogens and extra-limital (or newly recognized) occurrences of known blood borne pathogens have also been recently identified in Canadian cervids (including in BC) <sup>159, 160</sup>.</li> <li>• To date, the occurrence, distribution, and impact of blood borne pathogens have not been thoroughly evaluated in free-ranging caribou. However, Trypanosomes have been previously identified in the blood of woodland caribou from Alberta (prevalence 84%) <sup>157</sup> and in caribou elsewhere <sup>5, 45</sup>. <i>Babesia sp.</i> have recently been associated with the deaths of captive woodland caribou in the Northern USA <sup>161, 163</sup>.</li> <li>• As in other ungulates, blood borne pathogens may have the potential to cause a range of negative effects in boreal caribou. The importance of arthropod vectored blood borne pathogens for boreal caribou in NE BC is likely to increase as climate change supports the northward migration of vectors and/or the incursion of known disease carriers (e.g. white-tailed deer) into caribou range <sup>32</sup>.</li> <li>• New or previously unrecognized species of blood borne pathogens may also exist in boreal caribou.</li> </ul>

b) Vector Borne Nematodes; *Setaria* sp. and *Onchocerca* sp.

- Filarid nematodes of the genus *Setaria* are found as adults in the body cavities of a variety of ungulates in North America including caribou <sup>164</sup>. Adult females release microfilaria (larvae) that enter the blood stream where they are picked up and transmitted to a new host by blood feeding arthropods <sup>164</sup>. In heavy infections, migration and maturation of larvae may be associated with significant inflammation and tissue damage <sup>165</sup>.
- *Setaria tundra* has recently emerged as an important cause of epizootics of severe/fatal peritonitis in Scandinavian reindeer where outbreaks may occur following two consecutive warm summers <sup>165</sup>.
- *Setaria yehi* has also been associated with chronic peritonitis in Alaskan reindeer <sup>166</sup> and evidence of similar pathology likely caused by a *Setaria* sp. has recently been observed in captive caribou from NE BC <sup>167</sup>.
- The distribution, occurrence, and impact of *Setaria* sp. in boreal caribou from NE BC are unknown. However, as witnessed in Fennoscandia, *Setaria* infections may have severe impacts on *Rangifer* and establishing the status of this parasite in boreal caribou is important <sup>165</sup>.
- Moreover, the distribution, prevalence, intensity, and impact of *Setaria* infections in boreal caribou from NE BC may increase as climate change supports an increase the number of arthropod vectors and/or the seasonal duration of arthropod activity in the region <sup>165</sup>.
- Similar considerations may apply for *Onchocerca* sp., another Filarid nematode which may have the potential to cause severe disease in caribou and other free-ranging cervids <sup>reviewed 168</sup>.

**Table 4. Other health indices investigated as part of the Boreal Caribou Health Assessment Model in BCHRP Year 1. Indices were selected to provide an enhanced understanding of both the general health of boreal caribou in NE BC and/or biological mechanisms potentially influencing herd level health status.**

Health Parameter	Brief Background and Potential Significance for Boreal Caribou in Northeastern British Columbia
<p><b>Organ Function and General Health Status</b></p> <p>↓</p> <p><b>Serum Biochemistry and Complete Blood Counts (CBCs)</b></p>	<p><b>1) Background and Potential Significance:</b></p> <p>a) Serum Biochemistry</p> <ul style="list-style-type: none"> <li>• 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 <sup>e.g. 45</sup></li> <li>• This information may be particularly useful for the interpretation of pathogen based health testing results in individual caribou and for evaluating the general (baseline) herd level health status of boreal caribou in NE BC <sup>e.g. 45</sup>.</li> </ul> <p>b) Complete Blood Counts (CBCs)</p> <ul style="list-style-type: none"> <li>• Complete blood counts (CBCs) measure the numbers, types and morphology of 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 <sup>e.g. 169</sup>.</li> <li>• Like serum biochemistries, CBCs may be particularly useful for interpreting pathogen based health testing results in individual caribou and for evaluating the general health status of boreal caribou herds in NE BC <sup>e.g. 169</sup>.</li> </ul>

<p style="text-align: center;"> <b>Chronic Physiological Stress</b>  ↓  <b>Hair Cortisol Concentration</b> </p>	<p><b>1) Background and Potential Significance:</b></p> <ul style="list-style-type: none"> <li>• Full health assessments in wild animals are logistically challenging, expensive, and ideally require post mortem examinations. Practical, reliable, validated, and preferably minimally invasive biomarkers of health that effectively and consistently predict the health status of caribou are highly desirable as population level management (monitoring) tools. Integrated indices of health which may reflect both the general health status of individual caribou and biological mechanisms that may drive health related impacts on caribou populations (e.g. physiological stress, immunity, and nutritional status) may be especially informative.</li> <li>• Chronic physiological stress is increasingly recognized as a factor which may contribute to diminished health in free-ranging wildlife<sup>reviewed 156</sup> and a mechanistic linkage between chronic stress and diminished growth, immunity, reproduction, and survival is recognized in many species<sup>reviewed 156</sup>.</li> <li>• In wildlife, chronic stress may reflect the cumulative effects of natural stressors [e.g. predation risk, nutrition, life history demands (e.g. lactation), parasitism, weather, etc.] and human activity or other anthropogenic landscape attributes<sup>reviewed 156</sup>.</li> <li>• Chronic stress has also been demonstrated as a key driver of population dynamics in some free-ranging wildlife populations<sup>170-172</sup>.</li> <li>• The measurement of corticosteroids (e.g. cortisol, corticosterone) in hair is a rapidly emerging technique in wildlife health studies which has been previously validated in caribou/reindeer<sup>reviewed 156, 173, 174</sup> and may represent the best integrated measure of chronic physiological stress currently available.</li> <li>• Preliminary studies also suggest that hair cortisol concentration in caribou may be associated with parasite burden, diminished body condition, and anthropogenic landscape features in caribou habitat<sup>156, 174</sup>.</li> <li>• As such, hair cortisol concentration may represent an informative index of the general health status of individual boreal caribou in NE BC. It may also offer insight into biological mechanisms potentially linking recent landscape change with the reduced performance of boreal caribou populations in the region. Moreover, hair can be collected as a minimally invasive sample from live-captured caribou or can be easily obtained from caribou mortality sites<sup>reviewed 156</sup> which suggest that this technique may represent a particularly useful tool to monitor boreal caribou health now or in the future.</li> </ul>
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<p style="text-align: center;"><b>Immune Status</b> ↓ <b>Acute Phase Proteins</b> <b>e.g. Haptoglobin,</b> <b>Serum Amyloid A</b> <b>(SAA)</b> <b>and</b> <b>Genetic Markers of</b> <b>Immunity</b></p>	<p><b>1) Background and Potential Significance:</b></p> <p>a) Acute Phase Proteins</p> <ul style="list-style-type: none"> <li>• The first reaction of the body to invading pathogens (or to other factors such as injury, tumors, and immune disorders) is an innate, non-specific response called the acute phase response (APR)<sup>175</sup>. As part of the APR inflammatory cytokines released into the circulation at the site of insult travel to the liver where they stimulate the production and release of acute phase proteins (APPs) into the circulation<sup>175</sup>.</li> <li>• APPs such as haptoglobin and serum amyloid A (SAA) are important components of the APR and 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<sup>175-177</sup>.</li> <li>• 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 ruminants<sup>e.g. 178-183</sup>. As such, haptoglobin and SAA have been proposed as useful indicators of both the occurrence and severity of pathological conditions in these species.</li> <li>• Elevated haptoglobin levels have been used successfully as a screening tool to identify potentially sick free-ranging caribou in Alaska for further disease (Brucellosis) testing<sup>180, 181</sup> and may also be related to diminished body condition in this species<sup>180, 181</sup>. Likewise, increases in SAA levels have been recorded in association with the experimental administration of bacterial endotoxins and natural respiratory infections in captive reindeer<sup>182, 183</sup>.</li> <li>• All considered, haptoglobin and SAA may represent useful indicators of the general health status of boreal caribou in NE BC and may also serve as practical tools to monitor caribou health in the region now or in the future.</li> </ul> <p>b) Genetic Markers of Immunity</p> <ul style="list-style-type: none"> <li>• MHC (Major Histocompatibility Complex) molecules are essential components of the immune response that present antigens from invading pathogens to T-cells and control the cell and antibody mediated immune response<sup>184-186</sup>.</li> <li>• Variations in MHC alleles have been found to be associated with resistance to some pathogens and</li> </ul>
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	<p>survival in a variety of species including free-ranging ruminants<sup>e.g. 187-190</sup>.</p> <ul style="list-style-type: none"> <li>Genetic MHC markers have been developed for caribou<sup>191</sup> and may hold significant potential as a practical tool to evaluate relationships between the immune status, health, and fitness of boreal caribou in NE BC. The utility of this approach is further highlighted by the fact that analytical techniques are validated for use with genetic material obtained from fecal samples that can be collected non-invasively (i.e. without any direct contact with animals) from free-ranging caribou<sup>191</sup>.</li> </ul>
<p><b>Nutrition and Nutritional Status</b></p> <p>↓</p> <p><b>Body Condition Bone Marrow Fat Content Trace Nutrient Levels and Indices of Energy Balance</b></p>	<p><b>1) Background and Potential Significance:</b></p> <p>a) General</p> <ul style="list-style-type: none"> <li>The nutritional status of free-ranging ungulates is closely related to their general health status, fitness, and population performance<sup>e.g. 192-194</sup>.</li> <li>In the context of objectives related to the BCHRP, comparative evaluation of the body condition, body fat content (bone marrow)<sup>28, 29, 195, 196</sup> of live and dead boreal caribou (as well as caribou affected by various pathogens) will provide insight into the general health of individual boreal caribou and caribou herds in NE BC as well as potential biological mechanisms which may have been related to the higher than expected mortality of caribou observed in the region between December 2012 and July 2013 or may drive health related impacts on caribou populations. In domestic and free-ranging ungulates, trace nutrient levels are critically important (but often overlooked) determinants of immunity, health, growth, reproductive output, and survival<sup>e.g. 120, 197-203</sup> and warrant investigation in boreal caribou from NE BC.</li> </ul> <p>b) Assessment and Monitoring Tools</p> <ul style="list-style-type: none"> <li>An accurate and reliable serum based index of nutritional status would be a useful research and monitoring tool for boreal caribou in NE BC and elsewhere.</li> <li>The scope of available ancillary health and condition data collected (and to be collected) in the BCHRP may provide a unique opportunity to explore two potential biomarkers of nutritional status that have not been previously evaluated in caribou.</li> <li>Circulating levels of non-esterified fatty acids (NEFA) and beta-hydroxybutyrate (BHBA) are related to energy balance in domestic ruminants<sup>204, 205</sup> and elevated NEFA and BHBA levels in cattle are associated with an increased risk of clinical disease, decreased reproductive performance, and</li> </ul>



	<p>diminished milk yield<sup>e.g. 205-207</sup>.</p> <ul style="list-style-type: none"> <li>Increasing NEFA and BHBA levels may also provide evidence of subclinical nutritional stress before clinical manifestations are apparent<sup>204</sup>.</li> <li>All considered, NEFA and BHBA may represent useful indicators of the general health status of boreal caribou in NE BC and may also serve as practical tools to monitor caribou health in the region now or in the future.</li> </ul>
<b>Toxicology</b>	<p><b>1) Background and Potential Significance:</b></p> <ul style="list-style-type: none"> <li>A wide variety of environmental contaminants have been recorded in the tissues of free-ranging Canadian caribou<sup>e.g. 208-211</sup> and tissue levels of some contaminants in caribou are occasionally higher than those considered normal in domestic ruminants<sup>208</sup>.</li> <li>To date, no evidence of acute or chronic clinical disease directly related to toxins has been reported in free-ranging Canadian caribou<sup>e.g. 208-211</sup> and the levels of potential toxins in boreal caribou from NE BC are not well known.</li> <li>The subclinical effects of toxins are poorly understood in caribou and could be relevant in the context of cumulative effects on immunity, the stress response, and the overall health status of boreal caribou in NE BC<sup>118, 208</sup> For example, recent reports suggest that Cd and Se levels may be related to the intensity of <i>F. magna</i> infections in Quebec caribou<sup>118</sup>.</li> <li>Measuring toxins in boreal caribou from NE BC will also provide valuable baseline data for future monitoring programs.</li> </ul>

### 3.3 Pathogen and Health Testing

The BCHRP's health testing efforts are 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. UCVM and the CWHC are internationally recognized centres of expertise in research and diagnostics related to the health of free-ranging Northern ungulates and other wildlife. A primary goal of the BCHRP is to find and use the most up to date and innovative methods of evaluating the health of caribou with an aim to identify less costly, less invasive, less complicated and more efficient and accurate indicators of caribou herd health. As such, the BCHRP also partnered with a network of academic and commercial laboratories in Canada, the United States, and Europe using both established testing protocols and innovative techniques to study health and disease in free-ranging wildlife.

