British Columbia
Provincial Moose Research Project
Health Assessment Summary
2013 - 2018

Prepared for:

Government of British Columbia

Provincial Moose Research Project Team

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Executive Summary

The BC Moose Health Assessment Program was initiated in 2013 as part of a provincially coordinated moose research project aimed at evaluating the relationship between landscape change and moose survival in response to the decline of moose populations in some regions of British Columbia [reviewed 1-5]. The goals of this program were to: 1) develop fundamental moose health baselines for each of five study areas, 2) determine if infectious disease or non-infectious health determinants explain the fitness (survival and reproductive output) of individual moose and populations, and 3) provide recommendations to guide further moose health and population research in BC. This report is a summary of health testing results from 2013-2018 with interpretation to guide and refine moose health assessments as part of the ongoing British Columbia Provincial Moose Research Project (BCPMRP).

In total, 400 adult female moose and 60 calves of the year (captured in their first winter) have been live-captured, sampled, radio-collared, and monitored across five study areas to determine cause-specific mortality and annual survival rates (Figure 2). Investigation of 116 moose mortalities has identified health related factors as the probable cause of death in a higher than expected number of individuals (Figure 1).

Findings from some study areas are potentially concerning. Relatively low pregnancy rates and clear evidence of reproductive failure were observed in the Bonaparte study area. Variation in trace nutrient levels was apparent across BCPMRP study areas, with presumed suboptimal levels of some trace nutrients recorded in some individuals. Variation in exposure to selected pathogens was also identified, with high prevalence of some pathogens detected in some study areas. Gastrointestinal parasitism may be sporadically killing at least young moose in some study areas.

This investigation of moose health suggests that the occurrence and potential impact of selected health determinants may vary across BCPMRP study areas. However, no single factor can be identified as the cause of apparent differences in the overall health status of BC moose populations at the present time. Exposure to and/or infection with most of the viral, bacterial, and parasitic health determinants evaluated in this study are within ranges reported for moose populations elsewhere. Tick burden (infestation with the winter tick, *Dermacentor albipictus*) and body condition have emerged as important non-infectious determinants of health between years. Likewise, hair cortisol concentration (a probable biomarker of chronic physiological stress) may reflect the health status of moose in different study areas within years.

The limited scope of the current moose health initiative, informed by a previous Boreal Caribou Health Assessment model [70], cannot adequately evaluate potential sub-lethal or cumulative

effects of various health determinants on the fitness of individual moose or the performance of BC moose populations. Future research that would help inform management should include evaluation of the health status of BC moose within a cumulative effects framework and a continued refinement of the wild ungulate health assessment model. Continued monitoring of the moose health assessment model with enhancement of the number and type of health determinants and longitudinal monitoring at the individual and population level will provide the best opportunity to rigorously evaluate the relative importance of health as a driver of moose population dynamics in BC.

1.0 Background

In response to the decline of moose populations in some regions of British Columbia, the BC Ministry of Forests, Lands, Natural Resource Operations and Rural Development initiated a 5-year (December 2013–March 2018) provincially coordinated moose research project. The purpose of this project was to evaluate the relationship between landscape change and moose survival [reviewed 1-5]. To date, 400 adult female moose have been live-captured, radio-collared, and closely monitored across five provincial study areas in order to determine cause specific mortality and annual survival rates (Figure 2). In January 2017, 20 eight month old calves were also radio-collared and their survival monitored in the Bonaparte study area. In January 2018, an additional 40 eight month old calves were captured and radio-collared in the Bonaparte (n=20) and Prince George South (PG South; n=20) study areas.

Investigations of 116 moose mortalities have identified causes of death classified as: predation, health related (including apparent starvation, probable septicemia, peritonitis, and unknown health-related), hunting (licensed and unlicensed), natural accident, and unknown causes of death (Figure 1) [reviewed 4]. Health related factors have been identified as the probable or proximate cause of death in a higher than expected number of cases (Figure 1). Evidence of suboptimal pregnancy rates and poor body condition has been recorded in live-captured moose in some study areas. A more focussed moose health assessment was initiated in 2016 in response to these observations. This report summarizes health testing results with interpretation to guide and refine ongoing moose herd health assessments as part of the British Columbia Provincial Moose Research Project (BCPMRP).

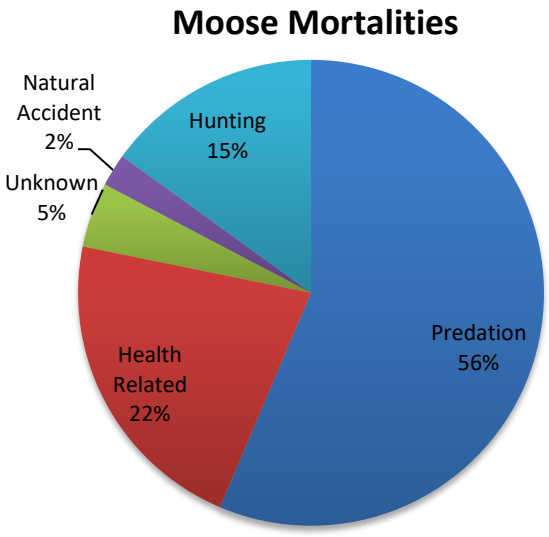

Figure 1. Proximate cause of death for moose cow mortalities in the BCMRP as of December 2018.

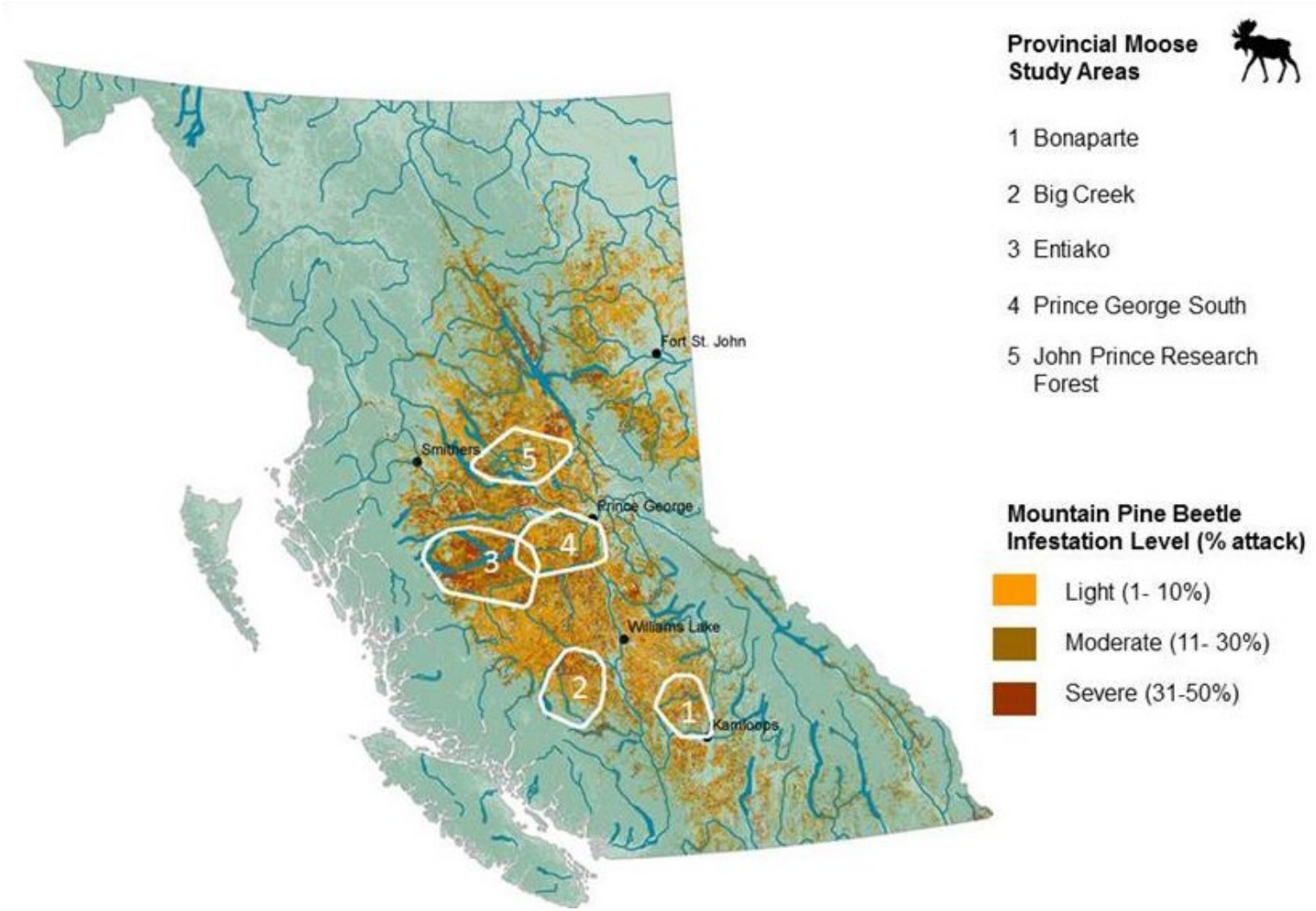

Figure 2. Provincial moose research study areas in British Columbia, Canada [from 3]

2.0 British Columbia Moose Health Assessment Program Objectives

The BC moose health assessment program currently has three primary objectives:

1) **Develop fundamental moose health baselines for each of the five BCPMRP study areas using a wild ungulate herd health assessment model.**
2) **Determine if selected infectious disease or non-infectious health determinants may explain non-predation related mortalities, poor body condition, or suboptimal pregnancy rates in any study area**.
3) **Provide recommendations to enhance the model of moose herd health assessment for the BCPMRP, including recommendations for continued monitoring and research gaps to help inform moose management decisions.**

3.0 Moose Health Program Methods

3.1 Moose Capture and Sampling

Detailed methods for moose capture, handling, radio-collaring and sample collection executed by the BCPMRP can be found in Kuzyk and Heard (2014), Kuzyk et al. (2015), Kuzyk et al. (2016), Kuzyk et al. (2017) and Kuzyk et al. (2018) [1-5]. A complete physical exam and health assessment was performed for each animal by the attending wildlife veterinarian or an experienced wildlife biologist, which included:

- A standardized set of biological samples, including: 30-40 cc of blood, feces, plucked hair, and a 6 mm skin biopsy (ear tag punch);
- A standardized palpation based scoring system to classify moose body condition as excellent, good, fair, poor or emaciated [2];
- Winter tick (*Dermacentor albipictus*) infestation scored as none, few, moderate or heavy by parting the hair and performing a manual and transect based search for ticks on the skin of the shoulder, back, rump and perineal region. Tick associated hair loss if present was also recorded;
- Reproductive status was recorded as presence or absence of a calf at heel. Lactation status was also evaluated; and
- Abnormalities, such as injuries, growths, dental pathology, jaw morphology, etc. were noted and pictures and/or samples were collected as necessary.

3.2 Pregnancy Assessment

The pregnancy status of all live-captured, adult female moose was determined by measuring serum progesterone (P4) and/or pregnancy specific protein B levels (PSPB). P4 testing yielded a larger than expected proportion of uncertain results in the initial project years and therefore in an attempt to clarify results, PSPB testing of archived serum samples was added and the testing protocol changed to that test.

3.3 Body Condition

Body condition score (BCS) is a course subjective measure on a four-point scale (0=emaciated, 1=poor, 2=fair, 3=good, 4=excellent) determined by palpating the fat cover over specific boney prominences. This system is used extensively in production animal systems and can be a good correlative measure of reproductive potential and health. The utility of body condition scoring depends on the level of training and experience of the operator. As a subjective measure, BCS was useful to assess patterns at the herd or area level.

3.4 Winter Tick

Winter tick (*Dermacentor albipictus*) burden was initially recorded on a four-point scale (none, few, moderate, heavy). These levels were measured inconsistently, therefore, an objective, standardized transect count of ticks was adopted.

3.5 Diagnostic Tests Used to Evaluate the Health Status of Live-Captured Moose

A variety of commercial and academic veterinary diagnostic laboratories were employed to test biological samples based on the laboratory's established record of health and disease testing in wildlife and/or expert knowledge in emerging topics in wild ungulate health (Appendix A). Serum, collected between January and March 2013 to 2018, was used to evaluate moose for exposure to or infection with selected viral and bacterial pathogens. Candidate pathogens and testing strategies were selected based on experience with health evaluations with Boreal Caribou, expert knowledge, emerging topics in moose health, an extensive literature review, and preliminary health testing during the early phases of the BCPMRP. The potential of the testing strategies to explain non-predation mortalities, such as drop-dead events (apparently healthy moose found dead with no evidence of trauma), poor body condition, and suboptimal pregnancy rates, was also considered. Finally, the quality and quantity of collected serum available as well as budgetary restraints influenced the specific viral and bacterial pathogen tests that were completed. Remaining unused samples were archived.

In order to build a more comprehensive baseline for moose health, moose calves of the year were sampled during capture in the Bonaparte study area in winter 2017, and Bonaparte and PG South study areas in winter 2018 [4,5]. Levels of selected trace nutrients were measured in serum samples and cortisol concentration was measured in hair samples of all ages of moose to investigate probable indicators of overall health and long-term physiological stress.

3.5.1 Viral Pathogens

Exposure to three viral pathogens was assessed; Parainfluenzavirus-3, Bovine Viral Diarrhea Virus, and Bovine herpesvirus-1 by serology (Appendix B).

Paramyxoviruses - Parainfluenzavirus-3

Parainfluenza-3 virus (PI3) is a paramyxovirus associated with cattle and causing mild to severe interstitial pneumonia which can lead to secondary bacterial infection and pneumonia. It is a common cause of morbidity and sometimes mortality in younger or stressed cattle. In an older study by Thorsen and Henderson (1971), exposure to PI3 was recorded in 14% of free-ranging moose from Alberta [8]. Many other serosurveys have been performed on wild ungulates; however the significance of exposure is unknown. No surveillance has been done in moose in BC exposure to this virus was evaluated.

Pestiviruses - Bovine Viral Diarrhea Virus

Pestiviruses (e.g. Bovine Viral Diarrhea Virus) are the causative agents of immunosuppression, mild to severe, or fatal respiratory and gastrointestinal disease as well as infertility, abortions, and neonatal morbidity and/or mortality in ungulates, primarily in domestic species [7, 9-11]. Immunosuppression related to infection with pestiviruses may also increase the susceptibility to or the severity of infections caused by other viruses, bacteria, and parasites [10]. Pestiviruses are transmitted horizontally (animal to animal contact) as well as vertically (mother to offspring) and persistent infections in offspring may occur with fetal exposure at specific times during gestation [11]. Persistently infected animals are considered to be of particular importance for the maintenance and transmission of this pathogen in and among ungulate herds [9,10]. Pestiviruses are also known to cross between species. In older studies, exposure to BVDV was recorded in 18% of moose from Alberta [8] and in 13% of moose in Alaska [12]. Antibodies against BVDV virus have been detected in a higher proportion, up to 43%, of European moose [13]. However, there is no known population effect from BVDV on moose.