A variety of enzyme-linked immunosorbent assays (ELISAs) and molecular (e.g. PCR, 16S sequencing, full genome sequencing) techniques along with general and selective bacterial culture protocols were employed as part of pathogen and health testing efforts in BCHRP Year 1 (Table 5). When they existed, The BCHRP focused on utilizing diagnostic tests previously evaluated and/or validated for use in caribou or reindeer. Where tests for targeted pathogens or other health indices had not been thoroughly evaluated in caribou (as is the case for many diagnostic tests for most wild species) technical advisors at collaborating laboratories were consulted to identify tests (most commonly for closely related pathogens of domestic ruminants) which due to the presence of similar antigens and immunological cross reactions would also have a high probability of success in caribou. In such cases, the approach to health testing also contained redundancy (e.g. results were confirmed by testing multiple samples from individuals and/or employing different assays in different collaborating laboratories) as well as the simultaneous testing (with blind submission) of known positive or negative controls. All diagnostic tests were performed by trained laboratory technicians, wildlife veterinarians, or board certified veterinary specialists.

**Table 5. Diagnostic tests employed for pathogen and health screening in BCHRP Year 1.**

<b>Pathogen or Health Index (Biological Sample Evaluated)</b>	<b>Diagnostic Test(s) Employed in BCHRP Year 1</b>	<b>Test(s) Previously Evaluated in Reindeer or Caribou? <sup>reference</sup></b>
Alphaherpesvirus (Serum)	LSIVet Bovine* IBR gB Blocking ELISA (Life Technologies Inc., Paris, France)	Yes <sup>212</sup>
Pestiviruses (Serum)	Synbiotics SERELISA BVD Kit (Synbiotics Corporation, Lyon, France)	Yes <sup>213</sup>
<i>Brucella suis</i> biovar 4 (Serum)	Protein A/G indirect ELISA for the detection of anti- <i>Brucella</i> antibodies (in house assay: Norwegian School of Veterinary Sciences, Tromsø, Norway)	Yes <sup>214</sup>
<i>Mycobacterium avium</i> ssp. <i>paratuberculosis</i> (Feces)	Quantitative PCR with a posteriori selective fecal culture (in house assays: University of Calgary Faculty of Veterinary Medicine and Canadian Wildlife Health Cooperative, Calgary, AB)	Yes <sup>71, 215</sup>
Miscellaneous Bacterial Pathogens (Tissue) ↓ Culture PCR 16S Sequencing Full Genome Sequencing	a) Direct PCR and selective tissue culture for <i>Erysipelothrix rhusiopathiae</i> followed by full genome sequencing of any <i>Erysipelothrix</i> isolates obtained (in house assays: University of Calgary Faculty of Veterinary Medicine and Canadian Wildlife Health Cooperative, Calgary, AB)	a) No <sup>#</sup>

Histopathology <sup>&amp;</sup>	b) General tissue culture (in house assays: Western College of Veterinary Medicine, Saskatoon, SK) employing: 1) aerobic culture on plain blood agar (a non selective medium), eosin methylene blue agar (a selective medium for gram negative organisms), colistin naladixic acid agar (a selective medium for gram positive organisms, and chocolate agar [a non selective medium to support growth of fastidious (hard to grow) microbes] followed by 16S sequencing to identify all bacterial isolates obtained; plus anaerobic culture on plain blood agar followed by 16S sequencing to identify all bacterial isolates obtained, 2) the selective culture protocol for <i>Erysipelothrix rhusiopathiae</i> used at UCVM , and 3) a selective (cold enrichment) culture protocol for the bacterial pathogen <i>Yersinia pseudotuberculosis</i>	b) No <sup>#</sup>
<i>Erysipelothrix rhusiopathiae</i> (Serum, Whole Blood, Carcass Fluids)	Indirect Protein A/G-HRP ELISA to detect antibodies against <i>Erysipelothrix</i> in muskox serum (in house assay: University of Calgary Faculty of Veterinary Medicine and Canadian Wildlife Health Cooperative, Calgary, AB)	No <sup>#</sup>
Protostrongylid Nematodes (Feces)	Fecal Baermann with a posteriori molecular (PCR) identification of dorsal-spine larvae (DSL) (in house assays: University of Calgary Faculty of Veterinary Medicine and Canadian Wildlife Health Cooperative, Calgary, AB)	Yes <sup>216</sup>
Abomasal Nematodes and Other Gastrointestinal Parasites	Fecal floatation with morphological identification of parasite eggs (in house assays: University of Calgary Faculty of Veterinary Medicine and Canadian Wildlife	Yes <sup>216</sup>

(Feces)	Health Cooperative, Calgary, AB)	
Trematodes ( <i>Fascioloides magna</i> ) (Feces)	Fecal sedimentation (Flukefinder®, Soda Springs, ID, USA) with morphological identification of fluke eggs (in house assays: University of Calgary Faculty of Veterinary Medicine and Canadian Wildlife Health Cooperative, Calgary, AB)	Yes <sup>216</sup>
<i>Besnoitia tarandi</i> (Serum)	Indirect ELISA for the detection of antibodies against <i>B. besnoiti</i> with a posteriori Western Blot under reducing conditions to detect specific antibodies against immunodominant antigens of <i>Besnoitia besnoiti</i> tachyzoites (in house assays: Complutense University, Madrid, Spain)	Yes <sup>217</sup>
<i>Toxoplasma gondii</i> (Serum)	ID Screen Toxoplasmosis Multispecies Indirect Elisa Kit (Innovative Veterinary Diagnostics, Grabels, France)	No <sup>#</sup>
<i>Neospora caninum</i> (Serum)	a) Indirect ELISA for the detection of antibodies against <i>N. caninum</i> with a posteriori Western Blot under reducing conditions to detect specific antibodies against immunodominant antigens of <i>N. caninum</i> tachyzoites (in house assays: Complutense University, Madrid, Spain)  b) Competitive ELISA for <i>Neospora caninum</i> antibody (Veterinary Medical Research and Development Inc., Pullman, Washington, USA).	a) Yes <sup>218</sup>  b) Yes <sup>53, 216</sup>

<p><i>Dermacentor albipictus</i> and Other Ectoparasites (Live or Dead Caribou)</p>	<p>Preliminary morphological identification of <i>Dermacentor albipictus</i> and other ectoparasites on live-captured caribou with collection of voucher specimens plus secondary (or confirmatory) morphological identification (in house assays: University of Calgary Faculty of Veterinary Medicine and Canadian Wildlife Health Cooperative, Calgary, AB) if required</p>	<p>Yes <sup>42</sup></p>
<p>Arthropod Vectored Blood Borne Pathogens (Whole Blood)</p>	<p>a) PCR for <i>Trypanosoma</i>, <i>Anaplasma</i>, <i>Babesia</i>, <i>Bartonella</i>, <i>Ehrlichia</i> in whole blood (in house assays: University of Calgary Faculty of Veterinary Medicine and Canadian Wildlife Health Cooperative, Calgary, AB)</p> <p>b) Knotts test for microfilaria in whole blood followed by a posteriori molecular (PCR) based identification of positive samples (in house assays: University of Calgary Faculty of Veterinary Medicine and Canadian Wildlife Health Cooperative, Calgary, AB)</p>	<p>a) No <sup>#</sup></p> <p>b) Yes <sup>115</sup></p>
<p>Chronic Stress (Hair)</p>	<p>Oxford EA-65 Cortisol Competitive EIA kit (Oxford Biomedical, Lansing, MI, USA)</p>	<p>Yes <sup>156, 173</sup></p>
<p>Haptoglobin (Serum)</p>	<p>a) Photometric test (in house assay: University of Guelph, Animal Health Laboratory, Guelph, ON)</p> <p>b) Tridelta Phase Colorimetric Haptoglobin Multispecies Assay Kit (Tridelta Development Ltd., Boonton, NJ, USA)</p>	<p>a) Yes <sup>219</sup></p> <p>b) Yes <sup>220</sup></p>

Serum Amyloid A (SAA) (Serum)	Tridelta Phase Serum Amyloid-A (SAA) Multispecies ELISA Kit (Tridelta Development Ltd., Boonton, NJ, USA)	Yes <sup>182, 183</sup>
Serum Biochemistry (Serum)	Bovine clinical diagnostic panel including photometric (+/- calculated) tests for: Calcium, Phosphorus , Ca:P Ratio, Magnesium, Sodium, Potassium, Na:K Ratio, Chloride, Carbon Dioxide, Anion Gap, Total Protein, Albumin, Globulin, Albumin: Globulin Ratio, Urea, Creatinine, Glucose, Cholesterol, Total Bilirubin, Conjugated Bilirubin, Free Bilirubin, Alkaline Phosphatase (ALP), Gamma-glutamyltransferase (GGT), Aspartate aminotransferase (AST), Creatine kinase (CK), and Glutamate dehydrogenase (GLDH), Non-esterified fatty acids (NEFA), Betahydroxybutyrate (BHBA) and haptoglobin (in house assays, University of Guelph, Animal Health Laboratory, Guelph, ON)	Yes <sup>219</sup>
Non-Esterified Fatty Acids (NEFA) (Serum)	Photometric test (in house assay: University of Guelph, Animal Health Laboratory, Guelph, ON)	No <sup>#</sup>
Beta-hydroxybutyrate (BHBA) (Serum)	Photometric test (in house assay: University of Guelph, Animal Health Laboratory, Guelph, ON)	No <sup>#</sup>
Trace Nutrient Status and Toxicology	a) Live caribou: serum-based evaluation of short-term trace nutrient and toxicology status (Be, Mg, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Sr, Mo, Cd, Sn, Sb, Ba, Tl, Pb, Bi, Hg,	a) Yes <sup>221</sup>

(Serum and Tissue)	<p>Vitamin A, Vitamin E) using High-Performance-Liquid-Chromatography (HPLC) (in house assays: Prairie Diagnostic Services Inc. Saskatoon, SK)</p> <p>b) Dead caribou: tissue (liver or kidney)-based evaluation of long-term trace nutrient and toxicology status (Be, Mg, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Sr, Mo, Cd, Sn, Sb, Ba, Tl, Pb, Bi, Hg, Vitamin A, Vitamin E) using High-Performance-Liquid-Chromatography (HPLC) HPLC (in house assays: Prairie Diagnostic Services Inc. Saskatoon, SK)</p>	b) Yes <sup>221</sup>
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\* The use of diagnostic tests designed for domestic ruminants is standard practice for health and disease testing in wild ungulates and is based on the presence of similar antigens on and immunological cross reactions between domestic and wild ungulate pathogens.