Alphaherpesviruses - Bovine herpesvirus-1, and Infectious Bovine Rhinotracheitis

Bovine herpesvirus-1 (BHV-1) is an alphaherpesvirus of cattle that causes an array of moderate to severe or fatal clinical diseases including infectious bovine rhinotracheitis (IBR). Exposure to or apparent infection with IBR has been recorded in a variety of wild ruminants [14, 15]. In three older studies from Alaska and Alberta, exposure to IBR was recorded in 6%, 14%, and 0% of moose [8, 12, 17]

3.5.2 Bacterial Pathogens

Exposure to *Mycobacterium avium ssp. paratuberculosis* and *Erysipelothrix rhusiopathiae* was investigated through serological testing.

Mycobacterium avium ssp. paratuberculosis

Mycobacterium avium ssp. paratuberculosis (MAP) infection causes Johne's disease, which is characterized by morbidity, progressive emaciation, and eventually death in moose [20, 21]. The clinical progression of disease may be rapid [20]. Horizontal transmission of MAP may occur through

contact with feces of an infected animal or through contaminated soil, forage, or water [18] and is therefore considered a disease of high density situations. Vertical transmission may also occur in utero or by ingestion of MAP in colostrum or milk after birth [19]. To date, exposure to or infection with MAP has not been recorded in free-ranging moose, however transmission through contact with infected livestock or other infected wildlife is possible [18].

Erysipelothrix rhusiopathiae

Erysipelothrix rhusiopathiae is a causative agent of chronic disease such as arthritis or endocarditis, subacute illness such as pneumonia, abortions, and acute or per acute fatal septicemia in domestic and wild ungulates [e.g. 22, 23, 24]. The ecology of *E. rhusiopathiae* in wildlife is not understood. Infection through ingestion of bacteria from the environment or via skin damage is the primary route in domestic species [22]. Asymptomatic carriers and clinically ill animals are believed to play important roles in transmission [22]. Clinical disease often occurs secondary to stress or compromised immunity in both carrier and newly infected animals [22]. *E. rhusiopathiae* may be a pathogen of emerging importance in wild ungulates of North America. It was recently identified as the agent most likely to be responsible for large scale disease outbreaks and mortality events in free-ranging muskox [25]. Evidence of exposure to and probable infection with *E. rhusiopathiae* has also been recorded in boreal and mountain caribou from BC and Alberta [26, 27]. *E. rhusiopathiae* has been identified as the cause of severe disease in free-ranging deer [28] and has been recovered from the carcasses of moose found dead (predated moose or unexplained mortalities) in Alberta and Ontario [27, 29].

Exposure to *Erysipelothrix rhusiopathiae* infection was determined using molecular (PCR) techniques in all years of the study. In 2018, mortality samples were tested using bacterial culture and molecular genetic techniques (PCR).

3.5.3 Gastrointestinal Parasites

The species of gastrointestinal nematodes infecting moose likely varies with the diversity of sympatric ungulates (both domestic and wild) encountered in an individual's home range [30]. Gastrointestinal nematodes of the subfamilies Ostertagiina, Trichostrongylinae, and Nematodirinae were the primary focus of fecal parasite investigation in the BCPMRP [groups reviewed in detail in 30]. These parasites have direct life cycles in that they infect a single species to complete their lifecycle. Adult worms are found in the host's gastrointestinal tract and eggs are shed in the feces. Eggs and/or larvae of these parasites can persist in the environment for extended periods of time, including over the winter.

In response to host and/or environmental factors, fourth stage larvae (L4) of some species of parasites undergo inhibited development in the gastrointestinal mucosa. Emergence of L4's from the mucosa may occur in response to improving environmental conditions and/or declining host condition in the spring [modified after 30] and is often associated with severe or fatal clinical disease [30]. Subclinical infections are also common and may adversely affect food intake, body condition, and reproduction.

The impacts of these nematodes are believed to be similar in domestic and wild ungulates, including moose [30].

Fecal flotation, the method used to test for Ostertagiinae, Trichostrongylinae, and Nematodirinae infections, is also able to reveal infections with other gastrointestinal nematodes such as Trichurinae, cestodes (tapeworms), and coccidia (enteric protozoa). *Trichuris* sp. is regularly recorded in peri-agricultural moose populations [31, 32, 33] while coccidia infections are relatively rare [30]. The effects of *Trichuris* sp. and coccidia infections in moose have not been thoroughly investigated; however, these are important pathogens of domestic livestock. Tapeworm infections (e.g. *Moniezia* sp.) are commonly recorded in moose but are not generally considered to adversely impact health or productivity unless burdens are exceptionally high [30-33].

Fecal sedimentation was used to identify infection with flukes (e.g. *Fascioloides* sp., *Paramphistomum* sp.) and Baermann testing was employed to identify infection with lungworms (e.g. *Dictyocaulus* sp.) and/or dorsal-spined larvae (DSLs) of lung and tissue dwelling Protostrongylid nematodes (e.g. *Varestongylus* sp. and *Parelaphostongylus* sp.). These parasites have been identified in moose with a range of clinical and subclinical consequences depending on the species and/or the intensity of infection [30-36].

3.5.4 Tissue Dwelling Protozoans

Two tissue dwelling protozoans were investigated, *Neospora caninum* and *Toxoplasma gondii*.

Neospora caninum

Neospora caninum is a protozoan parasite with a canid definitive host 736], (i.e. wolf, coyote, fox, or domestic dog) and a ruminant intermediate host [38]. Exposure to *N. caninum* has been recorded in 13% of free-ranging moose from Minnesota and 0.5% of moose in Alaska [38, 41], yet the impact of *N. caninum* in free-ranging moose populations is poorly understood. Horizontal transmission may occur when a moose ingests soil, water or forage contaminated with infective *N. caninum* oocysts shed in the feces of infected canids [39]. Vertical transmission of *N. caninum* may also occur. Depending on the age of the fetus, trans-placental infection may cause abortions (epidemic or endemic) or may result in the birth of un-infected calves, infected calves which are infected and unthrifty, or clinically normal but persistently infected calves [39, 40].

Toxoplasma gondii

Toxoplasma gondii is a protozoan parasite with a felid definitive host (i.e. lynx, cougar, bobcat, or domestic cat) and a wide variety of intermediate hosts including moose [42]. Intermediate hosts become infected with *T. gondii* after ingesting soil, water, or forage contaminated with oocysts shed in the feces of infected felids [42]. Vertical transmission in intermediate hosts occurs in utero [42].

Toxoplasma gondii causes a spectrum of diseases in intermediate hosts including anemia, pneumonia, enteritis, hepatitis, and encephalitis, congenital defects, abortions, still births, and weak neonates [42]. Exposure to *T. gondii* has been recorded in moose, ranging from 0 to >20% in free-ranging animals and appears to be highly variable across different populations. Evidence of congenital transmission of *T. gondii* has recently been recorded in wild moose from Minnesota [reviewed in 43]. However, to date, clinical disease associated with *T. gondii* has not been recorded in free-ranging moose and the effects of this parasite at the individual and population level are poorly understood.

3.5.5 Physiological Stress

Chronic physiological stress is increasingly recognized as a factor that can contribute to diminished growth, immunity, reproduction, and survival in many species [reviewed 44]. Chronic stress in wildlife may reflect the cumulative effects of predation risk, poor nutrition, reproduction and lactation costs, parasitism, inclimate weather, and human activity/disturbance or other anthropogenic landscape features and has been demonstrated as a key driver of population dynamics in some free-ranging wildlife populations [reviewed 44].The measurement of corticosteroids (e.g. cortisol, corticosterone) in hair is a rapidly emerging technique in wildlife health studies [reviewed 44] and may represent the best integrated measure of chronic physiological stress currently available.

3.5.6 Trace Nutrients

Adequate levels of trace nutrients are required in the diet of all animals to ensure their optimal physiologic function, growth, reproduction, and survival [e.g. 46]. Among wild ungulates, trace nutrients seem to play a particularly important role in the health of moose. Deficiencies in essential elements such as copper, selenium, cobalt, and iron have all been suggested as the primary cause or an integral component of a multifactorial set of causes leading to moose mortalities and/or poor performance in some moose populations [e.g. 47-53]. Less commonly, excesses of some trace nutrients such as molybdenum are believed to play a similar role [54]. In moose, trace nutrient levels are closely tied to the diversity and trace nutrient content of seasonal forage and may vary significantly over time and space [e.g. 55, 56]. This may be of particular relevance for moose inhabiting ranges where anthropogenic or natural landscape change such as from forestry practices or wildfires have influenced the type, distribution, quality, quantity, and/or phenology of forage plants available. The preliminary investigation of trace nutrient levels in BC moose for the BCPMRP was focused primarily on serum and tissue levels of selected elements: manganese, iron, cobalt, copper, zinc, selenium, and molybdenum.

Trace Nutrient Testing in Wild Ungulates

For most trace nutrients, low or high levels (deficiencies or toxicities) in animals are most accurately identified using liver samples. In studies of free-ranging ungulates, liver samples are often consumed by predators or scavengers before they can be collected. Trace nutrient levels can also be evaluated in kidney samples, which may provide another alternative for evaluating some elements.

Blood or serum may be an informative substitute for evaluating trace nutrients at the herd or population level when liver samples are not available or when testing live wildlife [46]. In this study all animals were evaluated with serum and when available, liver.

3.6 Moose Mortalities

All moose in the study were fitted with GPS radiocollars to allow rapid responses to mortality signals and facilitate accurate identification of the proximate and ultimate causes of moose death. A standardized mortality investigation protocol for recording and sampling moose mortality sites was implemented [reviewed in Kuzyk et al. (2016) and Kuzyk et al. (2017) [3-4].

Photographs were taken of the mortality sites and tissue samples collected and submitted to various diagnostic laboratories under the direction of a wildlife veterinarian. Health-related diagnostic testing was based on the probable cause of death and the condition and/or type of samples recovered. The type, quality, and quantity of samples obtained from moose mortality sites were highly variable and not all mortalities yielded usable tissue for health related diagnostics. Photographs assisted in determining the cause of death and interpretation of results of testing. Carnivore samples (hair, scat, swab from punctures on moose hide) were collected when present and needed to determine the carnivore species present at the mortality site.

4.0 Moose Health Program Results and Recommendations

4.1 Evaluation of BAM II

A combination of carfentanil citrate and xylazine hydrochloride was used to immobilize most moose in the early years of the study but due to lack of availability, starting in 2016, BAM II was used for the helicopter-based darting of adult female moose for the BCPMRP. BAM II is a compounded drug combination containing butorphanol (B), azaperone (A), and medetomidine (M). Immobilization is reversed with atipamezole and naltrexone. Anesthesia was extremely predictable and stable with the previous combination of carfentanil and xylazine, however this combination has a high risk to humans due to the opioid component.

The investigators that developed BAMII recommended a volume of 1.7 ml (IM) BAM II for an adult female moose , reversed with 3.4 ml atipamezole (IM) and 0.5 ml naltrexone (IM). Wildlife captured by aerial darting generally requires higher drug doses compared to ground darting due to the physiological responses associated with helicopter-based captures. Wildlife veterinary practitioners in the USA recommended using 2.8 ml BAM II (IM) reversed with 5.6 ml atipamezole (IM) and 0.5-1.0 ml naltrexone (IM) for aerial darting moose.

In 2016, 3.0 ml BAM II (IM), reversed with 6.0 ml atipamezole (IM) and 0.5 ml naltrexone (IM), was trialed as a moose aerial darting protocol for the BCPMRP. While this dose was effective in some

cases, demonstrating relatively short induction times, stable anesthesia and vital signs, as well as rapid recovery, many moose captured at this dose exhibited prolonged induction times, greater than 12 minutes. In addition, some moose were not adequately immobilized when approached on the ground with events such as too light dosing requiring top-up, vocalization, or spontaneous arousal with flight attempts or kicking. This was most apparent when dart placement was less than ideal (i.e. partial or full SQ injection or IM outside large muscles of the hind limbs) but also occurred in some animals where dart placement was ideal. The trial changed the BAMII volume and 3.0, 3.5, and 4.0 ml BAM II (IM) were used for 21 adult female moose captured in PGS and JPRF study areas. The results were more consistent anesthesia, improved induction times (6-8 minutes almost regardless of shot placement), stable vital signs under anesthesia, and notably better muscle relaxation. Recovery times were improved with only 5-6 minutes to fully ambulatory in most cases. Although induction and recovery times were similar to moose immobilized with 3.5 ml BAM II, the plane of anesthesia in moose immobilized with 4.0 ml BAM II was too deep with depressed heart and respiratory rates, poor oxygenation and relatively poor capillary refill times (>2.5 seconds) .

A volume of 3.5 ml BAM II (IM), reversed with 7.0 ml atipamezole (IM) and 1.0 ml naltrexone (IM) is currently recommended for adult female moose captured by aerial darting in BC. It is also recommended that oxygen is administered intranasally (at 2 - 5 L/min or at a rate sufficient to keep SP O2 >85%) to moose immobilized with BAM II. Pulse oximetry facilitates better monitoring of oxygen saturation and is also advised when using BAMII for anesthesia of wild ungulates.

4.2 Pregnancy Rates

The pregnancy rate of 377 moose in the study areas, from 2013 to 2018, was 79% (12 unclear results excluded). Pregnancy rates by study area (Figure 3) and year are detailed in Appendix C.

4.2.1 Evidence of Reproductive Failure in the Bonaparte Study Area

Pregnancy rates in the Bonaparte study area (Figure 4 and Appendix C) were below the average in all 5 years of the present study. Mortality site investigations also revealed other evidence of reproductive failure in moose from this study area in 2016 (Appendix D).

In spring 2016, three adult female moose were found moribund or dead with clear evidence of abortion and/or reproductive tract pathology. The pregnancy rate measured in moose from Bonaparte in 2016 was the lowest recorded for this study area in any year to date (47.2%, 95 C.I. 32.0%-63.0%, n=17/36). In 2017, 2 out of 22 serum samples tested from cow moose captured in the Bonaparte study area were classified as High Recheck using PSPB analysis. The most common cause for this designation is embryonic death or abortion occurring approximately in the week before testing. Anecdotal evidence of very early (peri-parturient) neonatal death and/or abortions may also be reflected in movement data recorded for some radio-collared cows in this study area, ie. pauses in movement indicating animals staying in an area for calving (C. Procter, pers. comm.). These findings suggest that a

health determinant is adversely affecting moose reproduction in the Bonaparte study area, however, no clear cause has yet to be determined. Evidence of suboptimal pregnancy rates and abortions may also be indicative of health challenges that could directly or indirectly threaten the viability, and subsequent recruitment, of neonatal or juvenile moose in this region. The apparently abrupt co-occurrence of poor pregnancy rates and sick and/or dead adult moose with reproductive abnormalities in the same year may indicate that an infectious cause is responsible. However, non-infectious (e.g. trace-nutrient abnormality) or multifactorial causes cannot be ruled out at the present time.

The below average pregnancy rates combined with other evidence of reproductive failure in moose from the Bonaparte study area indicate that further investigation is urgently needed in this region. A neonatal calf collaring study, while logistically challenging, could be particularly informative and a more thorough investigation of the reproductive health of cows is also necessary. For example, should this pattern continue, cervical culture for reproductive tract pathogens, further screening of serum and whole blood, complete blood counts and serum biochemistries should be considered. Where possible, reproductive tracts, including fetus(es) and placentas from moose mortalities could be assessed. For comparison, the same assessments could be performed in at least one "control" study area (e.g. JPRF) where pregnancy rates are consistently higher. PSPB testing is recommended over P4 testing for the BCPMRP going forward.

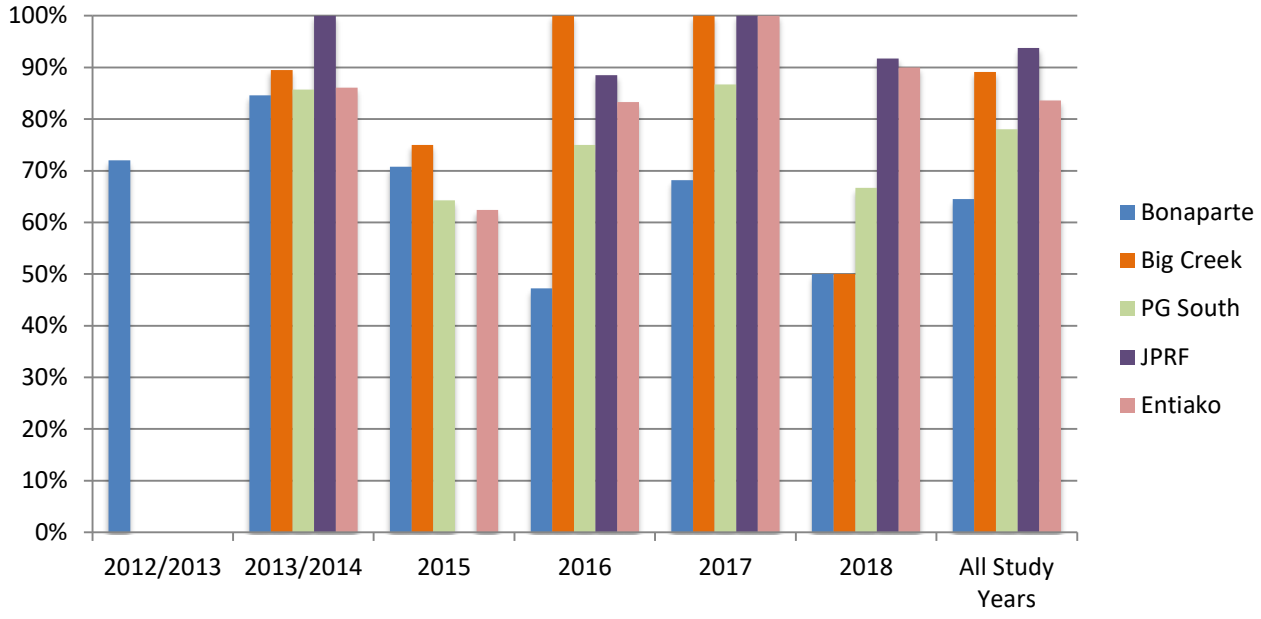

Figure 3. Pregnancy results for moose from winters 2012 to 2018.

4.3 Body Condition

The body condition scoring of moose cows across all study years show variation by region. A relatively high number of adult moose cows from the PG South study area were scored as fair, poor, or emaciated body condition at the time of capture (Figure 4). An ultrasound trial was conducted on a some moose cows captured in the PG South and JPRF study areas in winter 2017 to determine the feasibility of obtaining ultrasound based measurements of back fat from moose captured in late January and February. Rump fat was measured in a small number of animals, however, in most cows with calves and in many cows in fair, poor, or emaciated condition, no back fat could be measured.

Ultrasound measurement of back fat is recommended as an objective measure of body condition in moose. BCPMRP moose captures should be moved to the extent possible into December to provide more accurate data and a better likelihood of animals with fat. Unlike net-gunning, deep snow is not necessary for the safe and effective chemical immobilization of moose. Body condition scoring of all moose should be continued, with or without ultrasound measurements.

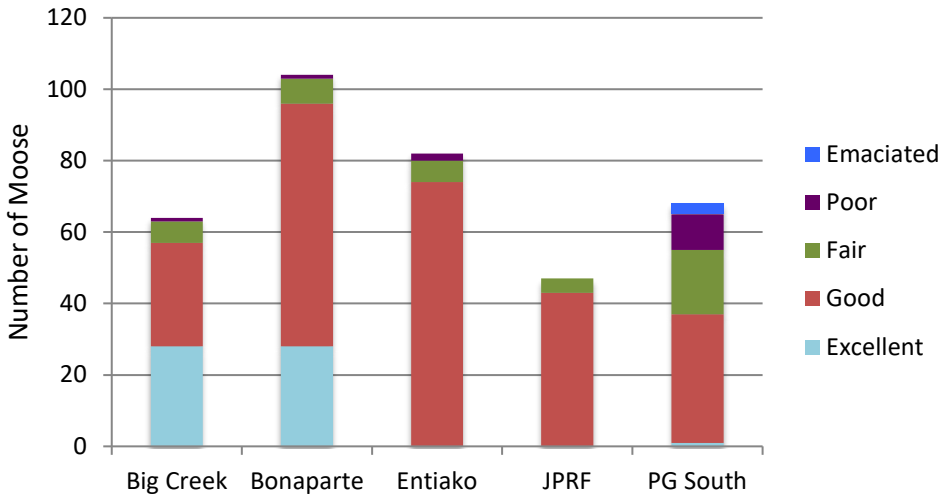

Figure 4. A comparison of field body condition scores in 366 adult female moose in winters 2013-2018.

Lactation status is a key determinant of winter body condition in wild ungulates and an important covariate in any analysis of moose body condition. A review of capture data recorded indicated that this index has been inconsistently recorded. While it is not consistent, some cows with calves at heel continue to lactate into the winter season. Extra effort is needed to record the lactation status of all cows, especially for those captured in early winter when lactation status is more likely.

4.4 Winter Tick

Tick burden scoring across all study years indicated a trend towards a relatively high number of adult cow moose from the PG South and Bonaparte study areas having moderate to heavy winter tick burdens (Figure 5). A variation in tick burden scores (TBS) in moose captured in the same study area

but in different years was also apparent with a trend towards moose captured in 2016 and 2017 having greater tick burdens than those in 2013/2014 and 2015. The TBS of most moose cows and calves captured in the Bonaparte study area in 2017 were similar. Overall, crude trends by population and year suggest that adult female moose from the PG South and Bonaparte study areas may suffer from the highest tick burdens.

Differences or changes in TBS across BC moose study areas likely reflect local and/or annual environmental conditions. As such, winter tick may be a key factor explaining apparent differences in moose health and productivity observed across study areas. Nonetheless, caution is warranted with all interpretations of TBS as success in identifying and accurately quantifying the presence of early life stages (larvae or nymphs) ticks is strongly influenced by the time of year, the stage size and experience of the investigator.

Estimating winter tick burden should remain an important part of all moose health assessments for the BCPMRP. To overcome the limitations of scoring tick burden, the standardized tick transect counting method should be used.

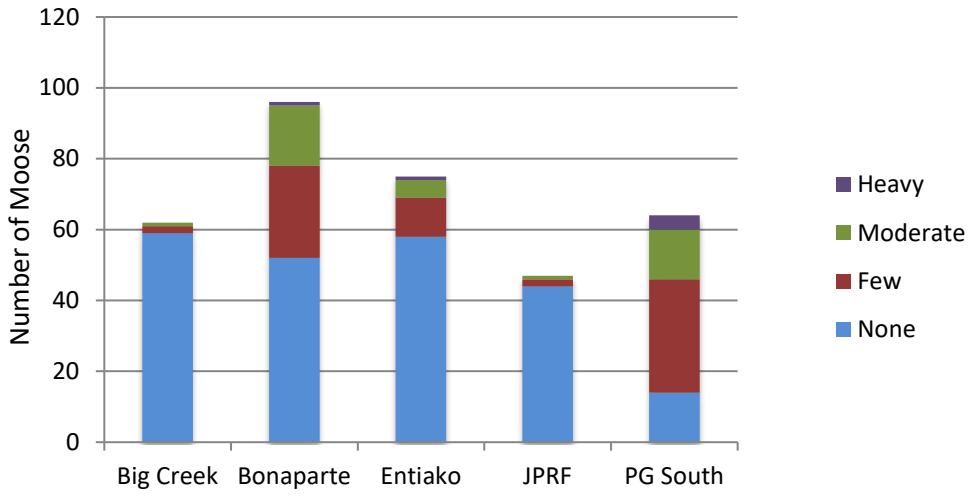

Figure 5. Winter tick (*Dermacentor albipictus*) burden scores by study area.

4.5 Diagnostic Health Testing

Trends in the prevalence of exposure to specific pathogens, including selected viruses, *Erysipelothrix rhusiopathiae*, *Neospora caninum*, and *Toxoplasma gondii* in BC moose are presented in Appendix E. All appear to have remained relatively constant throughout the current study. The exposure to *E. rhusiopathiae* increased from winter 2015 to winters 2017 and 2018 (1.9%, 17.5%, and 10.5% respectively), however, it is important to note that this indicates exposure to the pathogen rather than the disease occurrence. Caution in interpretation of serological findings is important as these are the

first sero surveys of BC moose. There is a low prevalence of exposure to some pathogens (paramyxoviruses and pestiviruses) and a relatively small sample size for some pathogens in some years. Different diagnostic tests used between study years may also account for some variation in results.

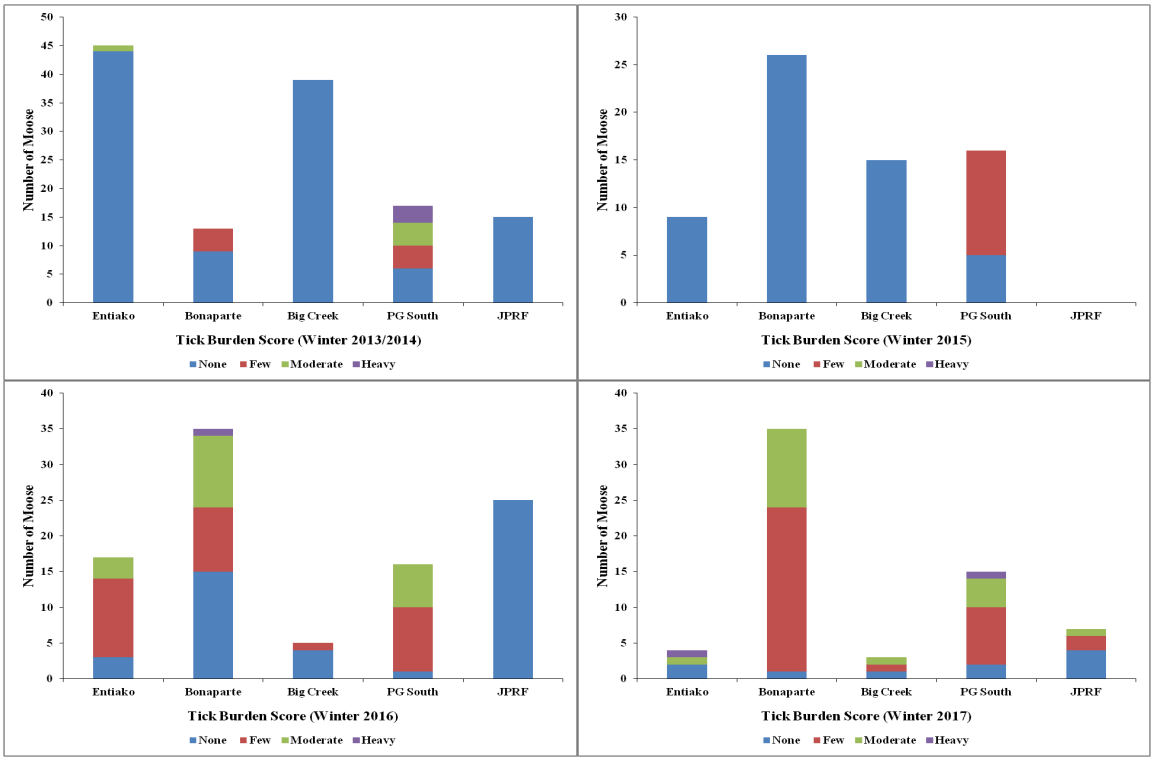

Figure 6. Winter tick (*Dermacentor albipictus*) burden scores by project year.

The exposure to pestiviruses, alphaherpesviruses, *E. rhusiopathiae*, *N. caninum*, and *T. gondii* in moose calves from the Bonaparte and PG South study areas is presented in Appendix F. The small number of moose calves tested precludes any meaningful interpretation. However, moose calves are clearly being exposed to *N. caninum* in the Bonaparte study area (12.8% prevalence). Infection with this parasite in utero may compromise the long-term viability of calves and comparing the survival rates of exposed versus unexposed calves over time is important, This result suggests that this parasite may be involved with the reproductive failure in the area, however the study of neonatal/newborn calves will be necessary to fully explore this topic. Exposure to *E. rhusiopathiae* was recorded in one calf in the Bonaparte study area and three calves in the PG South study area.

Monitoring of moose herd health is recommended as long as the BCPMRP is underway to strengthen baselines, what is “normal” and the variability over years and across different study areas. Longitudinal monitoring of the health status of individual moose (i.e. in animals recaptured and

sampled repeatedly, and tracked over multiple study years) is also strongly recommended. Recaptured animals provide unique opportunities to explore linkages between changing health status and fitness. Likewise, there may be opportunities to identify relationships between changing health status and the lifetime productivity of moose in study areas where calves are being collared. This approach has recently been explored in BC caribou health research programs and has been used successfully in programs for other species (e.g. grizzly and polar bears, bighorn sheep). The number of moose being captured and intensively tracked by the BCPMRP provides a unique opportunity to perform similar work in selected study areas where moose health appears to be compromised, such as the Bonaparte study area.

4.5.1 Viral Pathogens

Paramyxoviruses and Pestiviruses

Exposure to paramyxoviruses (PI3; 6.4%)) and pestiviruses (BVDV; 0.6%) in BC moose was low and within ranges recorded in moose populations elsewhere [9, 13] (Appendix G). Exposure to pestiviruses was only recorded in moose from the Bonaparte study area and exposure to paramyxoviruses was greater in moose from the Bonaparte study area than elsewhere, however its significance is unknown. As these viruses are associated with cattle, it may suggest greater contact with range cattle. There is little confirmatory evidence that these viruses cause significant disease in moose.

Testing for paramyxoviruses was only employed in the early years of the BCPMRP and was discontinued later due to budgetary restraints and a shift in focus towards pathogens more likely to directly influence moose survival and reproduction. Paramyxovirus testing could be reconsidered as part of an enhanced moose health assessment program exploring viral pathogen burden under a cumulative effects model of moose health. Pestivirus (BVDV) testing should be continued for all BC moose. Molecular studies may also be warranted to identify the specific pestivirus(es) infecting BC moose.