# Evaluation and/or validation of test(s) in caribou pursued as part of BCHRP.

& Histopathology will be performed on all tissues collected from dead caribou to evaluate and characterize pathology which may be due to infectious (viral, bacterial, parasitic) or other disease processes if the stage of sample decomposition permits.



## **4. Preliminary Results, Discussion, and Recommendations**

### **4.1 Considerations**

Pathogen and health testing results presented in Section 4.3 are those available as of December, 2014 and all results, interpretations, and recommendations presented therein should be considered preliminary. Ongoing work as well as additional research and in-depth analyses planned for BCHRP Years 2 and 3 (see Section 6) will provide greater understanding of specific pathogens as well as the relationships between pathogens, other health indices, caribou survival/reproduction, and larger scale (landscape level) factors necessary to critically evaluate the overall impact of health (and specific diseases) and more fully inform boreal caribou management initiatives in NE BC.

### **4.2 Terminology**

#### **4.2.1 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.

#### **4.2.2. 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). 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.

#### **4.2.3 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.

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.

## **4.3 Preliminary Results, Discussion, and Recommendations (Pathogens)**

### **4.3.1 Viruses**

#### **4.3.1.1 Alphaherpesvirus**

The overall prevalence of exposure to alphaherpesvirus among adult female boreal caribou captured in NE BC in 2012 and 2013 was 62% (n=101/162). However, the prevalence of exposure varied across NE BC with the highest (86%) (n=6/7) recorded in the Parker herd range [vs. Calendar 59% (n=16/27), Chinchaga 69% (n=35/36), Maxhamish 59% (n=16/27), Prophet 22% (n=2/9), and Snake-Sahtaneh 64% (n=36/56)].

With the exception of animals from the Prophet herd range, the prevalence of exposure to alphaherpesvirus recorded in boreal caribou from NE BC was higher than that recorded in other woodland caribou herds from NT (37.5%)<sup>45</sup>, AB (52%)<sup>46</sup>, and SK (55%)<sup>47</sup>. Although a larger sample size and broader analyses are necessary to fully interpret these findings, the relatively high and variable prevalence of exposure to alphaherpesvirus recorded in boreal caribou from NE BC may suggest that factors (e.g. chronic stress) influencing alphaherpesvirus transmission may be relatively prominent in this region and may also vary as a function of landscape level features encountered by different caribou herds. Given its potential to compromise the survival and reproductive success of caribou (see Section 3.2, Table 1), the exceptionally high prevalence of alphaherpesvirus recorded in the Parker herd range (population estimate n=13<sup>1</sup>) could be of particular concern. More research into the occurrence, distribution, and impact of alphaherpesvirus in boreal caribou from NE BC is warranted.

In 2014/2015 selected tissues will be obtained from caribou mortality sites (as available) and minimally invasive ocular, nasal and genital swabs will be collected from live boreal caribou (already being captured as part of ongoing collaring programs) in order to support the molecular identification of the specific alphaherpesvirus that occurs in boreal caribou from NE BC. Alphaherpesvirus will also be incorporated into research activities planned for BCHRP Years 2 and 3 which will 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 that are necessary to critically evaluate the overall impact of health and more fully inform boreal caribou management initiatives in NE BC (see Section 6).

#### **4.3.1.2 Pestiviruses**

The prevalence of exposure to pestiviruses among adult female boreal caribou captured in NE BC in 2012 and 2013 was 0.6% (n=1/161). The single seropositive animal was captured in the Maxhamish herd range in December, 2012. To our knowledge, this is the first record of exposure to pestiviruses in boreal caribou from NE BC.

Although n=109/161 (68%) of caribou were found to be seronegative, the serostatus of n=51/161 (32%) of caribou was unclear. In the ELISA employed for pestivirus testing in BCHRP Year 1, results were determined by comparing the relative binding success of anti-pestivirus antibodies in serum (test samples) and a positive control containing specific antibodies against the protein (antigen) from bovine viral disease virus (BVDV, a pestivirus of domestic cattle) bound to the wells of the test kit. Although this test has been used successfully in reindeer<sup>213</sup>, significant genetic and antigenic variability is a characteristic of ruminant pestiviruses both within species and among different species<sup>222, 223</sup>. It is probable that the pestivirus occurring in boreal caribou is antigenically different from BVDV and thus relatively poor specificity for the BVDV protein (antigen) coating the wells of the kit contributed to a relative decrease in the binding of serum antibodies (from the test samples) and the high number of uncertain results. Given considerations outlined in Section 3.2, Table 1, more research into the occurrence, distribution, and impact of pestiviruses on boreal caribou from NE BC is warranted. As a first step, an alternate assay will be employed in BCHRP Year 2 to re-evaluate serum samples collected from adult female boreal caribou in 2012 and 2013.

#### **4.3.2 Bacteria**

##### **4.3.2.1 *Brucella suis* biovar 4**

There was no evidence that any adult female boreal caribou captured in NE BC in 2012 and 2013 had been previously exposed to *Brucella* sp. (n=162/162 were seronegative). These findings suggest that *Brucella suis* biovar 4 is unlikely to occur in boreal caribou in NE BC at the present time. This is important baseline knowledge. Given the potential impact of *B. suis* biovar 4 for caribou (and other ungulates) *Brucella* testing (monitoring) will continue to as part of research efforts planned for BCHRP Years 2 and 3. The BCHRP working group also recommends that that personnel involved in mortality site investigations, the capture and handling of boreal caribou, or other field-based activities in NE BC remain vigilant for clinical signs associated with this disease (e.g. swollen joints) in caribou<sup>69</sup>.

##### **4.3.2.2 *Mycobacterium avium* ssp. *paratuberculosis* (MAP, Johne's, Paratuberculosis)**

The quantity of archived feces available from each caribou captured in 2012 and 2013 was highly variable and was a limiting factor in the allocation (and prioritization) of fecal samples for specific diagnostic tests. The accurate diagnosis of MAP in previously frozen (archived) fecal samples collected from free-ranging wildlife is difficult<sup>215</sup> and given the low prevalence of suspected infections in adjacent jurisdictions<sup>71</sup> and a lack of clinical signs associated with MAP (wasting and diarrhea) in boreal caribou captured in 2012 and 2013 this pathogen was deemed to be of a lower priority by the BCHRP working group in Year 1. However, quantitative PCR was used to evaluate fecal samples for MAP in a test group of n=36 adult female boreal caribou captured in NE BC in 2012 and 2013 for which an ample quantity of archived feces was available. This group included the single caribou noted to have “soft feces” at the time of capture as well as a selection of caribou which were visibly “healthy” at the time of capture, in poor body condition at the time of capture, exhibited relatively high haptoglobin levels (a nonspecific index of inflammation), or that died during the high mortality period occurring from December 2012 to July 2013. All samples tested negative and MAP will not be

directly considered as part of BCHRP research efforts in Year 2 and 3. Nonetheless, the ecology and impact of MAP in free-ranging caribou is poorly understood and the risk of MAP transmission should remain a consideration for management programs involving the translocation or confinement (e.g. maternal penning programs) of boreal caribou. As such, the BCHRP working group recommends that targeted MAP testing is still implemented in the feces of boreal caribou in particularly poor body condition or exhibiting signs of diarrhea (e.g. soft feces, fecal staining). Moreover, tissue samples that may support the molecular identification of this pathogen and histopathological diagnosis of related disease (e.g. mesenteric lymph nodes, sections of the gastrointestinal tract) should be considered important targets for collection from dead boreal caribou in NE BC whenever they become available.

#### 4.3.2.3 Miscellaneous Bacterial Pathogens (Tissues from Caribou Mortalities)

Testing for miscellaneous bacterial pathogens in tissue collected from dead boreal caribou in 2012 and 2013 is ongoing and involves methods reviewed in Section 3.3, Table 5. Highlights of results available as of December, 2014 are presented below.

Using PCR and selective culture protocols we identified the bacterial pathogen *Erysipelothrix rhusiopathiae* in the tissues of n=5 dead boreal caribou examined during the course of field operations in 2013 (Table 6).

Infection with *E. rhusiopathiae* may lead to a bacterial septicemia and can cause chronic disease (e.g. endocarditis, arthritis), subacute illness, and acute or per acute death in domestic and wild ungulates<sup>79, 224</sup>. Abortions caused by *E. rhusiopathiae* have also recently been recorded in sheep<sup>225</sup>. The ecology of *E. rhusiopathiae* in free-ranging wildlife is poorly understood however outbreaks of disease have been reported in a variety of species and infection is believed to occur primarily through ingestion of the bacteria<sup>79, 224</sup>. In some species, some animals carry *E. rhusiopathiae* in their tonsils (or other lymphoid tissues), pharynx, bile, and muscles without developing clinical disease<sup>79, 224</sup>. Bacteria secreted in the feces of these “healthy” carriers contaminate the environment (e.g. soil, water, fomites) and are believed to be of primary importance in the transmission of infections to naive hosts<sup>79, 224</sup>. Clinically ill animals also shed large numbers of bacteria in their feces, urine, and saliva which may enhance transmission during disease outbreaks<sup>79, 224</sup>. *E. rhusiopathiae* may also be transmitted by biting arthropods (insects, ticks, mites) and through the contamination of wounds<sup>79, 224, 226</sup>.

Although the pathogenesis of *E. rhusiopathiae* is unclear, multiple serotypes (strains) of the bacteria 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)<sup>e.g. 227-230</sup>. Clinical disease caused by *E. rhusiopathiae* is often associated with “stress” (e.g. environmental stressors, co-infections with other pathogens) or compromised immunity and may occur in both asymptomatic carriers and newly infected hosts<sup>79, 224</sup>. *E. rhusiopathiae* infections are also known to be transmitted between different species<sup>79, 224</sup>.

*Erysipelothrix rhusiopathiae* may be a pathogen of emerging importance for northern ungulates and has recently been identified as the agent most likely to be responsible for large

scale disease outbreaks and mortality events (which may be associated with recent population level declines) in free-ranging muskoxen in the Canadian Arctic <sup>87</sup>. *E. rhusiopathiae* has also been recorded as the cause of severe disease or death in free-ranging deer and moose <sup>88, 231</sup> and may have been responsible for a historical (1930's) outbreak of severe/fatal disease in semi-domesticated Scandinavian and Russian reindeer <sup>89</sup>. To our knowledge, BCHRP findings represent the first record of this pathogen in free-ranging caribou in North America and may provide important insight into the potential role of health in the unexpectedly high number of boreal caribou mortalities observed in NE BC between December 2012 and July 2013.

PCR and/or culture positive animals represented 63% (n=5/8) of boreal caribou mortalities examined in 2012 and 2013 from which usable tissue samples (for health and disease testing) were obtained and included n= 4 SCEK collared caribou found dead and an un-collared yearling male caribou found dying in NE BC in 2013 (Table 6, Fig. 2).