Alphaherpesviruses

The exposure to alphaherpesviruses was similar across all moose study areas (72.9% total). However, the prevalence of exposure recorded in BC moose appears to be far greater than that recorded in moose populations elsewhere [9, 13] (Appendix G). Previous studies on alphaherpesviruses in moose were completed in the 1970's and 1980's so differences in the type and quality of diagnostic tests employed may explain this result. The high prevalence of exposure to alphaherpesviruses noted in the BCPMRP is consistent with findings in other free-ranging cervids where exposure to and infection with host adapted alphaherpesviruses [e.g. cervid herpesvirus-2 (CvHV-2) in caribou, or elk herpesvirus (EHV-1) in elk 58, 59] are common.

Alphaherpesviruses are increasingly recognized as a potential cause of serious clinical disease in free-ranging ungulate populations and further characterization of exposure in archived serum from BC

moose is recommended. Molecular studies should also be pursued to determine the precise alphaherpesvirus(es) infecting BC moose, however there are challenges in finding researchers interested in working with these viruses and access to such facilities. Archives samples will be used when and if those partnerships can be developed.

4.5.2 Bacterial Pathogens

Mycobacterium avium ssp. paratuberculosis

Exposure to *Mycobacterium avium* ssp. *paratuberculosis* (Johne's Disease, MAP) was only evaluated in the early phases of the BCPMRP. There was no evidence of exposure to MAP in any BC moose(Appendix H). Clinical MAP infections have only been recorded in captive moose and it is recommended that testing efforts for this pathogen remain suspended unless clinical disease, such as diarrhea, weight loss or granulomatous thickening of the intestine in emaciated moose, is detected at a necropsy.

The enzyme-linked immunosorbant assay (ELISA) used to evaluate moose serum for exposure to MAP has marginal sensitivity and specificity in domestic species and is not validated for use in wildlife. If MAP testing is requested by the BCPMRP team, quantitative fecal PCR and fecal culture are superior diagnostic tests and should be considered.

Erysipelothrix rhusiopathiae - Serology

Exposure to *E. rhusiopathiae* among adult female moose was higher in the northern study areas, JPRF (28.6%), PG South (16.7%), and Entiako (16.7%), compared to the southern study areas, Bonaparte and Big Creek, where no evidence of exposure was recorded (Appendix H). In the northern study areas, prevalence of exposure in moose was similar to that recorded in boreal caribou from northeast BC in some years, approximately ~14% [60]. These findings may reflect differences in exposure and/or transmission rates related to variation in the diversity of sympatric species, environmental conditions, and/or other health determinants such as the levels of physiological stress, body condition, and co-infections, encountered by moose across BCPMRP study areas. It is important to recognize that nothing is currently known of the ecology of this organism in BC or most wild ecosystems.

Erysipelothrix rhusiopathiae - Tissue Culture

The culture of tissue samples collected from animals suspected of dying from infectious bacterial diseases may be informative. In free-ranging wildlife, the success of this approach is often limited by the relatively poor quality of tissues obtained from mortality site investigations. Accurately identifying fastidious and/or fragile primary bacterial pathogens such as *E. rhusiopathiae* is especially problematic and may be limited by the overgrowth of enteric, commensal, or environmental bacteria, biochemical inhibitors in decomposing carcasses, and/or environmental conditions such as freezing/thawing or heat. In addition, decomposition often limits the utility of performing the histopathologic analysis

necessary to definitively link the presence of a particular pathogen to clinical disease in and/or the death of an animal.

Bone marrow often remains relatively protected during the decomposition process and marrow culture has proven effective for identifying infections with and mortality caused by some bacterial pathogens many weeks to months after death (e.g. *Pasturella multocida* causing systemic avian cholera in waterfowl). Recent work in muskox, caribou, and moose indicates that this strategy can be effective for identifying systemic *E. rhusiopathiae* infections in wild ungulates [25-27]. *E. rhusiopathiae* has been cultured from bone marrow obtained from a small number of moose carcasses in BC. Bone marrow samples collected from 100 moose mortalities as part of the BPMRP have been submitted to the Western College of Veterinary Medicine, Saskatoon for *E. rhusiopathiae* culture (including genetic sequencing of isolates) to explore the prevalence of infection with and potential importance of this pathogen in BC moose. Polymerase chain reaction (PCR) testing (identification of pathogen genetic material) is also being employed as an ancillary test to identify *E. rhusiopathiae* DNA in moose bone marrow samples where the live bacteria is not recovered due to poor sample quality (see Appendix B).

In 2018, tissue culture was used to determine the presence of *E. rhusiopathiae* in mortality samples. The same samples were also tested using PCR for comparison but results were discrepant. Three of the 10 sample were positive using culture methods, and none were found positive using PCR. Only one of the culture positives was found to be serologically positive at capture. The discrepancy is considered due to the poor sensitivity of the tests and the volume of sample used by the different methods. PCR results only are included in the summary (Appendix E). Further research into *E. rhusiopathiae* and its relationship to moose health in BC is warranted.

4.5.3 Gastrointestinal Parasites

Overall, the prevalence of infection and diversity of gastrointestinal parasites identified in BC moose appears to be within ranges measured in moose populations elsewhere (Appendix I and J) [30-33]. The intensity of parasite infections in BC moose also appears to be low and within or below ranges measured in moose populations elsewhere [30-33]. Among BC moose, parasite diversity appears to be the greatest in the Bonaparte study area. This may reflect a greater diversity of sympatric species (both wild and domestic) encountered by moose in this region such as cattle [30-33]. This is the first large scale assessment of parasites in this species in BC so no trend interpretation is possible.

Gastrointestinal Nematodes

Nematodirinae infections were recorded in a relatively large number of BC moose and a similar proportion of moose from each study area (Appendix I). *Nematodirella alcidis* is the parasite most often found in the gastrointestinal tract of free-ranging North American moose [30]. There is evidence that infection with this parasite may be sporadically killing young moose from the Bonaparte study area. A severe infection with gastrointestinal nematodes (probable *Nematodirella alcidis*) was

identified as the most likely cause of a male calf's poor body condition and ultimate death. Case reviewed and photos in Appendix K.

Strongylid infections were only recorded in moose from the Bonaparte study area and at relatively low prevalence (Appendix I). Strongylid nematodes are rarely recorded in North American moose [30] but are regularly found in moose from Scandinavia where moose parasite diversity is relatively high and contact with domestic livestock is relatively common [61, 62]. The potential subclinical and clinical effects of infection with Strongylid nematodes in moose are likely similar to those caused by Nematodirids [61, 62].

Identification of Strongylid infections in the BCPMRP study has been based on egg morphology, but the precise species of Strongylid(s) could not be determined. *Ostertagia* sp. and *Marshallagia* sp. are Strongylid nematodes that have been previously recorded in North American moose [30]. *Trichuris* sp. infections were only recorded in moose from the Bonaparte study area and at relatively low prevalence. Infections have been recorded in peri-agricultural moose populations in Ontario and Alberta but appear to be rare elsewhere [30-33]. The effects of *Trichuris* sp. infections on free-ranging moose are not currently known. In domestic livestock *Trichuris* sp. often infect young animals and can cause significant disease (severe to fatal diarrhea) in heavily infected animals.

Cestodes

Tapeworm (*Moniezia benedeni*) infections were recorded in relatively few BC moose overall, and in a similar proportion of moose from each study area. These findings are consistent with moose populations elsewhere [30-33]. *Moniezia* sp. are not generally considered to be clinically significant in wild ungulates unless they occur in exceptionally large numbers. Nonetheless, sub-clinical effects of moderate to heavy infections are probable and may play a role when considering a cumulative effects model of moose health.

Lung and Tissue Dwelling Nematodes

No evidence of infection with lung and/or tissue dwelling Protostrongylid nematodes has been recorded in any BCPMRP moose tested to date. *Varestrongylus eleguneniensis* is a small Protostrongylid lungworm sometimes found in the airways or parenchyma of moose lungs. The significance of infection in moose is unknown at the present time [35].

No evidence of infection with lungworms was recorded in any fecal samples of BCPMRP moose (Appendix I). However, analysis of tissues obtained from dead moose in the Bonaparte study area indicate that lungworm infections (probable *Dictyocaulus* sp.) were relatively common and may be at least a contributing factor to some moose mortalities in this region. Evidence of verminous (parasitic) pneumonia most likely associated with *Dictyocaulus* sp. infection of lung tissue samples was recorded in 4 of 5 dead moose in the Bonaparte study area but no total assessment of lung burden was made. *Dictyocaulus* sp. are large and obvious nematodes found in the bronchi and bronchioles of the lungs.

Dictyocaulus sp. larvae and adults can cause significant lung damage and respiratory disease in heavily infected animals [30].

Of these cases, one was severe enough to be considered as the most likely cause of death in the affected moose calf. In contrast, 3 cases (1 adult cow and 2 calves) were considered mild to moderate or incidental (1 calf), possibly secondary to other factors such as poor condition (1 cow), or could not be determined (1 calf). Lungworm infections appear to be relatively common (prevalence up to 15-18%) in peri-agricultural moose populations [31-33] and the pattern of infection and disease observed in BC moose is consistent with the ecology of lungworm infections in other ungulates, the significance to overall health of the animal is poorly understood. Lungworm larvae are relatively delicate and can be destroyed when fecal samples are improperly stored or are frozen and thawed repeatedly. Deficiencies in sample processing and storage likely explain why no lungworm larvae were identified in fecal analyses (Appendix L).

Trematodes and Gastrointestinal Protozoans

No evidence of infection with Trematodes (flukes) or *Eimeria* sp. (gastrointestinal protozoans) has been recorded in any BCPMRP moose fecal sample examined to date. Infections with rumen (e.g. *Paramphistomum* sp.) or caecal flukes (*Zygocotyle lunata*) are sporadically recorded in moose across their distribution range but are not considered to be clinically significant [31]. Infections with giant liver fluke (*Fascioloides magna*) can cause severe or fatal disease in moose [34]. However, moose are dead-end hosts for this parasite and eggs are not typically found in the feces of infected animals [34]. *F. magna* is present in BC but no records in moose or other ungulates are reported in the BCPMRP study areas. *Eimeria* sp. is not generally found in North American moose but has been recorded in Scandinavian moose [30].

Recommendations for an Enhanced Parasitology Program in British Columbia Moose

The current parasite data is the beginning of a valuable baseline and results are currently pending for 175 fecal samples from BCPMRP moose. Continued monitoring of moose parasite burdens in all BCPMRP study areas is warranted. However, a modified testing strategy is recommended. Special attention should also be directed towards developing an enhanced parasitology program to improve understanding of parasite diversity, prevalence, intensity, and affects in the Bonaparte study area and any study area where moose calves are being captured. In all study areas, floatation and Baermann analysis should continue to be performed on all fecal samples collected from live-captured moose while sedimentation testing (for flukes) could be discontinued due to the low prevalence (0%) of fluke infections identified. Fecal samples from recently dead moose should also be evaluated, especially for mortalities occurring in spring through fall when burdens may be highest.

Genetic/DNA based identification (e.g. PCR for Protostrongylid DSLs, Nemabiome for Trichostrongylids etc.) of parasites identified in BC moose is strongly recommended as it will provide a more complete

picture of parasite diversity and potential health risks. These tests are somewhat costly and it may be best to limit this approach to the Bonaparte and another "control" study area where parasitism has not been identified as a potential threat to moose health.

Gastrointestinal parasite diversity and burdens, particularly Strongylate and Nematodirinae nematodes, can also be evaluated by opening the abomasum and anterior portion of the small intestine and collecting adult worms (in 70% ETOH at a 10:1 ratio) for later identification. This approach is very labour intensive and impossible to do properly in the field. If pursued, it is recommended that the abomasum and anterior small intestine (first 3-4 feet) be tied off with string then removed and frozen for later analysis. Like molecular/genetic identification of parasites, this approach may be best limited to the Bonaparte and a comparative study area. In all study areas it is still worth opening the abomasum and the first few feet of the small intestine in the field and collecting any obvious parasites in 70% ETOH. Likewise, samples of the intestinal tract and contents should be collected when any abnormalities (e.g. Appendix K Figure 14d, discolouration, abnormal contents etc.) are noted.

The eggs and/or larvae of many parasite species, such as Strongylid nematodes*, Dictyocaulus* sp. lungworms etc*.,* are adversely affected by freezing and freeze thaw cycles. Many BC moose fecal samples had been stored frozen for at least 12 months prior to analysis and some were inadequately packaged (i.e. in partially opened zip-lock bags compared to well-sealed whirl-paks). As a result, it is very likely that the results presented here underestimate the true diversity, prevalence, and intensity of gastrointestinal parasites in BC moose. The collection and rapid evaluation of fresh (or even formalin fixed) fecal samples would be more informative, particularly for the Bonaparte study area and moose calves (Appendix L).

The prevalence and intensity of many parasitic infections also vary seasonally. Non-invasive collection of moose fecal samples across seasons could supplement information gained from live-captured and dead animals. This work could even be combined with other potentially informative data that can be obtained from fecal samples (e.g. stress hormones, reproductive hormones/pregnancy status, diet, population genetics, microbiome etc.). Non-invasive fecal collection programs are used as part of community-based moose and caribou health assessment programs elsewhere and could provide similar opportunities for outreach and engagement with stakeholders in BC moose conservation.

The recommendations above require a strong collaboration with wildlife parasitologists and researchers, and increased resources and may be beyond the scope of the current program. Currently there are no such programs even for routine diagnostic work in Canada.

4.5.4 Tissue Dwelling Protozoans

Neospora caninum

The exposure to *N. caninum* recorded in BC moose was within the range recorded in moose populations elsewhere [38, 41] (Appendix M), and is similar across most study areas with the exception of PG South where exposure was not present. The probability of a female moose being non-pregnant or not having a calf at heel was unrelated to *N. caninum* exposure[1]. Exposure to *N. caninum* was recorded in 20% of moose calves from the Bonaparte study area in winter 2017, 5.0% of calves captured in the Bonaparte study area in winter 2018, and none of the calves from the Big Creek study area in winter 2018 (Appendix M).

Toxoplasma gondii

The exposure to *T. gondii* in BC moose was low and within the range recorded in moose populations elsewhere [43]. Exposure was seen in two moose from the Bonaparte study area and one in the PG South study area. Both of these Bonaparte moose were pregnant at the time of capture; their calves were collared and are still alive at 3 years of age. No evidence of exposure to *T. gondii* was recorded in any moose calf captured in the Bonaparte or Big Creek study areas (Appendix M).

Considerations and Recommendations for Tissue Dwelling Protozoans

At the population level, preliminary findings suggest that *N. caninum* and *T. gondii* are not related to decreased pregnancy rates in BC moose. However, antibody production (as measured with a single ELISA test in an individual moose) cannot precisely identify the timing of exposure, seroconversion and/or infection (Appendix B). As a result, adverse effects on the reproductive fitness of individual moose cannot be ruled out.

Although exposure to *N. caninum* does not appear to be related to the probability of a moose cow with a live calf in the winter, the actual impact of these parasites on the viability, survival, and recruitment of moose calves is not yet clear. Given the relatively high prevalence of exposure noted in moose calves from the Bonaparte study area this is an important research question. Continued monitoring of exposure to *N. caninum* and *T. gondii* is recommended for all BCPMRP study areas. In this context, evaluation of exposure and infection in neonatal calves (from known status cows) may provide better insight into the frequency of transmission during gestation and the long-term effects of exposure/infection on calf survival and recruitment. Monitoring exposure in older calves does not permit these linkages to be rigorously investigated (i.e. since exposure in utero vs. from the environment as a juvenile cannot be differentiated).

[1] Relative risk of not being pregnant if exposed: RR=1.47 (95% C.I. 0.74-2.91), P=0.274. Relative risk of not having a calf at heel if exposed: RR=0.748 (95% C.I. 0.486-1.15), P=0.186.

Monitoring changes in the serostatus of these and other pathogens in individual moose captured and sampled multiple times over a number of years is also strongly recommended. *N. caninum* and *T. gondii* should continue to be considered whenever abortions, other reproductive abnormalities, or unthrifty calves are identified in BC moose.

4.5.5 Physiological Stress

A wide range of hair cortisol (HCC) levels was recorded in BCPMRP moose (mean adult cows 4.21 pg/mg, range 0.42 pg/mg-21.14 pg/mg, n=119; mean calves 15.26 pg/mg, range 4.36 pg/mg-51.05 pg/mg, n=13). Findings suggest HCC varies with moose life history traits including age and reproductive status as well as with exposure to health determinants such as body condition and winter tick burden. HCC levels varied between study areas years within the same study area. These findings agree with HCC research in other species and support continued investigation of HCC as a health biomarker in BC moose.

Study Area

In winter 2013/2014 only, variation in HCC levels measured in BC moose was explained by study area[2]. HCC levels were highest in Entiako moose[3,4].

Collection Year

Variation in HCC levels in Entiako moose was explained by sample collection year[4]. HCC levels measured in winter 2013/2014 were lower than those measured in winter 2016.There was no difference in HCC levels measured in winter 2016 and 2017.

In the Bonaparte, PG South, and John Prince Research Forests (JPRF) study areas, HCC levels measured in moose in winter 2017 were greater than those measured in winter 2014[5,6,7].

Physiological Factors

HCC levels measured in Bonaparte moose calves in winter 2017 were higher than HCC levels in moose cows captured in the same study area and year[6]. HCC levels measured in moose cows that were not pregnant were marginally greater than HCC levels measured in moose cows that were pregnant[7]. HCC levels measured in moose cows with a live calf were greater than HCC levels measured in moose cows that did not have a calf at heel at the time of capture[8]. Variation in HCC levels was explained by moose

[2] One-way ANOVA, $F_{4,58}$=9.359, P<0.0001, n=28 Entiako, n=10 Bonaparte, n=10 Big Creek, n=5 PG South, n=10 JPRF
[3] Tukey- Kramer, P<0.05
[4] One-way ANOVA, $F_{2.47}$=4,472, P=0.017, n=28 winter 2013/2014, n=18 winter 2016, n=5 winter 2017
[5] Unpaired t-test, t_{22}=8.295, P<0.0001, n=10 2013/2014, n=14 2017
[6] Unpaired t-test, t_{25}=2.905, P=0.0076, n=13 calves, n=14 cows
[7] Unpaired t-test, t_{113}=1.703, P=0.050, n=20 open cows includes n=2 not pregnant/PSPB high recheck=evidence of abortion, n=97 pregnant cows
[8] Unpaired t-test, t_{113}=2.353, P=0.020, n=37 cows with a calf, n=78 cows with no calf

body condition score[9]. HCC levels were greatest in moose that were in poor condition or emaciated versus those in fair, good, or excellent condition[c]. There was no difference in HCC levels measured in moose classified as being in fair, good, or excellent condition. Variation in HCC levels was explained by moose winter tick burden score[10]. HCC levels were lowest in moose where no ticks were obvious versus those with few ticks or moderate to heavy burdens[c]. There was no difference in HCC levels measured in moose with few ticks or moderate to heavy burdens.

In summary, HCC is best considered as an annual, integrated measure of physiological stress (i.e. HCC is influenced by challenges faced by moose during the hair cycle that occurs between annual molts). As a result, the most meaningful comparisons across different moose populations are in the same sampling season. It must be noted that HCC is strongly influenced by collection, processing, and analytical methods, as well as by numerous biological covariates such as age and reproductive status [reviewed 44]. Caution is warranted when comparing results from hair cortisol studies using different methods and in interpreting the significance of results at the individual and population level. Hair cortisol holds significant promise as a biomarker of chronic stress in moose; however, further validation is required against life history traits, condition, and environmental parameters. This health measure is strongly recommended for the BCPMRP.

4.5.6 Trace Nutrients

Serum Trace Nutrient Levels in British Columbia Moose

Serum trace nutrient levels were measured in live-captured moose from the Big Creek, PG South, and JPRF study areas of British Columbia in winter 2017, and all study areas in 2018 (Appendix O, Table G). Normal ranges for serum trace nutrients are not available for moose and characterization of status was based on reference ranges for domestic ruminants (domestic cattle and/or sheep) and other wild cervids. Based on these values, evidence of probable trace nutrient deficiencies was identified in BC moose serum.

The proportion of likely deficient moose varied depending on the element evaluated [e.g. 0 % (iron) to ~ 100% (copper and selenium)] (Appendix O, Table H). Serum iron, copper, and zinc levels in BC moose were also compared to those measured in three American moose populations where health was considered to be suboptimal (Appendix O Table I). Differences were identified across populations. For example, copper levels measured in BC moose without symptoms were lower than those from an Alaskan moose population considered to suffer from clinical disease related to copper deficiency (Appendix O, Table I). Selenium levels were lower in Bonaparte moose calves compared to cows from the same study area. No BC moose cow had serum iron levels below a proposed pregnancy threshold

[9] One-way ANOVA, $F_{3,111}$=7.778, P<0.0001, n=11 emaciated/poor, n=15 fair, n=82 good, n=7 excellent
[10] One-way ANOVA, $F_{2,102}$=41.740, P<0.0001, n=65 no ticks obvious, n=15 few, n=10 moderate to heavy burden

of <0.83 ug/ml [identified in 53]. Variation in serum levels of zinc, copper, cobalt, and iron variation was explained by study area (Appendix O).

Zinc levels measured in moose from Big Creek and Bonaparte were lower than PG South and in the JPRF. Copper levels in moose from Bonaparte and Big Creek were lower than those in PG South study area and in the JPRF. Cobalt levels were lowest in the PG South study area.

Iron levels were lower in PG South and JPRF than Bonaparte and Big Creek study areas.

Tissue Trace Nutrient Levels in British Columbia Moose

Evidence of marginal or deficient levels of manganese, iron, cobalt, copper, and selenium were identified in liver samples obtained from dead moose in the Bonaparte, PG South, and Big Creek study areas (Appendix O, Table A). Liver and/or kidney mercury levels measured in selected samples were within normal limits (Appendix O, Tables A and B). Overall, liver levels of copper were lower in moose from PG South compared to Bonaparte. Evidence of marginal or deficient levels of manganese, iron, cobalt, copper, and selenium were also identified in kidneys of dead moose from the Big Creek and PG South study areas (Appendix O, Table B). One moose from PG South may have died as a result of copper toxicity (Case Study, Appendix P).

Classification of trace nutrient status using paired liver and kidney samples from the same moose did not always agree (Appendix O, Table C). Likewise, the relationship between liver and kidney levels of some elements was inconsistent indicating that caution may be warranted when using and interpreting data derived from kidney samples in moose.

Trace nutrient levels measured in post mortem liver and/or kidney samples collected from adult female moose in Bonaparte and PG South were compared to levels determined in free-ranging moose populations from Alaska and Minnesota, USA where moose health status and population performance are considered to be suboptimal (Appendix O, Tables D, E and F). Trace nutrient levels in BC moose appear to be within the ranges identified in these poorly performing American moose populations. However, differences in levels of specific nutrients were identified across populations. For example, levels of magnesium, manganese, iron, zinc, and molybdenum measured in BC moose livers were less than those measured in moose livers from a declining moose population in Minnesota, USA while liver levels of copper and selenium were similar. Conversely, liver copper levels measured in BC moose were higher than those measured in a poorly performing Alaskan moose population. Continued serum and tissue trace nutrient testing is recommended in order to determine the significance of levels relative to health outcomes.

4.6 Health Related Mortality Observations

4.6.1 Tumors

Several adult female moose from PG South and JPRF had cutaneous fibromas/fibropapillomas at the time of capture in winter 2017 (Figure 11). These tumors are relatively common in moose in BC [e.g. 66] and are caused by a papilloma virus. The virus is transmitted via infected skin cells by direct contact between animals or contaminated fomites (e.g. rub trees). Superficial wounds or cuts facilitate transmission. Papillomas are not generally considered to cause adverse effects in wildlife unless they are exceptionally large, numerous, or impede vision or movement. Many of the papillomas observed appeared abraded and were leaking serum and/or blood, likely from the moose recently scratching against trees. If a cumulative effects model of moose health is considered, it is doubtful these tumors are entirely inconsequential as constant wound healing represents an energetic demand not encountered by unaffected animals. An adult female moose from the PG South study area was also found with a large mass behind the left eye at capture (Figure 12). The undiagnosed mass was covered in hair, firm, and relatively immobile. The orbit and skull appeared to be involved. The most likely differential for this finding is an uncharacterized neoplasia (tumor).

Figure 7. Cutaneous papillomas on the shoulder of an adult female moose captured in the JPRF study area in winter 2017.

Figure 8. An uncharacterized mass associated with the orbit and skull of an adult female moose from the PG South study area in winter 2017.

4.6.2 Blind Circling Moose

In early October 2017 a young female moose with cloudy, white eyes was reported by a hunter near Kamloops. The moose was circling and appeared to be blind but was in otherwise good condition. Blind circling moose have been reported sporadically across western Canada. Cases may occur more frequently in peri-agricultural areas however, this may be an artefact of increased encounter rates. There are a number of hypotheses regarding the cause including bacterial and/or viral infections and centrally mediated processes (e.g. brain damage or dysfunction caused by an uncharacterized agent or process). Blind, circling moose are usually euthanized and a complete necropsy with comprehensive health testing is recommended whenever they are encountered according to a standard protocol by the WAFWA Wildlife Health Working Group. The protocol for investigating these cases can be obtained from the BC Wildlife Health Program.

5.0 Summary

Evaluation of health data from BC live-captured moose as well as mortality event investigations, suggests that the occurrence and potential impact of selected health determinants may vary across BCPMRP study areas. Findings suggest that exposure to and/or infection with most of the viral, bacterial, and parasitic health determinants evaluated to date are within ranges reported in moose populations elsewhere [9,13,22,31]. Although there is clear evidence that some determinants, such as gastrointestinal parasitism, may be sporadically killing at least young moose in some study areas, no single factor can be identified as the cause of apparent differences in the overall health status and/or performance of BC moose populations at the present time. The scope of the current moose health initiative, and relatively small sample sizes in some years, cannot adequately evaluate the potential sub-lethal or cumulative effects of various health determinants on the fitness of individual moose or the performance of BC moose populations.

Findings from some study areas are potentially concerning. The relatively low pregnancy rates and evidence of reproductive failure in Bonaparte cow moose are perhaps the most notable examples. More in-depth investigation of moose reproductive health and moose calf health and survival is needed in this study area, including investigation into what habitat and environmental features may be contributing to the higher tick burden, poor body condition, and evidence of physiological stress in this study area.

Although trace nutrient requirements and metabolism in moose are not well characterized, we now have sufficient samples to estimate baseline values for BC moose and identify outliers. Levels of some elements appear to be sub-optimal with a variation in trace nutrient levels apparent across BCPMRP study areas. Continued research into nutrition, trace nutrient levels and their impacts on BC moose health and fitness is warranted. Integration of this work with studies evaluating the diversity, quality, quantity, and phenology of moose forage available in different BCPMRP study areas is strongly recommended.

Continued monitoring of selected moose pathogens and parasites assessed in this report as part of comprehensive moose herd health baselines is encouraged for all BCPMRP study areas. This should include the evaluation of additional ermerging infectious and non-infectious health determinants, along with more in-depth exploration of the current selected determinants. To reduce costs, an expanded initiative could be limited to one or two key study areas where moose health or productivity currently appears to be most compromised (e.g. Bonaparte and PG South), with comparison to a “control” area where these parameters appear to be more optimal (e.g. JPRF).

The development of community and/or harvester based moose health assessment and sampling programs is encouraged. These initiatives could engage First Nations and stakeholders in BC moose health assessments and management and increase samples sizes for moose health research. Pursuing collaborative research with other moose health programs (e.g. NWT Moose Health Assessment Program) may also be beneficial.

In summary, it is recommended to enhance the collection and analysis of health determinants measured in moose and combine longitudinal monitoring at the individual and population level to rigorously evaluate the importance of health as a driver of moose population dynamics in BC. Longitudinal moose health monitoring is recommended for all BCPMRP study areas using the same diagnostic testing methods (i.e. use the same tests run at the same labs) so data is directly comparable. Recapture of individual moose should include re-sampling and tracking over years, especially in areas where the health status of moose appears to be suboptimal (Bonaparte and PG South). The British Columbia Boreal Caribou Health Program [27] provides a model for wild ungulate health assessment for this moose study.

5.1 A Recommendation for a Cumulative Effects Model of Moose Health in British Columbia

To date, the health assessment strategy employed by the BCPMRP has focused on identifying a series of health determinants, including several key pathogens and parasites, considered to potentially impact moose survival or reproduction. However, considering moose health in the context of a cumulative effects framework (Figure 13) is likely to yield a more complete understanding of how moose health, individual fitness, and population performance are linked. Evaluating moose health in this manner is complex but may be a more effective strategy to identify the most important threats and to develop potential management strategies to mitigate risk or respond to emerging challenges [67].

Evaluating the health status of BC moose within a cumulative effects framework is strongly recommended. For example, under such a model, moose from the Bonaparte appear to be most at risk. Other potential health determinants that can fit under the model, including the diversity of sympatric species, including domestic livestock, anthropogenic or natural landscape characteristics, were not evaluated as part of this review. However, the Bonaparte moose population appears to be challenged by the greatest total burden of pathogen/disease related factors affecting moose survival or reproduction and the least resilient and sustainable population in the study.

The health status of the moose reflects the cumulative effects of natural and anthropogenic challenges acting on individual moose and moose populations [67]. Extrinsic determinants of health (outer circles) interact with each other and intrinsic biological characteristics (middle circle) to determine the health profile of moose (inner circle). This health profile is dynamic and may vary across time and space. It is

directly linked to the fitness (reproduction and survival) of individual animals and the performance (reproductive and survival rates, juvenile recruitment) of moose populations as well as to the resilience and ability of each to cope and remain sustainable with natural or anthropogenic challenges.