**Table 6. *Erysipelothrix rhusiopathiae* PCR, and selective tissue culture results for n=12 boreal caribou (*Rangifer tarandus caribou*) from Northeastern BC, Canada that died in 2013 and were examined in BCHRP Year 1. PCR and/or culture positive animals are highlighted in yellow.**

<b>Caribou Identification Number</b>	<b>Herd Range</b>	<b>Date Mortality Investigated and Suspected Cause of Death*</b>	<b><i>Erysipelothrix rhusiopathiae</i> PCR Status</b>	<b><i>Erysipelothrix rhusiopathiae</i> Culture Status</b>
Un-collared 545M	Snake-Sahtaneh	April 2, 2013 Undetermined (~ intact carcass)	Negative**	Positive
Un-collared 198M	Snake-Sahtaneh	February 25, 2013 Wolf kill	Negative	Negative
SK011	Parker	September 15, 2013 Wolf Kill	Positive	Positive
SK022	Snake-Sahtaneh	July 28, 2013 Wolf kill	No sample (dry)	No sample (dry)
SK039	Chinchaga	May 16, 2013 Undetermined (possible road kill)	Negative <sup>#</sup>	Negative <sup>#</sup>
SK069	Snake-Sahtaneh	May 24, 2013 Undetermined	Negative**	Positive
SK072	Snake-Sahtaneh	September 7, 2013 Undetermined (suspect died/scavenged)	No sample (dry)	No sample (dry)
SK075	Snake-Sahtaneh	July 28, 2013 Undetermined (~ intact carcass)	Negative <sup>&amp;</sup>	Negative <sup>&amp;</sup>
SK106	Calendar	July 28, 2013 Undetermined (suspect died/scavenged)	Positive	Positive
SK117	Calendar	July 28, 2013 Undetermined	No sample (dry)	No sample (dry)
SK133	Calendar	July 28, 2013 Undetermined (suspect died/scavenged)	Positive	Positive

SK159	Chinchaga	September 10, 2013 Wolf kill	No sample (dry)	No sample (dry)
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\* As determined during field-based mortality site investigation.

\*\* PCR false negatives (where PCR result is negative and culture of same sample is positive) are likely attributed to difficulties noted in processing and extracting DNA from caribou tissue (especially bone marrow). PCR inhibitors in autolyzed tissue may also have been important in some samples <sup>232</sup>.

# Marginal sample (~dry) but tested

& Poor quality sample (significant autolysis) but tested

# and & The success of the selective culture protocol employed in this study depended on the presence of live *E. rhusiopathiae* in tissue samples and is likely to have been reduced by environmental exposure, putrefaction, and sample storage conditions (especially freezing > 3 months <sup>233, 234</sup>) in some cases. As such, highly suspect animals (especially those found dead and intact in late spring or summer) that cultured negative are not necessarily *E. rhusiopathiae* free (e.g. SK075).



Figure 2. Preliminary Case Report: *Erysipelothrix rhusiopathiae* in an un-collared yearling male boreal caribou (*Rangifer tarandus caribou*) found moribund in the Snake-Sahtaneh herd range of Northeastern BC in March, 2013. On March 30, 2013 an un-collared yearling male boreal caribou was observed (from the air) alive but moribund in close proximity to a group of caribou containing collared animals. The animal 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. Despite some scavenging, the carcass was relatively intact and there was no indication of predator attack (ante mortem wounding etc.)<sup>44</sup>. These findings are consistent with death related to infectious disease. *Erysipelothrix rhusiopathiae* was cultured from multiple organs (femur marrow, lung, liver, and skeletal muscle) providing preliminary evidence that this bacterial pathogen may have been the cause of death in this animal (histopathology results pending as of December, 2014). Preliminary (gross<sup>235, 236</sup>) evaluation of tissue and bone marrow fat suggested that this caribou was in poor to moderate condition (bone marrow fat content analysis pending as of December, 2014) while 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. Photo Credit: D. Culling and B. Culling, Diversified Environmental Services Inc., Fort St. John, BC.



In free-ranging wildlife, moribund animals are rarely observed due to their quick removal by predators and scavengers and when found to test positive for certain disease agents may represent the “tip of the iceberg” and point to the occurrence of a broader disease outbreak. Although additional research is required, the relatively high number of PCR and/or culture positive caribou (including the rare finding of a moribund animal) identified in BCHRP Year 1 suggests that an outbreak of disease caused by *Erysipelothrix rhusiopathiae* could have been a factor in the relatively high number of caribou mortalities recorded NE BC in 2013. This hypothesis may also be supported by the retrospective identification of n=7 boreal caribou mortalities from 2013 that should be considered suspect cases (i.e. where carcasses were relatively intact with no sign of predation and/or death occurred in late spring and summer) but for which no tissue samples were available<sup>237</sup>.

Changes in serum antibody titres and/or the prevalence of exposure to a pathogen over time can help to confirm the occurrence of disease outbreaks in wildlife. As a follow up to tissue-based findings, the BCHRP also employed a novel ELISA (originally developed for muskoxen) 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). Approximately 30% of caribou captured in 2012/13 and 2013/2014 had been previously exposed to this pathogen (were seropositive). Seroprevalence often increases after an outbreak of infectious disease. However, given that it is likely many caribou infected with *E. rhusiopathiae* may die (or are killed by predators) before antibodies can be produced, this finding neither supports nor refutes the possible occurrence of an *E. rhusiopathiae* outbreak in 2013. Nonetheless, this pattern may suggest that exposure to *E. rhusiopathiae* was relatively common among SCEK boreal caribou in 2012/2013 (and 2013/2014) and that some caribou may be exposed to this pathogen and recover and/or may carry subclinical infections. Moreover, the prevalence of exposure to *Erysipelothrix* 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)] which could indicate that factors (e.g. chronic stress, immune status, inter-specific carriers, risk) influencing *Erysipelothrix* transmission or the occurrence of clinical disease may vary as a function of landscape level features encountered by different herds.

Seropositive (SK069, SK133, Table 6) and seronegative (SK011, SK106, Table 6) caribou were identified among PCR/culture positive mortalities and preliminary (gross) analysis of bone marrow fat (using CARMA scores<sup>235, 236</sup>) indicated that some positive caribou had been in good condition while others were in relatively poor condition prior to death (detailed bone marrow fat content analysis pending as of December, 2014). Together these findings may indicate that both chronic and acute disease processes related to *E. rhusiopathiae* are acting on caribou in NE BC. Moreover, *E. rhusiopathiae* was cultured from the tissues of a caribou (SK011, Table 6) believed to have been killed by wolves, which may indicate that this pathogen could have the potential to enhance predation risk.

All considered, evidence gathered in BCHRP Year 1 suggests that *E. rhusiopathiae* may have played an important role in the higher than expected mortality of SCEK boreal caribou recorded in NE BC in 2013 (either as a direct cause of death or indirectly as a cause of morbidity and increased predation risk). If true, this pathogen could threaten the long-term sustainability of boreal caribou in the region. Nonetheless, more research is required to improve our

understanding of the source, ecology and potential impact of *E. rhusiopathiae* in boreal caribou before management initiatives in NE BC can be more fully informed.

*Erysipelothrix rhusiopathiae* will be a major focus of BCHRP Years 2 and 3 and research efforts will include continued (and enhanced) evaluation of serum and tissue samples obtained from live-captured and dead boreal caribou as well as an experimental study to explore the timing and duration of the *E. rhusiopathiae* specific antibody response in *Rangifer* (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 or spatial (landscape level) factors which may influence the transmission of *E. rhusiopathiae* and/or the occurrence of disease caused by this pathogen will also be initiated. The full genomes of *E. rhusiopathiae* isolates obtained from culture positive boreal caribou will also be sequenced to explore the relationship between isolates from caribou and those obtained from other species.

In the context of *E. rhusiopathiae*, preliminary suggestions for management include a recommendation to closely track caribou survival (e.g. though the enhanced use of GPS collars) and to rapidly evaluate, extensively sample, and immediately test tissues (serology, PCR, culture, and histopathology) collected from caribou mortalities for this pathogen (and other health related considerations). The investigation of *E. rhusiopathiae* in other species (e.g. rodents, birds, carnivores, other ungulates) found in NE BC may also provide important insight into the occurrence and distribution of carriers that may transmit the infection to boreal caribou and is encouraged. An unexpectedly high number of boreal caribou mortalities were also recorded in adjacent jurisdictions of the Northwest Territories<sup>238</sup> (and possibly Alberta<sup>239</sup>) in 2012/2013. The initiation of a formal inter-jurisdictional collaboration to explore *E. rhusiopathiae* (and other health related considerations) in boreal caribou from BC, NT, and AB is also recommended to enhance baseline knowledge as well as broader management, conservation, and recovery efforts in these areas.

### **4.3.3 Parasites**

#### **4.3.3.1 Parasites Found in Caribou Feces**

The quantity of archived feces available from each caribou captured in 2012 and 2013 was highly variable and was a limiting factor in the allocation (and prioritization) of samples for specific diagnostic tests. As such, the prevalence and species of nematode, cestode, and trematode parasites found in the feces of boreal caribou from NE BC were first evaluated in a test group of n=36 animals captured in 2012 and 2013 for which an ample quantity of archived feces were available. This group included the single caribou noted to have “soft feces” at the time of capture as well as a selection of caribou which were visibly “healthy” at the time of capture, in poor body condition at the time of capture, exhibited relatively high haptoglobin levels (a nonspecific index of inflammation), or that died during the high mortality period occurring from December 2012 to July 2013.

##### **4.3.3.1.1 Gastrointestinal Parasites (Focus Abomasal Nematodes)**

Few gastrointestinal parasites were recorded in the pilot study. 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. Similarly, only 8% (n=3/36) of caribou had cestode eggs (tapeworm; *Moniezia* sp.) in their feces. Although the intensity of *Moniezia* sp. infections in n=2/3 caribou was relatively high (18 and 21 eggs/ gram feces respectively) this parasite is not considered to be clinically significant in free-ranging caribou. Although cestode, *Nematodirus*, and *Marshallagia* eggs are relatively resistant, *Ostertagia* eggs are adversely affected by freezing and freeze thaw cycles. All fecal samples had been stored frozen for at least 12 months prior to analysis and it is likely that these results underestimate the true prevalence and intensity of *Ostertagia* infections in boreal caribou from NE BC. Similar considerations are likely for other parasites (e.g. *Dicytocaulus*, a lung worm) which may also be found in caribou feces. Given preliminary results and the amount of feces available from untested animals no further efforts were made to evaluate these parasites in BCHRP Year 1. However, the collection and evaluation of fresh, or formalin fixed, fecal samples is recommended to more accurately establish baselines and to enhance our understanding of factors influencing the herd level health status of boreal caribou in NE BC.

It should also be noted that, as for many other parasites of caribou, the prevalence and intensity of abomasal nematode infections may vary seasonally and with the life history stage of infected hosts<sup>5</sup>. Only fecal samples obtained from adult female caribou captured in winter/late spring were considered in BCHRP Year 1. Opportunistic (or directed) analysis of fecal samples collected from adult female caribou throughout the year and from male and juvenile caribou are recommended to further enhance baseline knowledge and our understanding of the occurrence and impact of gastrointestinal nematodes and other parasites which may be found in caribou feces. As a general consideration, similar studies are also recommended for other pathogens and health indices being evaluated as part of the BCHRP.

#### **4.3.3.1.2 Protostrongylid Nematodes**

Dorsal-spined Protostrongylid larvae (DSLs) were recorded in 25% (n=9/36) of fecal samples examined in the pilot study and further evaluation of feces from an additional n=121 caribou found the prevalence of DSLs in the feces of adult female caribou captured in NE BC in 2012 and 2013 to be 35% (n=55/157) overall. The prevalence of DSLs also varied 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)]. The average intensity of infection was 25 DSLs/gram feces (range < 1 - 191 DSLs/ gram feces) and was similar in all herd ranges except the Snake-Sahtaneh where the average intensity of DSLs was 43 DSLs/gram feces (range <1 -188 DSLs/gram feces). If preliminary results reflect broader patterns, these findings may suggest that factors influencing Protostrongylid transmission may vary as a function of landscape level features encountered by different boreal caribou herds in NE BC.