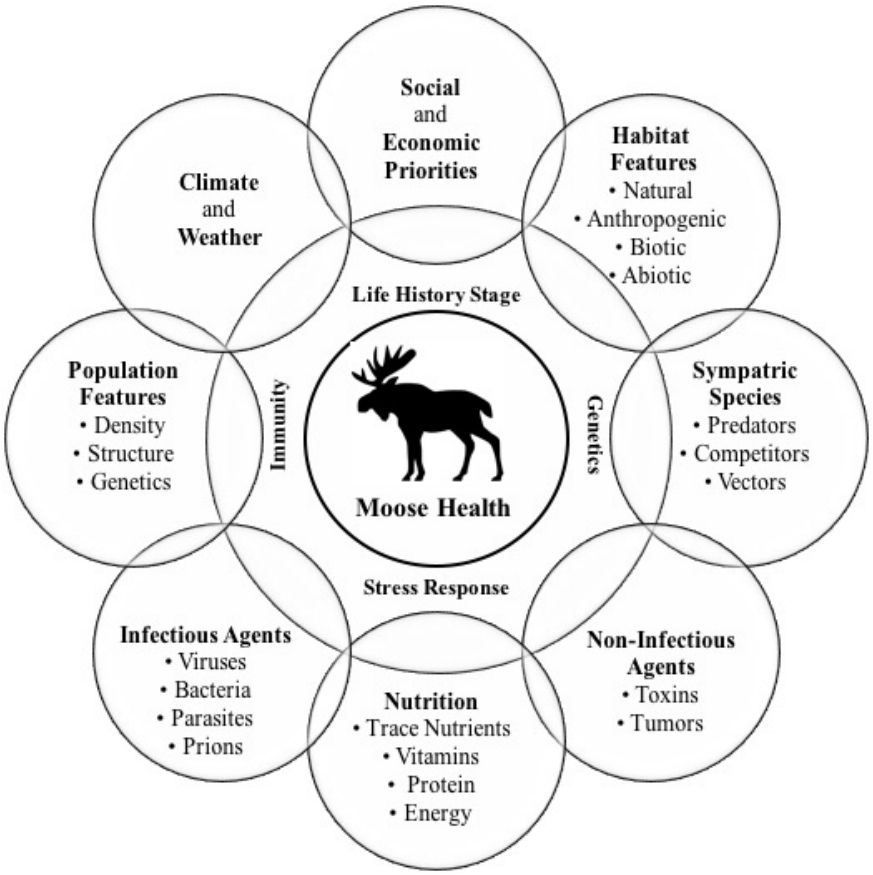

Figure 9. A cumulative effects model for moose health.

The health status of the moose reflects the cumulative effects of natural and anthropogenic challenges acting on individual moose and moose populations [67]. Extrinsic determinants of health (outer circles) interact with each other and intrinsic biological characteristics (middle circle) to determine the health profile of moose (inner circle). This health profile is dynamic and may vary across time and space. It is directly linked to the fitness (reproduction and survival) of individual animals and the performance (reproductive and survival rates, juvenile recruitment) of moose populations as well as to the resilience and ability of each to cope and remain sustainable with natural or anthropogenic challenges.

5.2 Future Health Sampling and Research Direction

The assessment of BC moose health and associated provincial research were designed from one developed for boreal caribou as a wild ungulate herd health model [69]. For both species little health testing had been performed in BC and the model was created to develop a baseline dataset, valuable for long-term health research and herd monitoring. Knowledge gaps identified in this report can be used to further research efforts to inform management decisions. The following is a summary of specific recommended areas of future research:

1. Continue to measure and monitor moose pathogens, parasites, and health determinants impacting female and calf moose survival and reproduction.

2. If there are confirmed effects, enhance and focus diagnostic testing in those study areas , and include at least one control study area for comparison.

3. Include longitudinal monitoring of individual moose by re-capturing and sampling animals to evaluate changes in health determinants.

4. Include the assessment of weather, sympatric species, landscape and other environmental factors and associate with health determinants and their variation.

5. Continue to focus on objective measures of nutrition and tick burdens:
    a. Ultrasound determination of back fat is recommended to determine body condition of live animals, with capture dates moved earlier.
    b. A standardized tick counting protocol has already replaced tick scoring.

6. Ensure that lactation status is recorded for captured cow moose. Lactation status is a key determinant for winter body condition in wild ungulates and an important parameter in seeking to understand factors influencing moose body condition.

7. Continue measuring trace nutrient levels and consideration of their impacts on moose health and fitness. This is an area with potential experimental application.

8. Employ genomic/DNA/RNA based identification of:
    a. parasites identified in BC moose - strongly recommended for a complete picture of parasite diversity and potential health risks.
    b. gene transcription - with cortisol measures (fecal and hair) determines herd stress levels and relates to cumulate effects.

9. Continue and enhance investigations of moose reproductive health and moose calf health and survival as possible. See above for the need for an improved understanding of the habitat and environmental features contributing to health (ie. the tick burdens and associated physiological stress in some study areas).

10. Record and relate specific maternal factors (e.g. maternal body condition, HCC, and trace nutrient profile) with calf survival. Associate these factors with body condition and forage quality research underway.

11. Continue to use new methodologies wherever possible to enhance assessments of determinants of health.

12. Implement community/harvester based moose health assessment sampling where there is interest for engagement and to increase sample size.

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Appendix A - Viral and bacterial pathogens evaluated

Pathogens, parasites, and non-infectious health determinants evaluated in live-captured moose from British Columbia, Canada.

Health Determinant	Diagnostic Test	Testing Location [a]
Viral Pathogens		
Paramyxoviruses [Parainfluenzavirus-3 (PI3)] [b]	ELISA and/or VNT [c]	AHC
Pestiviruses [Bovine Viral Diarrhea Virus (BVDV)]	ELISA and/or VNT	AHC and PDS
Alphaherpesviruses [Bovine herpesvirus-1, (IBR)]	ELISA	AHC and PDS
Bacterial Pathogens		
Mycobacterium avium ssp. *paratuberculosis* [b]	ELISA	AHC
Eysipelothrix rhusiopathiae[d]	ELISA, PCR, culture	CWHC, UCVM, WCVM
Gastrointestinal Parasites		
Gastrointestinal Nematodes	Fecal Flotation	CWHC and UCVM
Cestodes (Tapeworms)	Fecal Flotation	
Coccidia (Enteric Protozoans)	Fecal Flotation	
Lungworms	Baermann	
Protostrongylid DSLs	Baermann	
Trematodes (Flukes)	Fecal Sedimentation	
Tissue Dwelling Protozoan Parasites		
Neospora caninum	ELISA	PDS
Toxoplasma gondii	ELISA	WCVM
Non-Infectious Health Determinants		
Hair Cortisol Concentration (Physiological Stress Levels)	ELISA	WCVM
Serum Trace Nutrient Levels (Mn, Fe, Co, Cu, Zn, Se, Mo)	High-Performance Liquid Chromatography	PDS

[a] BC Animal Health Centre, Abbotsford, BC (AHC), Prairie Diagnostic Services Inc., Saskatoon, SK (PDS), Western College of Veterinary Medicine, Saskatoon, SK (WCVM), Canadian Wildlife Health Cooperative, Calgary, AB (CWHC), University of Calgary Faculty of Veterinary Medicine, Calgary, AB (UCVM), Western College of Veterinary Medicine, Saskatoon, Saskatchewan (WCVM)

[b] Denotes pathogen investigated only in the early stages of the BCPMRP.

[c] Enzyme-linked immunosorbent assay (ELISA), virus neutralization test (VNT).

[d] ELISA was performed in 2015 and 2017 at CWHC and UCVM respectively. PCR and culture was performed at WCVM in 2018.

Appendix B - Terminology

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.

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.

Note on Serological Testing in Wild Ungulates

There are few serological tests validated for wildlife and the use of diagnostic tests designed for domestic ruminants is standard practice for health and disease testing in free-ranging ungulates. This practice is based on the presence of similar antigens on and immunological cross reactions between domestic and wild ungulate pathogens that infect related species (e.g. ruminants). Accordingly, serological evidence of “exposure” in moose may reflect: 1) genuine exposure to livestock pathogens; and/or 2) exposure to moose specific pathogens closely related to those of domestic livestock. For example, moose from areas with few cattle that test positive for exposure to infectious bovine rhinotracheitis (IBR) are more likely infected with a moose specific alphaherpesvirus than bovine herpesvirus-1 (the alphaherpesvirus responsible for IBR in livestock). Where moose and cattle frequently overlap it is possible that seropositive animals may have been exposed to either (or both) viruses. In animals with active or latent

infections, molecular or genetic testing (e.g. PCR or genetic sequencing) may be used to more accurately identify the specific pathogen in question.

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 from materials present in the samples. The presence of live pathogens in a tissue sample is not required to obtain a PCR positive result.

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

For most pathogens, linking PCR and/or culture results to clinical disease and/or mortality requires histopathology confirming there are structural changes in the tissues associated with the infection

Appendix C – Detailed Pregnancy Results

Pregnancy Status of n=438 adult female moose live-captured in British Columbia, Canada in winter 2012/2013, 2013/2014, 2015, 2016, 2017, and 2018 [a].

Year	All Study Areas	Bonaparte	Big Creek	PG South	JPRF	Entiako
Pregnancy Rate and 95% C.I. All Study Years [b]	**78.8%** (n=297/377)	**64.5% [c]** (n=78/121) (55.6%-72.4%)	**89.1% [g]** (n=49/55) (78.2%-94.9%)	**78% [i]** (n=46/59) (65.9%-86.7%)	**93.8%** (n=43/48) (83.2%-97.9%)	**83.6% [l]** (n=61/73) (73.4%-90.3%)
Pregnancy Rate and 95% C.I. Winter 2012/2013	-	**72%** (n=18/25) (52.4%-85.7%)	-	-	-	-
Pregnancy Rate and 95% C.I. Winter 2013/2014	**88.6%** (n=109/123)	**84.6% [d]** (n=11/13) (57.7%-95.7%)	**89.5% [h]** (n=34/38) (75.9%-95.8%)	**85.7% [j]** (n=12/14) (60.1%-96%)	**100%** (n=15/15) (79.6%-100%)	**86.1% [m]** (n=37/43) (72.7%-94.5%)
Pregnancy Rate and 95% C.I. Winter 2015	**68.5%** (n=37/54)	**70.8% [e]** (n=17/24) (50.8%-85.1%)	**75%** (n=6/8) (40.9%-92.9%)	**64.3% [k]** (n=9/14) (38.8%-83.4%)	-	**62.5%** (n=5/8) (30.1%-86.3%)
Pregnancy Rate and 95% C.I. Winter 2016	**71.3%** (n=72/101)	**47.2%** (n=17/36) (32.0%-63.0%)	**100%** (n=5/5) (56.6%-100%)	**75%** (n=12/16) (50.5%-89.8%)	**88.5%** (n=23/26) (71%-96%)	**83.3%** (n=15/18) (60.8%-94.2%)
Pregnancy Rate and 95% C.I. Winter 2017	**82.7%** (n=43/52)	**68.2% [f]** (n=15/22) (47.3%-83.6%)	**100%** (n=4/4) (51.1%-100%)	**86.7%** (n=13/15) (62.1%-96.2%)	**100%** (n=7/7) (64.5%-100%)	**100%** (n=4/4) (51.1%-100%)

Pregnancy Rate	**83.3%**	**50%**	**50%**	**66.7%**	**91.7%**	**90%**
and 95% C.I.	(n=30/36)	(n=3/6)	(n=1/2)	(n=4/6)	(n=11/12)	(n=9/10)
Winter 2018		(11.8%-88.2%)	(9.4%-99.2%)	(22.3%, 95.7%)	(61.5%, 99.8%)	55.5%-99.7%

[a] 2012/2013 captures occurred December 2012 and January, 2013 onward. 2013/2014 captures occurred December 2013 and January, 2014 onward. 2015 captures occurred January, 2015 onward. 2016 captures occurred January, 2016 onward. 2017 captures occurred January, 2017 onward. 2018 captures occurred January, 2018 onward. Subsequent captures occurred January of the year onwards.

[b] All study years and all radio-collared adult female moose captured in the study area.

[c] Calculation **does not include** n=3 samples where pregnancy status could not be clearly determined using progesterone testing and could not be clarified with follow up PSPB testing (no sample remaining or discordant result). See d, e following for details.

[d] Calculation **does not include** n=1 sample where pregnancy status could not be clearly determined using progesterone testing and could not be clarified with follow up PSPB testing (no sample remaining or discordant result).

[e] Calculation **does not include** n=2 samples where pregnancy status could not be clearly determined using progesterone testing and could not be clarified with follow up PSPB testing (no sample remaining or discordant result).

[f] Calculation **includes** n=2 samples classified as "high recheck" based on PSPB testing (interpreted as: not pregnant with evidence of early embryonic death). If these samples are removed from analysis pregnancy rate for Bonaparte in the winter of 2017 increases slightly to 75%, n=15/20, 95% C.I. 53%-88.8%.

[g, h] Calculation **does not include** n=2 samples where pregnancy status could not be clearly determined using progesterone testing and could not be clarified with follow up PSPB testing (no sample remaining or discordant result).

[i] Calculation **does not include** n=5 samples where pregnancy status could not be clearly determined using progesterone testing and could not be clarified with follow up PSPB testing (no sample remaining or discordant result). See j, k following for details.

[j] Calculation **does not include** n=3 samples where pregnancy status could not be clearly determined using progesterone testing and could not be clarified with follow up PSPB testing (no sample remaining or discordant result).

[k] Calculation **does not include** n=2 samples where pregnancy status could not be clearly determined using progesterone testing and could not be clarified with follow up PSPB testing (no sample remaining or discordant result).

[l, m Calculation] **does not include** n=2 samples where pregnancy status could not be clearly determined using progesterone testing and could not be clarified with follow up PSPB testing (no sample remaining or discordant result).

Appendix D – Evidence of Reproductive Failure

Evidence of reproductive failure in n=2 adult female moose from the Bonaparte Study Area, British Columbia in spring 2016.

				Health Status at Capture				
Moose WHID	**Capture Year**	**Date of Death (2016)**	**Description**	**Body Condition Score**	**Tick Burden Score**	**Calf at Heel**	**Pregnancy Status (PSPB)**	**Exposure to** **a) *Neospora caninum*** **b) *Toxoplasma gondii*** **c) Pestiviruses (BVDV)** **d) Alphaherpesviruses (IBR)**
16-7184	2016	March 7	Found alive and moribund with aborted fetus nearby [a]	Good	None Obvious	No	Pregnant	Negative NA Negative Negative
16-7200	2016	April 26	Found dead with bloody mucus discharge from vagina	Excellent	Moderate	No	Pregnant	Negative NA Negative NA

[a] The fetus and tissues from this cow were received by the wildlife health group and testing laboratory in poor condition. Molecular testing (performed at the National Institutes of Health, Bethesda, MD, USA) on the recovered fetus was inconclusive. Initial results suggested a parasitic cause of abortion/fetal death, either *Neospora* or *Toxoplasma.* However, follow-up work identified only fungal DNA in the remaining tissue samples and the appearance of the moose fetus was not consistent with fungal abortion. Inconclusive findings were most likely due to the poor quality of tissues available for testing. **This case highlights the CRITICAL IMPORTANCE of rapidly collecting (and properly handling, storing, and shipping) a full suite of tissue (including blood and any intact reproductive tract, placenta, and fetus) from moose where health appears to have been a contributing factor to reproductive failure or death. Blood can be collected from immobilized sick moose as with live captured cows or from the body cavity or heart immediately after euthanasia by gunshot. In some instances, usable blood can also be collected from the heart of recently dead moose.**

Appendix E – Trends in Prevalence of Viruses and Parasites in Adult Female Moose

Trends in prevalence of exposure to paramyxoviruses [e.g. parainfluenza-3 (PI3)], pestiviruses [e.g. bovine viral diarrhea virus (BVDV)], alphaherpesviruses [BHV-1, infectious bovine rhinotracheitis (IBR)], *Erysipelothrix rhusiopathiae*, *Neospora caninum,* and *Toxoplasma gondii* recorded in adult female moose live-captured in British Columbia, Canada in winter, 2012/2013, 2013/2014, 2015, 2016, 2017, and 2018.

	Prevalence and 95% Confidence Interval					
Sampling Year	**Paramyxoviruses**	**Pestiviruses**	**Alphaherpesviruses**	***Erysipelothrix rhusiopathiae***	***Neospora caninum***	***Toxoplasma gondii***
Winter 2012/2013 [a]	-	6.7% [g] (n=1/15) (1.2%-29.8%)	60.0% [g] (n=9/15) (35.8%-80.2%)	-	14.3% [g] (n=3/21) (5.0%-34.6%)	-
Winter 2013/2014 [b]	2.4% (n=3/123) (0.8%-6.9%)	0.8% (n=1/123) (0.1%-4.5%)	87.5% (n=7/8) (52.9%-97.8%)	-	13.3% (n=6/45) (6.3%-26.2%)	-
Winter [c] **2015**	8.4% (n=5/59) (3.8%-18.4%)	0% (n=0/59) (0%-6.1%)	-	1.9%[h] (n=1/52) (0.3%-10.1%)	-	-
Winter [d] **2016**	9.9% (n=10/101) (5.4%-17.3%)	0% (n=0/101) (0%-3.7%)	68.8% (n=11/16) (44.4%-85.8%)	-	10.9% (n=11/101) (6.2%-18.5%)	-
Winter [e] **2017**	-	0% (n=0/20) (0%-16.1%)	80.0% (n=16/20) (58.4%-91.9%)	17.5%[h] (n=7/40) (8.8%-32.0%)	11.5% (n=6/52) (5.4%-23.0%)	-
Winter [f] **2018**	-	-	-	10.5%[h] (n=8/76) (4.7%, 19.7%)	1.3% (n=1/77) (0%, 7.0%)	1.3% (n=1/77) (0%, 7.0%)

[a] Captures occurred December 2012 and January – March 2013.

[b] Captures occurred December 2013 and January – March 2014.

[c] Captures occurred January - March 2015.

[d] Captures occurred January – to March 2016.

[e] Captures occurred January – March 2017.

[f] Captures occurred January – March 2018.

[g] Data from Bonaparte study area only

[h] Reported results from PCR, culture was also done in 2018 and yielded 30.0% (3/10).

Appendix F – Trends in Prevalence of Viruses and Parasites in Moose Calves

Prevalence of exposure to pestiviruses [e.g. bovine viral diarrhea virus (BVDV)], alphaherpesviruses [e.g. BHV-1, infectious bovine rhinotracheitis (IBR)], *Erysipelothrix rhusiopathiae*, *Neospora caninum,* and *Toxoplasma gondii* recorded in moose calves of the year live-captured in the Bonaparte and PG South study areas of British Columbia in winter 2017 and 2018.

Study Area	Prevalence and 95% Confidence Interval in Bonaparte Moose Calves				
	Pestiviruses	**Alphaherpesviruses**	***Erysipelothrix rhusiopathiae***	***Neospora caninum***	***Toxoplasma gondii***
Bonaparte	0% (n=0/4) (0%-49.0%)	0% (n=0/4) (0%-49.0%)	5.3% (n=1/39) (1.0%-24.6%)	12.8% (n=6/39) (2.0%-23.7%)	0% (n=0/39) (0%-%)
PG South	-	-	14.3% (n=3/21) (3.0%, 36.3%)	0% (n=0/21) (0%, 16.1%)	0% (n=0/21) (0%, 16.1%)

Appendix G – Prevalence of Viral Pathogens

Prevalence of exposure to paramyxoviruses [e.g. parainfluenza-3 (PI3)], pestiviruses [e.g. bovine viral diarrhea virus (BVDV)], and alphaherpesviruses [e.g. BHV-1, infectious bovine rhinotracheitis (IBR)] recorded in adult female moose live-captured in British Columbia, Canada [a].

Viral Pathogen	Prevalence and 95% C.I. Province Wide [b]	Prevalence and 95% C.I. Bonaparte Study Area [c]	Prevalence and 95% C.I. Big Creek Study Area [c]	Prevalence and 95% C.I. PG South Study Area [c]	Prevalence and 95% C.I. JPRF Study Area [c]	Prevalence and 95% C.I. Entiako Study Area [c]
Paramyxoviruses	6.4% (n=18/283) (4.1%-9.8%)	20% (n=15/76) (12%-30%)	1.9% (n=1/54) (0.3%-9.8%)	0% (n=0/49) (0%-7.3%)	0% (n=0/33) (0%-10.4%)	2.8% (n=2/71) (0.8%-9.7%)
Pestiviruses	0.6% (n=2/322) (0.2%-2.3%)	2.1% (n=2/96) (0.6%-7.3%)	0% (n=0/56) (0%-6.4%)	0% (n=0/56) (0%-6.4%)	0% (n=0/35) (0%-9.9%)	0% (n=0/75) (0%-4.9%)
Alphaherpesviruses	72.9% (n=43/59) (60.4%-82.6%)	63.9% (n=23/36) (47.6%-77.5%)	100% (n=2/2) (34.2%-100%)	80% (n=12/15) (52.8%-92.9%)	100% (n=2/2) (34.2%-100%)	100% (n=4/4) (51%-100%)

[a] See section 4.5.1.

[b] All study years and all radio-collared adult female moose captured in all study areas.

[c] All study years and all radio-collared adult female moose captured in the listed study area.

Appendix H – Prevalence of Bacterial Pathogens

Prevalence of exposure to the bacterial pathogens *Mycobacterium avium ssp. paratuberculosis* (Johne's Disease, MAP) and *Erysipelothrix rhusiopathiae* recorded in adult female moose live-captured in British Columbia, Canada.

Bacterial Pathogen	Prevalence and 95% C.I. Province Wide [a]	Prevalence and 95% C.I. Bonaparte Study Area [b]	Prevalence and 95% C.I. Big Creek Study Area [b]	Prevalence and 95% C.I. PG South Study Area [b]	Prevalence and 95% C.I. JPRF Study Area [b]	Prevalence and 95% C.I. Entiako Study Area [b]
Mycobacterium avium ssp. paratuberculosis	0% (n=0/277) (0%-1.2%)	0% (n=0/76) (0%-7.3%)	0% (n=0/54) (0%-7.3%)	0% (n=0/49) (0%-7.3%)	0% (n=0/33) (0%-10.4%)	2.8% (n=0/65) (0%-5.6%)
Erysipelothrix rhusiopathiae	8.1% (n=8/99) (4.2%-15.1%)	0% (n=0/46) (0%-7.7%)	0% (n=0/10) (0%-27.8%)	16.7% (n=5/30) (7.3%-33.6%)	28.6% (n=2/7) (8.2%-64.1%)	16.7% (n=1/6) (3.0%-56.4%)

[a] All study years and all radio-collared adult female moose captured in all study areas.

[b] All study years and all radio-collared adult female moose captured in the listed study area.

Appendix I – Prevalence of Gastrointestinal Parasites

Prevalence of infection with gastrointestinal parasites recorded in adult female moose live-captured in British Columbia, Canada in winter 2013/2014, 2015, and 2016.

Parasite	Prevalence and 95% C.I. Province Wide [a]	Prevalence and 95% C.I. Bonaparte Study Area [b]	Prevalence and 95% C.I. Big Creek Study Area [b]	Prevalence and 95% C.I. PG South Study Area [b]	Prevalence and 95% C.I. JPRF Study Area [b]	Prevalence and 95% C.I. Entiako Study Area [b]
Fluke Eggs	0% (n=0/218) (0%-1.7%)	0% (n=0/52) (0%-6.9%)	0% (n=0/53) (0%-6.8%)	0% (n=0/36 (0%-9.6%)	0% (n=0/39) (0%-9.0%)	0% (n=0/38) (0%-9.2%)
Strongylate Eggs	3.7% (n=8/218) (1.9%-7.1%)	15.4% (n=8/52) (8.0%-27.5%)	0% (n=0/53) (0%-6.8%)	0% (n=0/36 (0%-9.6%)	0% (n=0/39) (0%-9.0%)	0% (n=0/38) (0%-9.2%)
Nematodirinae Eggs	59.6% (n=130/218) (53%-65.9%)	36.5% (n=19/52) (24.8%-50.1%)	45.3% (n=24/53) (32.7%-58.5%)	50% (n=18/36) (34.4%-65.5%)	46.2% (n=18/39) (31.6%-61.4%)	55.3% (n=21/38) (39.7%-69%)
***Trichuris* sp. Eggs**	4.1% (n=9/218) (2.2%-7.7%)	17.3% (n=9/52) (9.4%-29.7%)	0% (n=0/53) (0%-6.8%)	0% (n=0/36) (0%-9.6%)	0% (n=0/39) (0%-9.0%)	0% (n=0/38) (0%-9.2%)
Tapeworm (*Moniezia benedeni*) Eggs	4.6% (n=10/218) (2.5%-8.2%)	5.8% (n=3/52) (2.0%-15.6%)	2.0% (n=1/53) (0.3%-10%)	8.3% (n=3/36) (2.9%-21.8%)	7.7% (n=2/39) (2.7%-20.3%)	2.6% (n=1/38) (0.5%-13.5%)
***Eimeria* sp.**	0% (n=0/218)	0% (n=0/52)	0% (n=0/53)	0% (n=0/36)	0% (n=0/39)	0% (n=0/38)

	(0%-1.7%)	(0%-6.9%)	(0%-6.8%)	(0%-9.6%)	(0%-9.0%)	(0%-9.2%)
Parasite	**Prevalence and 95% C.I. Province Wide [a]**	**Prevalence and 95% C.I. Bonaparte Study Area [b]**	**Prevalence and 95% C.I. Big Creek Study Area [b]**	**Prevalence and 95% C.I. PG South Study Area [b]**	**Prevalence and 95% C.I. JPRF Study Area [b]**	**Prevalence and 95% C.I. Entiako Study Area [b]**
Lung Worm Larvae	0% (n=0/218) (0%-1.7%)	0% (n=0/52) (0%-6.9%)	0% (n=0/53) (0%-6.8%)	0% (n=0/36) (0%-9.6%)	0% (n=0/39) (0%-9.0%)	0% (n=0/38) (0%-9.2%)

[a] All study years and all radio-collared adult female moose captured in all study areas.

[b] All study years and all radio-collared adult female moose captured in the listed study area

Appendix J – Intensity of Infection with Gastrointestinal Parasites

Intensity of infection with gastrointestinal parasites recorded in adult female moose live-captured in British Columbia, Canada in winter 2013/2014, 2015, and 2016.

Parasite	Mean Intensity (Eggs/g Feces) and 95% C.I. Province Wide [a]	Mean Intensity (Eggs/g Feces) and 95% C.I. Bonaparte Study Area [b]	Mean Intensity (Eggs/g Feces) and 95% C.I. Big Creek Study Area [b]	Mean Intensity (Eggs/g Feces) and 95% C.I. PG South Study Area [b]	Mean Intensity (Eggs/g Feces) and 95% C.I. JPRF Study Area [b]	Mean Intensity (Eggs/g Feces) and 95% C.I. Entiako Study Area [b]
Strongylate Eggs	0.56 n=8 (0.23-0.91)	0.56 n=8 (0.23-0.91)	- [c]	- [c]	- [c]	- [c]
Nematodirinae Eggs [d]	1.62 n=130 (0.23-0.91)	0.88 n=19 (0.47-1.30)	1.45 n=24 (0.79-2.11)	0.71 n=18 (0.17-1.25)	1.24 n=18 (0.69-1.80)	1.91 n=21 (1.11-2.72)
***Trichuris* sp. Eggs**	0.82 n=9 (0.18-1.46)	0.82 n=9 (0.18-1.46)	- [c]	- [c]	- [c]	- [c]
Tapeworm (*Moniezia benedeni*) Eggs	28.8 n=10 (18.12-39.48)	29.85 n=3 (0-66.05)	43.49 n=1 -	16.98 n=3 (0-48.95)	41.18 n=2 (0-183.16)	21.5 n=1 -

[a] All study years and all radio-collared adult female moose captured in all study areas.

[b] All study years and all radio-collared adult female moose captured in the listed study area.

[c] Prevalence of infection, 0 % in samples tested. [d] No difference (One-way ANOVA, P>0.05) in intensity recorded across study area

Appendix K – Case Review of Infection with Gastrointestinal Nematodes

On April 30, 2017, BCPMRP researchers in the Bonaparte study area received a mortality signal from the radio-collar of a male moose calf (WHID 16-9060). The calf was captured on February 26, 2017. A mortality site investigation was initiated early on May 1, 2017 and the dead calf was located near the edge of a small pond in a 5-7-year-old cut-block (Figure 10a). The carcass was still warm. Winter tick burden was classified as few to moderate with mild (5-20%) tick associated hair loss. The calf was in poor body condition with evidence of regurgitation (rumen contents) around the mouth and nose (Figure 10b). There was a mild rectal prolapse with a small amount of blood leaking from the anus (Figure 10c). Lymph nodes were enlarged and the small intestines appeared to contain a copious amount of dark liquid (Figure 10d).

Examination of bone marrow confirmed that the calf was in very poor condition (serous atrophy of marrow fat, Figure 10e). There were no signs of predation or accident. "Health" (probable infectious agent) was identified as the most likely cause of death and a full suite of tissues were collected and to submitted.

This moose was found to have extremely large numbers of nematodes (Nematodirinae, probable *Nematodirella alcidis*) within the submitted section of small intestine. The worms were too numerous to count with well over 100 in 10 mL of intestinal content. The heavy parasite burden was deemed to be the most likely cause of the poor body condition and dark liquid intestinal contents observed. The moose also had mild verminous (parasitic) pneumonia but the clinical significance of this infection was unclear as a small section of lung was available for histopathology. No pathogenic bacteria were cultured. The heavy gastrointestinal parasite load was the most likely cause of poor condition and death.