Molecular identification of DSLs is ongoing. All larvae examined to date have been identified as the caribou muscle worm *Parelaphostrongylus andersoni*. This is important baseline knowledge and the occurrence, prevalence, intensity, and identity of DSLs will continue to be evaluated as part of research planned for BCHRP Years 2 and 3.

#### **4.3.3.1.3 Giant Liver Fluke (*Fascioloides magna*)**

No fluke eggs were identified in n=157/157 fecal samples collected from adult female boreal caribou captured in NE BC in 2012 and 2013. These findings suggest that *Fascioloides magna* is unlikely to occur in boreal caribou in NE BC at the present time. This is important baseline knowledge. Given the potential importance of this parasite for caribou (and moose), continued monitoring of caribou fecal samples (and livers as available) will occur as part of research planned for BCHRP Years 2 and 3. The BCHRP working group also recommends that *F. magna* infection be explored in other ungulates (particularly elk and deer) inhabiting boreal caribou range in NE BC.

#### **4.3.3.2 *Besnoitia tarandi***

The overall prevalence of exposure to *B. tarandi* in adult female boreal caribou captured in NE BC in 2012 and 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) may be relatively prominent in NE BC and may also vary as a function of landscape level features encountered by different boreal caribou herds.

*Besnoitia tarandi* will continue to be evaluated as part of research planned for BCHRP Years 2 and 3. To our knowledge this is the first time a serological assay has been used to assess *B. tarandi* in a free-ranging boreal caribou population and additional research is necessary to characterize the relationship between exposure to this parasite and the occurrence of related clinical disease. As such, the BCHRP working group recommends that personnel involved in mortality site investigations, the capture and handling of boreal caribou (or other field-based activities in NE BC) remain vigilant for and document the occurrence of pathology associated with this disease (e.g. hair loss, skin crusting) in caribou<sup>124</sup>. Microscopic evaluation of skin samples from the anterior aspect of the mid-third portion of the metatarsus has been proposed as a standardized comparative indicator of the density of *B. tarandi* infections in *Rangifer* and the targeted collection of metatarsi from boreal caribou mortality sites is also recommended<sup>122</sup>.

#### **4.3.3.3 *Toxoplasma gondii***

Testing for the protozoan parasite *Toxoplasma gondii* is ongoing and preliminary results are anticipated in early January, 2015.

#### **4.3.3.4 *Neospora caninum***

*Neospora caninum* is a protozoan parasite carried by canids (wolves, coyotes, foxes, dogs) which may infect caribou and is known to cause abortions (endemic or outbreaks) and the production of weak calves in other ungulates (see Section 3.2, Table 3). Using an ELISA based protocol [see Section 3.3, Table 5 *Neospora* test a)] preliminary results suggested up to 22% (n=32/148) of adult female boreal caribou captured in NE BC in 2012 and 2013 may have been exposed to this parasite. However, a posteriori testing using a more specific test (Western Blot

for specific antibodies against immunodominant antigens of *N. caninum* tachyzoites) confirmed only n=2/32 potential positives. Given this marked discrepancy and the potential importance of this parasite for caribou all serum samples were re-evaluated with a second ELISA [see Section 3.3, Table 5 *Neospora* test b)]. There was good agreement between results obtained using this test and via Western Blot and n= 2/148 caribou were identified as positive using the second ELISA including 1 of 2 positive controls (previously confirmed via Western Blot) plus an additional animal which had been deemed likely to be positive with the first ELISA but could not be confirmed by Western Blot. N=2/2 negative controls (previously confirmed via Western Blot) were also determined to be negative using the second ELISA.

All considered, we found evidence that 2% (n=3/148) of adult female boreal caribou captured in 2012 and 2013 in NE BC had been previously exposed to *N. caninum*. The overall prevalence of *N. caninum* in boreal caribou from NE BC is within the range previously recorded in other free-ranging caribou herds<sup>e.g. 22, 53, 139, 140, 141</sup>. A single *N. caninum* positive animal was identified in each of the Parker, Calendar, and Chinchaga herd ranges. If preliminary findings are indicative of broader patterns, the prevalence of exposure to *N. caninum* may be greatest (14%, n=1/7) in boreal caribou from the Parker herd range [vs. Calendar (5%) (n=1/20), Chinchaga (3%) (n=1/34), Maxhamish (0%) (n=0/27), Prophet (0%) (n=0/8), Snake-Sahtaneh (0%) (n=0/52)], which may suggest that factors [e.g. prevalence of the parasite in wolves or other canids, persistent and trans generational infections in caribou ] influencing *N. caninum* transmission in caribou may be relatively prominent in this region and may also vary as a function of landscape level features encountered by different boreal caribou herds in NE BC.

*Neospora caninum* 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. The overall pregnancy rate for adult female boreal caribou captured in 2012 and 2013 in NE BC was 83% (n=135/163). Preliminary analysis indicated that the relative risk of “not being pregnant” may have been 4.2 times greater (Z=3.185, 95% C.I. 1.7-10.2 times greater, P=0.001) in *N. caninum* positive caribou than in *N. caninum* negative caribou. If these preliminary results reflect broader patterns, *N. caninum* may be an emerging threat to the reproductive success of boreal caribou in NE BC. Moreover, this parasite may already be of concern for caribou inhabiting the Parker herd range (population estimate, n= 13<sup>1</sup>) given that it is known to cause persistent (life-long) infections as well as repeated episodes of reproductive failure across multiple generations in other ungulates (see Section 3.2, Table 3).

Additional research and broader analyses are required to improve our understanding of the ecology and overall impact of *N. caninum* in boreal caribou from NE BC. The acquisition and testing of additional biological samples (serum and tissues) from live and dead boreal caribou inhabiting the Parker herd range and juvenile caribou (calves) are particularly important. *N. caninum* will be incorporated into research activities planned for BCHRP Years 2 and Year 3. A research program to evaluate the prevalence of *N. caninum* in wolves (a host which may transmit the infection to caribou) in NE BC is also being developed. Wolves are infected with *N. caninum* when they feed on ungulates tissues containing parasite cysts. As such, moose, elk, and

deer may have the potential to serve as important maintenance hosts for *N. caninum* in NE BC and an evaluation of this parasite in these species is recommended.

#### **4.3.3.5 Ectoparasites (*Dermacentor albipictus*, *Hypoderma tarandi*, Others)**

Winter ticks and/or tick associated hair loss/breakage (ranging from mild to severe) were recorded in 16% (n=26/163) of adult female boreal caribou captured in NE BC in 2012 and 2013<sup>240</sup>. The prevalence of winter tick (or related pathology) was highest in the Calendar herd range (33%) (n=9/27), lowest in the Maxhamish herd range 8% (n=2/26), and similar in all others [Chinchaga 11% (n=4/37), Parker 15% (n=1/7), Prophet 11% (n=1/9), and Snake-Sahtaneh 16% (n=9/56)]. These findings may indicate that factors [e.g. microclimate, overlap with other carriers (especially moose)] influencing *D. albipictus* transmission may vary as a function of landscape level features encountered by different herds.

Although more detailed analyses are required, preliminary findings suggest that both serum amyloid A (SAA) levels (Mann-Whitney U=1256.5, P=0.018) and hair cortisol concentration (Mann-Whitney U=1231.0, P=0.011) were higher in boreal caribou with hair loss (or hair breakage) related to infestation with winter ticks compared to those without. If preliminary results reflect broader patterns, these findings may indicate that infestation with winter tick is associated with chronic stress and inflammation in boreal caribou from NE BC. Some caribou with evidence of winter tick related hair loss were found to be in poor body condition suggesting that, as in other ungulates, this parasite may also have the potential to adversely affect boreal caribou survival (or reproduction). In addition, *D. albipictus* is known to carry and may transmit blood borne pathogens (e.g. *Anaplasma*)<sup>146</sup> that may cause disease in ungulates. Anecdotal reports of winter ticks on boreal caribou from NE BC may be increasing<sup>42</sup> and *Dermacentor albipictus* will be incorporated into research activities planned for BCHRP Years 2 and Year 3. Winter ticks collected from live-captured and dead boreal caribou in 2014/2015 will also be examined as possible carriers of *Erysipelothrix rhusiopathiae* and other potential pathogens of caribou.

No evidence of *H. tarandi* infections or other ectoparasites were recorded in boreal caribou captured in NE BC in 2012 or 2013<sup>42</sup>.

#### **4.3.3.6 Arthropod Vectored Blood Borne Parasites (*Babesia*, *Anaplasma*, *Ehrlichia*, *Bartonella*, *Trypanosoma*, *Setaria*, *Onchocerca*)**

Testing for arthropod borne blood parasites is ongoing and preliminary results are anticipated in early 2015.

### **4.4. Preliminary Results, Discussion, and Recommendations (Other Health Indices)**

#### **4.4.1 Serum Biochemistry**

The quantity of archived serum available from each caribou captured in 2012 and 2013 was a limiting factor in the allocation of samples for specific diagnostic tests. As such, serum biochemistry was evaluated in a pilot study of n=75 adult female boreal caribou captured in NE

BC in 2012 and 2013. This group included caribou which were collared in 2012 and 2013 and that remained alive at the start of the BCHRP (fall 2013) or had died during the high mortality period occurring from December 2012 to July 2013.

**Table 7. Serum biochemical parameters for n=75 free-ranging, adult female boreal caribou captured in 2102 and 2013 by net gun in Northeastern BC, Canada.**

<b>Parameter</b>	<b>Mean</b>	<b>Median</b>	<b>Range (95% C. I.)</b>	<b>S.D.</b>
Calcium (mmol/l)*not normally distributed	2.48	2.48	2.44 - 2.52	0.16
Phosphorus (mmol/l)	1.99	2.00	1.91 - 2.08	0.37
Ca:P Ratio	1.30	1.25	1.22 - 1.39	0.36
Magnesium (mmol/l)*	1.08	1.10	1.059 – 1.101	0.092
Sodium (mmol/l)	141.75	143.00	140.13 – 143.36	7.021
Potassium (mmol/l)*	8.58	5.60	7.068 – 10.092	6.56
Chloride (mmol/l)	94.72	95.00	94.01 - 95.43	3.10
CO <sub>2</sub> (mmol/l)	6.16	6.00	5.48-6.84	2.95
Anion (mmol/l)	49.59	49.00	48.30-50.88	5.60
Na : K Ratio	23.80	26.00	21.23-26.37	11.17
Total Protein (g/l)	69.92	69.00	68.64-71.20	5.57
Albumin (g/l)	42.54	43.00	41.84-43.23	3.03
Globulin (g/l)	27.39	26.00	26.10-28.67	5.66
Albumin : Globulin Ratio	1.62	1.63	1.54-1.70	0.36
Urea (mmol/l)*	1.48	1.30	1.34-1.61	0.57
Creatinine (mmol/l)	212.13	205.00	204.67-219.60	32.39
Glucose (mmol/l)	6.68	6.60	6.23-7.13	1.96
Cholesterol (mmol/l)	1.16	1.15	1.11-1.21	0.21
Total Bilirubin (umol/l)*	1.32	1.00	1.17-1.47	0.66
Conjugated Bilirubin (umol/l)*	0.80	1.00	0.71-0.89	0.40
Free Bilirubin (umol/l)*	0.55	0.00	0.35-0.74	0.86
Alkaline phosphatase (ALP) (U/l)	54.96	55.00	49.91-60.01	21.93
Gamma-glutamyltransferase (GGT) (U/l)*	19.73	17.00	16.16-23.31	15.52
Aspartate aminotransferase (AST) (U/l)	73.24	69.00	68.50-77.98	20.58
Creatine kinase (CK) (U/l)	238.65	206.00	210.12-267.19	123.86
Glutamate dehydrogenase (GLDH) (U/l)*	2.36	2.00	1.78-2.94	2.53



In general, serum biochemical parameters recorded in adult female boreal caribou from NE BC were similar to those previously recorded in adult female boreal from the Northwest Territories (also captured by net-gun)<sup>45</sup>. In BCHRP Years 2 and 3 serum biochemical panels will be measured in all caribou captured as part of collaring programs in 2014 and 2015 and results will be further evaluated in the context of individual animal health pending results of disease testing.

Logistical considerations prevented the collection of samples (fresh blood preserved in EDTA) required for CBCs in BCHRP Year 1. However, blood smears will be collected from all caribou captured in 2015 to support this analysis.

#### **4.4.2 Chronic stress (Hair Cortisol Concentration)**

A wide range of hair cortisol levels were recorded in n=163 adult female boreal caribou captured in NE BC in 2012 and 2013 (mean 3.70 pg/mg, range 0.16 - 47.94 pg/mg). Overall, hair cortisol concentrations recorded in boreal caribou from NE BC were higher (Kruskal-Wallis KW=18.968,  $P < 0.0001$ ) than that those previously determined using the same assay in n=24 captive reindeer and caribou from Alaska and n=97 free-ranging caribou from Greenland<sup>156</sup>. Multivariate analyses are necessary to gain meaningful insight into factors that may explain hair cortisol concentrations measured in boreal caribou<sup>156</sup> and will occur in BCHRP Years 2 and 3. Hair cortisol concentration will continue to be evaluated in samples collected from live-boreal caribou captured in NE BC in 2014 and 2015 as well as any caribou mortalities that occur in either year.

#### **4.4.3 Immunity**

##### **4.4.3.1 Haptoglobin**

A range of haptoglobin levels were recorded in n=152 adult female boreal caribou captured in NE BC in 2012 and 2013 (mean 0.15 g/l, range 0.11-0.39 g/l). Haptoglobin levels in all caribou examined fell within the test range (0.00-0.50 g/l) considered to be normal in domestic ruminants<sup>219</sup>. Preliminary findings also suggested that haptoglobin levels were similar (Kruskal-Wallis KW=9.112,  $P=0.105$ ) among all boreal caribou herds in NE BC. In depth analyses are ongoing and haptoglobin levels will continue to be evaluated in the context of individual caribou and herd health and landscape level features in BCHRP Years 2 and Year 3. The initial focus of this work will be a pilot study to evaluate an alternate haptoglobin assay which has been used successfully in reindeer<sup>220</sup> but requires less serum than the test employed in BCHRP Year 1. To provide further context for results obtained from boreal caribou, we will also evaluate haptoglobin (and other health indices) in captive reindeer (maintained at the University of Calgary) with known clinical histories.

##### **4.4.3.2 Serum Amyloid A (SAA)**

A wide range of SAA levels were recorded in n=160 adult female boreal caribou captured in NE BC in 2012 and 2013 (mean 70.88 ug/ml, range 0.00 - 412.80 ug/ml). Overall, SAA levels in boreal caribou from NE BC were lower than those reported for captive reindeer administered

bacterial endotoxins in experimental studies or affected by acute bacterial infections<sup>182, 183</sup>. However, SAA levels in boreal caribou exceeded or were similar to levels reported in other ungulates harbouring chronic viral or bacterial infections<sup>179</sup>. We also determined SAA levels in n=3 captive caribou bulls from NE BC which had evidence of moderate to severe chronic peritonitis and peri-hepatitis (likely due to *Setaria* sp. infection) at post mortem. SAA levels in these animals were recorded at 192.50 ug/ml, 198.13 ug/ml, and 206.20 ug/ml respectively. SAA levels in 18% (n=29/163) of boreal caribou exceeded 100 ug/ml. SAA levels were higher (one-way Analysis of Variance (AVOVA)  $F_{(5,159)} = 9.778$ ,  $P < 0.0001$ , Tukey-Kramer:  $P < 0.05$ ) in boreal caribou from the Calendar herd range compared to those from the Chinchaga, Maxhamish, Parker, Prophet and Snake-Sahtaneh ranges in which SAA levels were similar (Tukey-Kramer:  $P > 0.05$ ). Interestingly, caribou from the Calendar range also had the highest prevalence DSLs (48%) (most likely *P. andersoni*, the caribou muscle worm) and the highest prevalence (33%) of pathology relate to winter ticks. These pathogens may both cause chronic inflammation in caribou and may explain (at least in part) the relatively high SAA levels recorded in this area. If preliminary findings reflect broader patterns, SAA may hold promise as a tool to evaluate and monitor in boreal caribou from NE BC. SAA levels will continue to be evaluated in the context of individual caribou and herd health in BCHRP Years 2 and Year 3.

#### **4.4.3.3 Genetic Markers of Immunity**

Discussions to initiate a formal research collaboration to explore MHC based analyses in boreal caribou from NE BC occurred in BCHRP Year 1 and this program is currently under development. Fecal and tissues samples from all boreal caribou that were captured or died in 2012 and 2013 have been archived for this and other genetic analyses which are anticipated to begin in BCHRP Year 2. Samples from all caribou captured or dying in 2014 and 2015 will also be archived for genetic analyses.

#### **4.4.4 Nutrition**

##### **4.4.4.1 Body Condition and Bone Marrow Fat Content**

BCHRP collaborators Drs. J. and R. Cook [North American Council for Air and Stream Improvement (NCASI), La Grande, Oregon, USA) are leading an ongoing study to evaluate the role of nutrition and body condition in the population dynamics of boreal caribou in NE BC<sup>241</sup>. As part of this program body condition was assessed using ultrasonography to measure rump fat thickness, combined with a body condition score (BCS) obtained by palpating the rump in n=113/164 boreal caribou captured as part of the radio-collaring program in 2012 and 2013 and all n=41 caribou captured in 2014<sup>42</sup>. Body condition will also be assessed in the majority of caribou anticipated to be captured in 2015. Body condition and body fat levels derived from this work as well as the fat content of marrow collected from the femurs of dead boreal caribou<sup>195, 196</sup> are being explored in the context of pathogens and other health indices as part of the BCHRP's ongoing research efforts and will continue in BCHRP Years 2 and 3.

##### **4.4.4.2 Non-Esterified Fatty Acids (NEFA) and Betahydroxybutyrate (BHBA)**

Serum NEFA and BHBA levels were evaluated as part of serum biochemical analysis undertaken in the pilot study of n=75 adult female boreal caribou captured in NE BC in 2012 and

2013 (see 4.4.1). This group included caribou which were collared in 2012 and 2013 and that remained alive at the start of the BCHRP (fall 2013) or had died during the high mortality period occurring from December 2012 to July 2013. A range of NEFA (mean 0.70 mmol/l, range: 0.20-1.80 mmol/l) and BHBA (mean 592.78 umol/l range: 281.00-1060.00 umol/l) levels were recorded in boreal caribou. NEFA levels in many caribou were greater than those considered normal (0.10-0.37 mmol/l) in domestic ruminants<sup>219</sup> while BHBA levels in most caribou were within the range (324.00-1296.00 umol/l) considered normal in domestic ruminants<sup>219</sup>. Preliminary analyses to evaluate the utility of NEFA and BHBA as potential biomarkers of individual and herd health in boreal caribou are ongoing.

#### 4.4.4.3 Trace Nutrients

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 [an un-collared yearling male found dying in March, 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.

Iron deficiency in domestic ruminants is associated with diminished growth, reduced immune function, morbidity and death<sup>197, 201</sup>. A variety of adverse effects have also been reported in domestic and wild ruminants with marginal to deficient copper levels including: diminished body condition, poor hair coats, hoof deformities, alterations in estrous cycle length, anestrus, early embryonic loss, increased prevalence of ovarian cysts, diminished immune function, and death<sup>197-199, 202, 203</sup>.

Infections causing anemia (e.g. blood borne parasites), chronic blood loss (e.g. due to gastrointestinal parasitism or other diseases), and inflammation are considered among the most common causes of iron deficiency in ruminants<sup>197</sup>. Iron deficiency has also been recorded in semi-domestic reindeer in poor nutritional condition<sup>169</sup> and suboptimal hepatic copper concentrations are considered a marker of insufficient dietary intake of this element<sup>197</sup>. Preliminary (gross) evaluation of bone marrow fat suggested that SK075 was indeed emaciated (results of fat content analysis pending as of December, 2014). However, the relative importance of pathogens or diet (nutrition) as factors contributing to the trace nutrient deficiencies recorded in this caribou is the subject of ongoing research efforts.

Overall, these findings represent important baseline information and suggest diet (nutrition), disease or a combination of both factors might have played a role in some boreal caribou deaths occurring in NE BC between December 2012 and July 2013. However additional tissue samples collected from all boreal caribou herds in NE BC are required to establish trace nutrient baselines for the region and for individual animals of varying health and nutritional status. Tissue-based trace nutrient testing will continue as part of research planned for BCHRP Years 2 and 3 if and when appropriate samples (liver and kidney) are collected from boreal

caribou mortality sites in NE BC. In addition, trace nutrient levels will be evaluated in the serum of a test sample of boreal caribou which were collared in 2012 and 2013 and that remained alive at the start of the BCHRP (fall 2013) or died during the high mortality period occurring from December 2012 to July 2013. Similar testing will occur in tissue and blood samples collected as part of research activities occurring in 2014 and 2015. The collection of serum requires the live-capture and handling of caribou and obtaining liver or kidney samples from caribou mortality sites is often difficult (i.e. since they are usually consumed by predators and scavengers). As such, the BCHRP is also exploring analysis of bone and hair as techniques to evaluate and monitor the trace-nutrient status of boreal caribou in NE BC.

#### **4.4.5 Toxicology**

Levels of lead (Pb), mercury (Hg), and other potential toxins [also considered trace nutrients; e.g. Selenium (Se)] were measured in liver samples obtained from n=2 boreal caribou found dead in the Snake-Sahtaneh range in NE BC in 2013 [an un-collared yearling male found dying in March, 2013 (Fig 2.) and SK075 an adult female found dead (and intact) in July, 2013 during the high mortality period]. With the exception of low iron and marginal copper levels in the adult female, all potential toxins which were examined were within normal limits in both animals. These findings represent important baseline information however additional tissue samples collected from all boreal caribou herds in NE BC are required to establish toxicology baselines for the region. Toxicology testing will continue as part of research planned for BCHRP Years 2 and 3 if and when appropriate tissue samples (liver and kidney) are collected from boreal caribou mortality sites in NE BC.

### **5. General Recommendations**

Most pathogens that are being explored as part of the BCHRP may also infect and/or cause disease in wildlife other than boreal caribou. In this context, the BCHRP working group recommends establishing direct research partnerships with First Nations and other land-user groups in NE BC to enhance disease surveillance, sample collection, and testing efforts in species such as moose (or other ungulates) and wolves (or other carnivores) which live in caribou habitat and that may harbour caribou pathogens but may be encountered (or harvested) more frequently<sup>242-244</sup>. The development of formal partnerships with other research programs involved in the capturing and handling of live wildlife or mortality site investigations are also recommended. Such initiatives would greatly enhance our understanding of factors influencing health and disease in boreal caribou in NE BC and would also provide meaningful insight into the health status of other wildlife in the region (which may ultimately inform management and conservation efforts for these species).

In addition, the BCHRP working group recommends directly engaging First Nations and other stakeholders in community-based health surveillance and monitoring efforts for boreal caribou in NE BC. For example, the development and distribution of educational materials related to diseases of concern for caribou and a standardized method of reporting observations made when caribou are encountered would be informative as would traditional knowledge regarding the occurrence or historical trends of certain caribou pathogens in NE BC. Community-based sampling initiatives (e.g. employing non-invasive fecal sampling) have been developed and implemented successfully in other caribou research programs<sup>e.g. 245</sup> and when

combined with observational data could provide an invaluable means to evaluate the health status of adult female boreal caribou in seasons or locations not currently under intensive study as well as the health status of other caribou (bulls, juveniles, calves) not currently considered as part of the BCHRP. Moreover, non-invasive fecal surveys also provide informative (and complementary) data regarding the genetic background, sex, age class, reproductive status, diet, and stress levels of individual caribou as well as the structure, size, and trends of caribou herds e.g. 173, 246-250. The direct consideration of health in ongoing BCIP research programs evaluating the survival (and recruitment) of boreal caribou calves in NE BC would also be valuable as would inter-jurisdictional collaborations with boreal caribou research programs in the Northwest Territories and Alberta. Health assessment and sampling recommendations developed for the BCHRP could be integrated into other programs with minimal effort and would enhance baseline knowledge as well as broader management, conservation, and recovery efforts in these areas. Similarly, strategies to evaluate, monitor, and protect the health of boreal caribou developed as part of the BCHRP will also benefit other woodland caribou conservation initiatives in BC and elsewhere and provide a starting point for similar studies in other species-at-risk.

## **6. Future Directions: Research Objectives for BCHRP Years 2 and 3**

6.1 Build on BCHP Year 1 findings to establish comprehensive boreal caribou health baselines and identify populations which may be “at risk” from compromised health in NE BC.

Blood, hair, feces and tissue samples collected as part of the ongoing research and management programs (e.g. radio-collaring efforts, mortality site investigations, opportunistically) in winter 2013/2014 and summer 2014 (already archived at the University of Calgary) and in winter 2014/2015 and summer 2015 (anticipated as part of collar monitoring maintenance, and replacement programs) will be evaluated using techniques developed in BCHP Year 1. Results will be incorporated into the existing health database and used to determine the health status of live and dead boreal caribou in NE BC.

An additional two years of herd health testing will constitute a short-term longitudinal boreal caribou health monitoring program. This will strengthen baseline knowledge of boreal caribou health in NE BC in an unprecedented manner. We will enhance our understanding of relationships among health indicators and refine our understanding of what may be “normal” and the variability around “normal” in different years and across different herd ranges<sup>13</sup>. When evaluated in conjunction with available ancillary biological data (e.g. body condition, pregnancy status, survival), the comprehensive baselines will also help identify “at risk” populations where compromised health may already threaten boreal caribou fitness or abundance. Establishing comprehensive health baselines is also an important consideration for many potential boreal caribou management and recovery strategies (e.g. maternal penning programs, predator exclosures, predator/alternate prey control programs, and captive breeding or relocation programs) and will provide vital information for use in future environmental impact assessments (EIAs) or other monitoring initiatives.

6.2 Enhance our understanding of the significance of exposure to *Erysipelothrix rhusiopathiae* in boreal caribou.

To better understand the importance of *Erysipelothrix rhusiopathiae* in boreal caribou, research on a captive reindeer model is needed. Administration of vaccines is a common experimental technique used to artificially stimulate and track the immune response of animals exposed to specific pathogens<sup>53</sup>. This technique has been used successfully in caribou<sup>53</sup> and a vaccination trial in captive animals is necessary to evaluate the timing and duration of the antibody response in caribou or reindeer exposed to *Erysipelothrix rhusiopathiae*. Such an experiment is critical to accurately establish accurate cut offs for positive (exposed) and negative (unexposed) individuals and is vital to enhance our understanding of results obtained from the free-ranging boreal caribou (see Section 4.3.2.3). Access to handling facilities and captive reindeer maintained at the University of Calgary, Faculty of Veterinary Medicine provide the only opportunity to pursue this important initiative in Canada. Following protocols developed by S. Kutz et al. for a similar trial in captive muskoxen we will vaccinate n= 12 captive reindeer with a commercially available *Erysipelothrix* vaccine and n=3 reindeer (controls) with physiological saline. Blood will be collected at week 0 (prior to vaccination) and at 4 (booster may also be given in week 4), 8, 12, and 24+ weeks post vaccination. This time frame reflects the period from probable spring/summer exposure to *Erysipelothrix* to late winter capture and handling when seropositive animals were identified. The timing and duration of the antibody response will be evaluated with a novel *Erysipelothrix* ELISA developed at the University of Calgary<sup>251</sup>. Antibody levels will also be compared in fresh serum and serum derived from blood dried on filter paper. Evaluation of blood collected on filter paper has proven to be an effective and practical tool (requiring no sample processing in the field and can be stored dry at room temperature) for monitoring other pathogens in free-ranging caribou<sup>53</sup>. Ongoing work at the University of Calgary also suggests that using filter paper to collect “muscle fluids” from caribou carcasses may hold promise as a field friendly technique to identify exposure to this pathogen. Captive animal work at the University of Calgary provides an unparalleled opportunity to validate laboratory testing results for a free-ranging wild species in a proven facility.

### 6.3 Evaluate health biomarkers as potential tools for monitoring boreal caribou health in NE BC.

Full health assessments of wild animals are logistically challenging, expensive, and ideally require post mortem examinations. Practical, reliable, validated, and preferably minimally invasive biomarkers of health that effectively and consistently reflect health are highly desirable as a population level management tool for many species but particularly caribou. For example, feather corticosteroids have been used as both retro and prospective indicators of fitness in birds<sup>252</sup>. The large number (n>200) of adult female caribou captured as part of the BCHP, longitudinal monitoring of these animals, and available ancillary data relating to body condition, pregnancy status, and survival provide an unprecedented opportunity to rigorously evaluate selected integrative health biomarkers as potential boreal caribou health monitoring tools. Candidate markers including: hair cortisol concentration (an index of chronic physiological stress), genetic immune markers, haptoglobin and serum amyloid A (SAA) (indices of inflammation and immunity), and non-esterified fatty acids (NEFA) and beta-hydroxybutyrate (BHBA) (indices of energy balance) (see Section 3.2, Table 4) will be evaluated as predictors of caribou health. Specifically, these indices will be examined with respect to infection or exposure to disease, body condition, survival, and reproductive success (data compiled in Objective 6.1) to determine if they accurately reflect individual animal health.

#### 6.4 Evaluate relationships between boreal caribou health and larger scale (landscape level) features in NE BC.

Existing data gathered from current research partners and through new collaborations will be used to evaluate selected temporal and spatial relationships between caribou health and larger-scale (landscape level) factors (e.g. weather, population genetics, habitat quality and use, predation risk, natural or anthropogenic disturbance). This approach will allow us to determine if integrative health biomarkers (listed in 6.3) and the health status of individual caribou and caribou herds are linked with environmental conditions in NE BC. Landscape level analyses will also increase our understanding of boreal caribou ecology, the cumulative effects of environmental change on boreal caribou populations, and the relative importance of health and disease as potential drivers of boreal caribou population performance in NE BC. Like comprehensive health baselines and longitudinal monitoring, landscape level analyses may also offer insight into proactive solutions to monitor and maintain healthy caribou populations which could inform boreal caribou management and conservation programs in BC and elsewhere.

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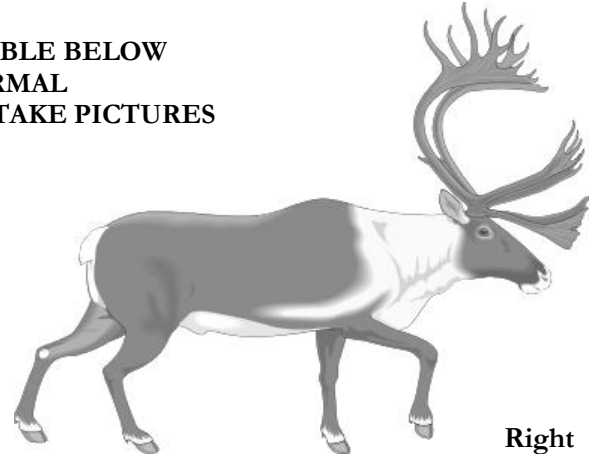
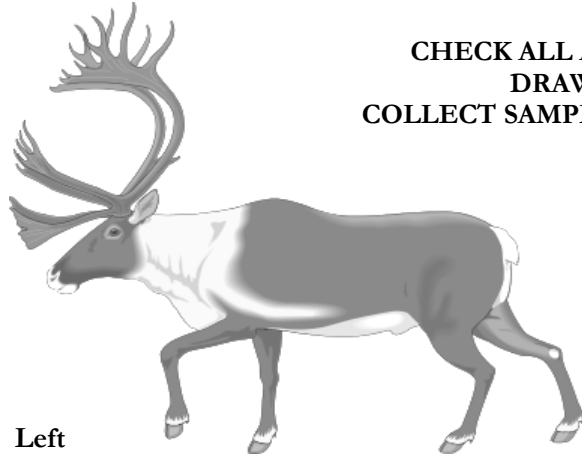
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## LIVE-CAPTURED CARIBOU: HEALTH EVALUATION AND SAMPLE COLLECTION

PERSONNEL: \_\_\_\_\_  
 DATE (d/m/y): \_\_\_\_\_ POPULATION UNIT: \_\_\_\_\_  
 CAPTURE SITE: UTM zone: 10U ☐ 11U ☐ UTM east: \_\_\_\_\_ UTM north: \_\_\_\_\_  
 ANIMAL ID: \_\_\_\_\_ COLLAR (type/freq.): \_\_\_\_\_ EAR TAG: \_\_\_\_\_  
 RECAPTURE: No ☐ Yes ☐  
 SEX: M ☐ or F ☐ → Calf at heel: No ☐ Yes ☐ → Lactating: No ☐ Yes ☐ TIME CAPTURED: \_\_\_\_\_  
 FIELD AGE (incisor wear): Yearling ☐ 2-3 ☐ 3-5 ☐ 4-6 ☐ 6-8 ☐ 8-10 ☐ 10-12 ☐ 12-15 ☐ RELEASED: \_\_\_\_\_

**1. GENERAL APPEARANCE:** Normal ☒ Abnormal ☐



**CHECK ALL APPLICABLE BELOW**  
**DRAW ABNORMAL**  
**COLLECT SAMPLES and TAKE PICTURES**

BODY CONDITION and MORPHOMETRICS		OCULAR		NASAL		ORAL CAVITY	
Palpation	<input type="checkbox"/>	Normal	<input type="checkbox"/>	Normal	<input type="checkbox"/>	Normal	<input type="checkbox"/>
Ultrasound	<input type="checkbox"/>	Eyes cloudy	<input type="checkbox"/>	Ulcers	<input type="checkbox"/>	Growths/swelling	<input type="checkbox"/>
Body condition excellent	<input type="checkbox"/>	Eyes swollen	<input type="checkbox"/>	Nasal discharge	<input type="checkbox"/>	Ulcers (palate/lips/gums)	<input type="checkbox"/>
Good	<input type="checkbox"/>	Ocular discharge	<input type="checkbox"/>	Unilateral circle L <u>or</u> R	<input type="checkbox"/>	Swabs collected	<input type="checkbox"/>
Fair	<input type="checkbox"/>	Unilateral circle L <u>or</u> R	<input type="checkbox"/>	Bilateral	<input type="checkbox"/>		
Poor	<input type="checkbox"/>	Bilateral	<input type="checkbox"/>	Clear	<input type="checkbox"/>	Abnormal tooth wear	<input type="checkbox"/>
Est. % body fat: _____		Clear	<input type="checkbox"/>	Purulent	<input type="checkbox"/>	Tooth injury	<input type="checkbox"/>
Body mass (kg): _____		Purulent	<input type="checkbox"/>	Rumen contents	<input type="checkbox"/>	Impaction	<input type="checkbox"/>
		Swabs collected	<input type="checkbox"/>	Swabs collected	<input type="checkbox"/>	Other	<input type="checkbox"/>
Body length (cm): _____		<i>Besnoitia</i> (head/eyelids)	<input type="checkbox"/>	Other	<input type="checkbox"/>		
Neck circumf. (cm): _____		Other	<input type="checkbox"/>				
Metatarsal length (cm): _____							

HAIR and SKIN		MUSCLE and LIMBS		REPRODUCTIVE		GASTROINTESTINAL	
Normal <input type="checkbox"/>	<input type="checkbox"/>	Normal <input type="checkbox"/>	<input type="checkbox"/>	Normal <input type="checkbox"/>	<input type="checkbox"/>	Normal <input type="checkbox"/>	<input type="checkbox"/>
Hair loss/break (draw extent) <input type="checkbox"/>	<input type="checkbox"/>	Muscle wasting <input type="checkbox"/>	<input type="checkbox"/>	Lactating <input type="checkbox"/>	<input type="checkbox"/>	Bloat <input type="checkbox"/>	<input type="checkbox"/>
Injury/wounds (body/head) <input type="checkbox"/>	<input type="checkbox"/>	Swollen joints <input type="checkbox"/>	<input type="checkbox"/>	Mastitis <input type="checkbox"/>	<input type="checkbox"/>	Mild <input type="checkbox"/>	<input type="checkbox"/>
Skin growths <input type="checkbox"/>	<input type="checkbox"/>	<i>Besnoitia</i> cysts (limbs) <input type="checkbox"/>	<input type="checkbox"/>	Vulvovaginitis <input type="checkbox"/>	<input type="checkbox"/>	Moderate <input type="checkbox"/>	<input type="checkbox"/>
Skin lesions <input type="checkbox"/>	<input type="checkbox"/>	Injury/wounds (limbs) <input type="checkbox"/>	<input type="checkbox"/>	Orchitis <input type="checkbox"/>	<input type="checkbox"/>	Severe <input type="checkbox"/>	<input type="checkbox"/>
Swabs collected <input type="checkbox"/>	<input type="checkbox"/>			Balanoposthitis <input type="checkbox"/>	<input type="checkbox"/>	Fecal staining <input type="checkbox"/>	<input type="checkbox"/>
Ectoparasites (hide/ears) <input type="checkbox"/>	<input type="checkbox"/>	Hoof deformities <input type="checkbox"/>	<input type="checkbox"/>	Discharge <input type="checkbox"/>	<input type="checkbox"/>	Diarrhea <input type="checkbox"/>	<input type="checkbox"/>
Warbles <input type="checkbox"/>	<input type="checkbox"/>	Interdigital lesions <input type="checkbox"/>	<input type="checkbox"/>	Clear <input type="checkbox"/>	<input type="checkbox"/>	Sample collected <input type="checkbox"/>	<input type="checkbox"/>
Est. number: _____ <input type="checkbox"/>	<input type="checkbox"/>	Swabs collected <input type="checkbox"/>	<input type="checkbox"/>	Purulent <input type="checkbox"/>	<input type="checkbox"/>	Other <input type="checkbox"/>	<input type="checkbox"/>
Sample collected <input type="checkbox"/>	<input type="checkbox"/>	Other <input type="checkbox"/>	<input type="checkbox"/>	Swabs collected <input type="checkbox"/>	<input type="checkbox"/>		
Other <input type="checkbox"/>	<input type="checkbox"/>			Other <input type="checkbox"/>	<input type="checkbox"/>		

**2. SAMPLES TO COLLECT:**  
(also see reverse)

- a) Blood: (3) Red top serum tubes ☐
- b) Blood: (2) Purple top EDTA tubes and (2-4) blood smears ☐
- c) Fecal pellets: (as many as possible, per rectum or fresh off snow) ☐
- d) Plucked hair: [min. 100 mg (~ coin envelope), top shoulder] ☐
- e) Ectoparasites: (as identified, any and all life stages) ☐
- f) Swabs: (wounds/ulcers/discharge) (2) DNA ☐ (2) culture ☐
- g) Pictures abnormal: (with scale, wide angle and close up) ☐

## This image shows a blank sheet of white paper with horizontal ruling lines. The lines are evenly spaced and run across the width of the page. There are no margins, text, or other markings on the paper.

SAMPLE	SAMPLE PROCESSING AND STORAGE
Blood: Red top serum tubes	Centrifuge → separate serum <u>and</u> buffy coat into separate cryovials → store frozen (-20°C)
Blood: Purple top (EDTA) tubes and blood smears	Store (1) purple top cool/not frozen (4°C) → store (1) purple top frozen (-20°C) → make smears ASAP (use cooled purple top)→ air dry (do not fix) → store smears at room temp.→ submit smears along with the cooled purple top for CBC with differential ASAP
Fecal pellets	Store frozen (-20°C) in tightly sealed whirl-pack
Plucked hair	Store room temp. in dry paper envelope in dark
Ectoparasites	Store half sample frozen (-20°C) and half in 70% ETOH at room temp.
Swabs	Collect as directed and submit for diagnostic testing ASAP

## CARIBOU MORTALITY SITE INVESTIGATION

**PERSONNEL:** \_\_\_\_\_

**DATE** (day/month/year): mortality signal: \_\_\_\_\_ death: \_\_\_\_\_ site investigated: \_\_\_\_\_

**POPULATION UNIT:** \_\_\_\_\_

**MORTALITY SITE:** UTM zone: 10U ☐ 11U ☐ UTM east: \_\_\_\_\_ UTM north: \_\_\_\_\_

**ANIMAL ID:** \_\_\_\_\_ collared: No ☐ Yes ☐ → collar type: \_\_\_\_\_ ear tag: \_\_\_\_\_

**SEX:** F ☐ M ☐ **AGE CLASS** (cheek teeth): calf (< 1 yr) ☐ sub adult (1-3 yrs) ☐ adult (≥ 3 yrs) ☐

**FIELD AGE** (incisor wear): Yearling ☐ 2-3 ☐ 3-5 ☐ 4-6 ☐ 6-8 ☐ 8-10 ☐ 10-12 ☐ 12-15 ☐ 15+ ☐

### SITE EVALUATION

\*check all applicable AND describe\*

**1. HABITAT TYPE:** \_\_\_\_\_

**2. WEATHER:** ambient: \_\_\_\_\_ snow depth (cm): \_\_\_\_\_ ice crusting: none ☐ mild ☐ moderate ☐ marked ☐

**3. PICTURES:** landscape (aerial) ☐ habitat (cardinal directions + undisturbed carcass) ☐ other ☐ \_\_\_\_\_

**4. CARCASS POSITION:** intact ☐ curled up ☐ disarticulated ☐

**5. CARCASS CONDITION:** fresh ☐ bloated ☐ active decay (maggots) ☐ advanced decay (rotten no maggots) ☐ dry ☐

**6. EVIDENCE ACCIDENT or HUMAN ACTIVITY:** No ☐ Yes ☐ \_\_\_\_\_

**7. LINEAR FEATURES:** No ☐ Yes ☐ → distance to nearest (m): \_\_\_\_\_ type: \_\_\_\_\_

**8. EVIDENCE STRUGGLE:** No ☐ Yes ☐ → tufts hair ☐ blood trails ☐ broken branches ☐ other ☐ \_\_\_\_\_

**9. EVIDENCE PREDATORS or SCAVENGERS (SITE):** No ☐ Yes ☐ → sighting ☐ tracks ☐ scat ☐ hair ☐

collar chewed ☐ decomposition odour on collar ☐ cache(s) ☐ bed(s) ☐ other ☐ \_\_\_\_\_

**10. SAMPLES:** pictures taken (scale) ☐ predator/scavenger fecal samples collected ☐ hair samples collected ☐

**DESCRIPTION:** \_\_\_\_\_

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### SITE DIAGRAM

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## EXTERNAL EXAM

\*check all applicable AND describe\*

1. GENERAL BODY CONDITION: poor ☐ fair ☐ good ☐ excellent ☐
  2. HEAD and ORAL CAVITY: ocular discharge ☐ nasal discharge ☐ regurgitation ☐ ulcers ☐ skin thickening or crusting ☐ dental disease ☐ other ☐ \_\_\_\_\_
  3. HAIR, SKIN, LIMBS and HOOVES: ectoparasites ☐ → samples collected ☐ active moult ☐ hair loss ☐ skin lesions or crusting ☐ swollen joints ☐ abnormal hoof growth ☐ inter digital lesions ☐ other ☐ \_\_\_\_\_
  4. REPRODUCTIVE and GASTROINTESTINAL: lactating ☐ pregnant ☐ mastitis or orchitis ☐ ulcers/lesions ☐ fecal staining ☐ diarrhea ☐ other ☐ \_\_\_\_\_
  5. EVIDENCE PREDATION or SCAVENGING (CARCASS): No ☐ Yes ☐ → bite marks or other wounds ☐ ante mortem haemorrhage ☐ other ☐ \_\_\_\_\_
  6. SAMPLES: pictures taken (scale) ☐ wound swabs collected (predator DNA) ☐ → no. and location(s) \_\_\_\_\_
  7. PREDATOR or SCAVENGER SUSPECTED: grizzly bear ☐ black bear ☐ wolf ☐ cougar ☐ other ☐ \_\_\_\_\_
- DESCRIPTION (e.g. lesions: location and severity/ predation: wound type and location(s), pattern of consumption):

### DIAGRAM

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## INTERNAL EXAM

\*check all applicable AND describe/place samples in SEPARATE whirl-packs/MINIMUM 100g per tissue\*

1. BODY FAT (omentum, heart, kidney): absent ☐ moderate ☐ plentiful ☐
2. SAMPLES CHEST CAVITY: heart blood ☐ cavity fluid ☐ filter paper ☐ red top ☐ number collected: \_\_\_\_\_  
intact thoracic pluck ☐ heart ☐ lungs (cranial, middle and caudal lobes) ☐
3. SAMPLES ABDOMINAL CAVITY: liver ☐ spleen ☐ kidney ☐ rumen contents: absent ☐ moderate ☐ plentiful ☐  
→ sample collected ☐ abomasum and first 3 feet small intestines ☐ mesenteric lymph node(s) ☐ formed feces in colon: No ☐ Yes ☐ → collect (minimum 40 pellets) ☐ reproductive tract (uterus, ovaries, mammary glands or testes, fetus) ☐
4. SAMPLES SKIN, MUSCLE AND BONE: hair (minimum 100 hair shafts, ideally from shoulder) ☐ skin/muscle ☐  
warbles: No ☐ Yes ☐ → est. number: \_\_\_\_\_ head and upper neck ☐ mandible(s) ☐ metatarsus ☐ femur (s) ☐  
intact limbs (best) ☐ → FL ☐ FR ☐ HL ☐ HR ☐ intact hide (or 20 cm<sup>2</sup> section hide from neck) ☐
5. ABNORMAL FINDINGS (parasites, signs of disease, injury other than predation): No ☐ Yes ☐ → pictures taken (scale) ☐ sample(s) collected (lesion with normal tissue interface) ☐

### DIAGRAM

DESCRIPTION: \_\_\_\_\_

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