Nematodirella alcidis is the most commonly recorded gastrointestinal parasite in moose from North America but the intensity of infections is usually relatively low [29]. Likewise, pathology and clinical disease associated with *N. alcidis* in moose (although suspected) have not been previously reported [29]. The probable lifecycle of *N. alcidis* is reviewed in detail in section 3.5.3 and findings in this moose calf likely represent the effects of heavy infection burden obtained in spring through fall, 2016, larval stasis in winter, and masse emergence of L4 larvae in spring 2017.

Photos: C. Procter

Figure 10. Field necropsy photos of a male moose calf, WHID 16-9060 from the Bonaparte study area.

A review of Bonaparte mortality data sheets identified a second moose mortality (adult cow, WHID 16-7202, died March 29, 2016) where very similar clinical signs were recorded (e.g.

discharge from the nose and mouth, rectal prolapse). Although tissues from this moose were not available for further analysis, it is possible that gastrointestinal parasitism played a role in this mortality, however, some clinical signs in this case were more consistent with the "reproductive mortalities" described in Appendix C (also in the Bonaparte study area in spring 2016). Similar mortalities have not been recorded in other BCPMRP study areas. The two cases may indicate that the risk of heavy infections with *N. alcidis* is relatively high for moose in the Bonaparte.

Appendix L – Collection and Submission of Fecal Samples for Parasitology

For sample integrity, a whirl-pak (or similar) should be used to collect moose fecal samples. Do not use Ziploc bags. The best analysis is done from chilled, not frozen feces. . Remove as much air as possible from the whirl-pak by moving the pellets to the bottom of the bag and rolling the bag to squeeze out as much air as possible. This creates an anaerobic environment that prevents development (hatching) of parasite eggs and degradation of parasite larvae. Samples can be maintained at 4˚C (fridge temperature) before shipping to a diagnostic lab within 3 days (maximum/including shipping time). For shipping, samples should be in a cooler with but not on ice packs. Testing must be prearranged with the BC Wildlife Health Group if this approach is to be employed as there are few labs performing these tests currently. Since samples are more often frozen, freeze them as soon after collection as possible and avoid freeze/thaw as it will damage or destroy many parasite eggs/ larvae and will bias results. Self-defrosting freezers should also be avoided.

Appendix M – Prevalence of Tissue Dwelling Parasites

Prevalence of exposure to the protozoan parasites *Neospora caninum* and *Toxoplasma gondii* recorded in adult female moose live-captured in British Columbia, Canada.

Protozoan Parasite	Prevalence and 95% C.I. Province Wide [a]	Prevalence and 95% C.I. Bonaparte Study Area [b]	Prevalence and 95% C.I. Big Creek Study Area [b]	Prevalence and 95% C.I. PG South Study Area [b]	Prevalence and 95% C.I. JPRF Study Area [b]	Prevalence and 95% C.I. Entiako Study Area [b]
Neospora caninum	10.2% n=26/256 (6.7%, 14.5%)	13.3% n=13/98 (7.3%, 21.6%)	11.4% n=4/35 (3.2% 26.7%)	0% n=0/42 (0%, 8.4%)	16.2% n=6/37 (7.8%-31.1%)	9.4% n=3/32 (2.0%, 25.0%)
Toxoplasma gondii	3.9% n=3/77 (0.8% 11.0%)	7.1% n=2/28 (0.8%, 23.5%)	0% n=0/7 (0%, 40.9%)	4.8% n=1/21 (0.2%, 23.8%)	0% n=0/7 (0%-35.4%)	0% n=0/14 (0%, 23.2%)

[a] All study years and all radio-collared adult female moose captured in all study areas.

[b] All study years and all radio-collared adult female moose captured in the listed study area.

Prevalence of exposure to the protozoan parasites *Neospora caninum* and *Toxoplasma gondii* recorded in moose calves live-captured in British Columbia, Canada.

Protozoan Parasite	Prevalence and 95% C.I. Province Wide [c]	Prevalence and 95% C.I. Bonaparte Study Area	Prevalence and 95% C.I. Big Creek Study Area
Neospora caninum	8.3% n=5/60 (2.8%, 18.4%)	12.8% n=5/39 (4.3% 27.4%)	0% n=0/21 (0%, 16.1%)
Toxoplasma gondii	0% n=0/60 (0%, 6.0%)	0% n=0/39 (0%, 9.0%)	0% n=0/21 (0%, 16.1%)

[c] 2017 and 2018 and all radio-collared moose calves captured in Bonaparte and Big Creek study areas.

Appendix N - Hair Collection Protocols for Live-Captured and Dead Moose

Hair is an extremely valuable biological sample and is relatively non-invasive. The uses for this sample extend beyond stress and other hormones. Stable isotope, trace nutrient, toxicology, and genetic information can also be obtained from hair samples and the evaluation of new health biomarkers in wildlife hair is the subject of ongoing research. Archived hair samples also take up little space and can be stored at room temperature.

A review of hair samples collected across BCPMRP study areas indicated that an insufficient or marginal quantity of moose hair was collected at many moose captures. The site of hair collection was also not specified in most cases. Hair samples should also be collected from all live and dead moose.

Figure 12. Hair sample collection protocol for live-captured moose.

Collect hair from the top of the shoulder (yellow arrow, Figure 12) in an area as dry and as free of contaminants (blood, dirt etc.) as possible. Fill a large coin envelope by plucking [with needle

nose pliers or hemostats] to ensure undamaged, intact hairs with roots are obtained (Figure 16). Label all samples with moose WHL ID #, date of collection, and body site of collection. Ensure samples are dry before long-term storage and note on labels if samples were collected from wet or dirty animals. Wet or damp hair samples should be gently blotted, not wiped, with paper towel then air dried before transferring to a new envelope for long-term storage. Air dry wet or damp hair samples out of direct sunlight and protect from heat (i.e. not near a wood stove, hot windowsill, on a truck dashboard etc.). For long-term storage keep moose hair samples at room temperature in a dry, non-Manila, paper envelope protected from heat, light, and moisture. Silica desiccant can be kept in the same general storage container (i.e. if storing many envelopes containing hair in a Rubbermaid etc.).

Figure 13. Example of hair sample size to collect from dead moose.

For mortalities, when possible, samples should be collected from the same location and in the same manner as for live-captured moose. When this is not possible the label should clearly indicate what region of the carcass the hair sample came from. Labels should also clearly

indicate that the hair sample was obtained from a dead moose and the known or suspected degree of contamination with blood.

Appendix O – Trace Nutrient Tables for Moose Mortalities

Table A. Magnesium (Mg), manganese (Mn), iron (Fe), cobalt (Co), copper (Cu), zinc (Zn), selenium (Se), molybdenum (Mo), and mercury (Hg) levels measured in post mortem liver samples collected from n=11 radio-collared moose and n=3 opportunistically encountered moose in British Columbia, Canada.

			Trace Mineral Concentration and Interpretation [a]									
Moose WHID or ID	**Study Area**	**Age-Sex Class**	**Mg (ppm)**	**Mn (ppm)**	**Fe (ppm)**	**Co (ppb)**	**Cu (ppm)**	**Zn (ppm)**	**Se (ppm)**	**Mo (ppm)**	**Hg (ppb)**	**Cause of Death**
16-7186 [b]	Bonaparte	Adult Cow	223.6 HN	2.37 N	601.1 HN	58.9 N	36.7 N	31.4 N	**0.261** [d] **M**	1.39 N	76.4 N	Not Available
16-7200 [b]	Bonaparte	Adult Cow	203.0 HN	4.28 N	**36.0 D**	50.2 N	157.9 N	85.2 N	**0.140 D**	2.57 HN	7.54 N	Health
16-9063 [b]	Bonaparte	Calf Female	248.3 HN	2.91 N	288.1 N	60.0 N	154.9 N	54.0 N	0.910 N	1.55 HN	Not Measured	Apparent Starvation
16-9064 [b]	Bonaparte	Calf Female	218.7 HN	3.37 N	212.3 N	77.3 N	186.1 N	74.7 N	1.35 N	1.74 HN	Not Measured	Predation
16-9065 [b]	Bonaparte	Calf Female	159.4 N	5.34 N	143.1 N	125.5 HN	111.6 N	106.0 HN	0.862 N	1.69 HN	Not Measured	Apparent Starvation
16-9066 [b]	Bonaparte	Calf Male	184.7 N	2.86 N	179.7 N	63.7 N	171.3 N	79.8 N	1.23 N	1.71 N	Not Measured	Predation
14-5254 [b]	Big Creek	Adult Cow	212.4 HN	3.53 N	230.1 N	202.6 HN	204.9 N	43.4 N	1.16 N	3.45 HN	34.2 N	Gunshot
14-5290 [b]	PG South	Adult Cow	243.4 HN	**1.86 M**	**87.2 M**	103.5 HN	92.3 N	259.3 HN	1.42 N	2.69 HN	11.8 N	Apparent Starvation
14-5298 [b]	PG South	Adult Cow	183.9 HN	2.92 N	**83.0 M**	78.9 N	80.5 N	28.7 N	1.90 HN	1.59 HN	10.4 N	Predation

Moose WHID or ID	Study Area	Age-Sex Class	Mg (ppm)	Mn (ppm)	Fe (ppm)	Co (ppb)	Cu (ppm)	Zn (ppm)	Se (ppm)	Mo (ppm)	Hg (ppb)	Cause of Death
14-5020 [c]	PG South	Adult Cow	296.6 HN	7.71 HN	292.9 N	51.7 N	72.0 N	105.1 HN	0.602 N	1.40 N	19.7 N	Not Available
14-5022 [c]	PG South	Adult Cow	149.5 N	5.85 N	**57.2 D**	37.5 N	**5.76** [d] **D**	57.4 N	0.617 N	0.355 N	14.1 N	Not Available
AN542 [c]	PG South	Adult Cow	170.9 N	2.48 N	359.5 N	48.8 N	73.3 N	94.2 HN	0.719 N	1.21 N	5.61 N	Not Available
14-4525 [b]	Entiako	Adult Cow	140.2 N	**0.118 D**	1561.0 HN	**4.41 D**	**0.691 D**	25.5 N	**0.263 M**	0.056 N	1.33 N	Not Available
17-9544 [b]	Entiako	Adult Cow	180.8 N	2.91 N	324.1 N	97.1 N	103.1 N	21.0 M	**0.205 M**	1.49 HN	12.5 N	Not Available
17-10555	PG South	Calf	**50.0 M**	**0.184 M**	894.3 N	1.88 HN	**1.51 D**	**3.14 D**	**0.170 D**	0.019 N	3.98 N	Predation
16-7139	PG South	Adult Cow	293.1 HN	**2.86 M**	588.4 N	77.9 N	75.9 N	46.8 N	**0.185 D**	1.66 HN	37.4 N	Predation
16-7196	Bonaparte	Adult Cow	292.4 HN	5.31 N	316.5 N	154.2 HN	152.0 N	55.7 N	1.42 N	2.85 HN	11.3 N	Health Related
17-10140	Bonaparte	Calf	243.6 HN	3.96 N	136.0 N	167.9 HN	215.6 HN	97.6 N	**0.274 M**	1.96 HN	10.8 N	Predation
14-4504	PG South	Adult Cow	209.7 HN	11.2 HN	357.4 N	68.5 N	95.5 N	42.0 N	0.519 N	2.24 HN	64.6 N	Predation
17-10149	PG South	Calf	222.0 HN	4.06 N	343.0 N	103.6 HN	99.3 N	103.8 HN	**0.121 D**	1.25 N	40.2 N	Health Related
16-9067	Bonaparte	Calf	215.8 HN	4.57 HN	**31.0 D**	37.0 N	10.0 HN	72.7 N	**0.305 D**	0.622 N	78.1 N	Health Related
16-9063	Bonaparte	Calf	175.3 N	2.55 N	**43.2 M**	41.0 N	8.73 HN	45.1 HN	**0.553 M**	0.691 N	54.3 N	Health Related

17-10164	PG South	Calf	223.7 HN	3.81 N	232.4 N	143.9 HN	41.2 N	32.1 N	**0.418** **M**	1.81 HN	23.6 N	Licensed Hunting
16-9083	Entiako	Adult Cow	244.6 HN	3.94 N	**51.7** **M**	41.7 N	7.77 HN	0.201 HN	**0.247** **D**	0.917 N	96.4 N	Predation
16-9064	Bonaparte	Calf	212.2 HN	2.97 N	**48.1** **M**	46.6 N	7.41 HN	75.9 HN	**0.578** **M**	0.664 N	609.7 HN	Health Related

[a] To determine trace nutrient status project data was compared against reference ranges for moose liver at Prairie Diagnostic Services Inc. Saskatoon, SK using values from: Puls, R. (1994). Mineral Levels in Animal Health, 2nd Edition. Sherpa International, Clearbrooke, BC (Dr. B. Blakely, Western College of Veterinary Medicine, Toxicology Department, personal communication, 2017). Status designated as: Toxic (T), High Normal (HN), Normal (N), Marginal (M), Deficient (D). NB: Analytical methods and reference ranges used to measure and interpret trace nutrient levels in moose tissue in other studies may be different. Caution is warranted when comparing and interpreting results generated here with those reported elsewhere.

[b] Radio-collared moose tracked as part of the BC Provincial Moose Research Program.

[c] Moose tissue sample collected opportunistically (e.g. found dead, road kill, other research etc.) in study region but moose not tracked as part of the ongoing BC Provincial Moose Research Program.

[d] Error in classification noted in raw data provided by testing laboratory. Interpretation corrected using other results from the same report and/or reference ranges reported in O'Hara et al. (2001) where marginal to deficient levels in liver and kidney: Cu <10-25 ppm and Fe <30 ppm.

Table B. Magnesium (Mg), manganese (Mn), iron (Fe), cobalt (Co), copper (Cu), zinc (Zn), selenium (Se), molybdenum (Mo), and mercury (Hg) levels measured in post mortem kidney samples collected from n=4 radio-collared moose and n=4 opportunistically encountered moose in British Columbia, Canada.

			Trace Mineral Concentration and Interpretation [a]									
Moose WHID or ID	**Study Area**	**Age-Sex Class**	**Mg (ppm)**	**Mn (ppm)**	**Fe (ppm)**	**Co (ppb)**	**Cu (ppm)**	**Zn (ppm)**	**Se (ppm)**	**Mo (ppm)**	**Hg (ppb)**	**Cause of Death**
14- 5254 [b]	Big Creek	Adult Cow	187.2 HN	3.58 N	**42.3 D**	96.9 HN	**4.63** [d] **D**	31.1 N	0.886 N	0.739 N	35.2 N	Gunshot
14-4501[b]	PG	Adult Cow	142.0 N	2.71 N	**33.9** [d] **D**	31.1 N	**66.8 T**	126.2 HN	0.533 N	0.468 N	22.8 N	Health
14- 5290 [b]	PG	Adult Cow	176.7 N	**1.51 M**	**28.8 D**	37.6 N	11.8 N	251.3 HN	0.733 N	0.489 N	12.7 N	Apparent Starvation
14- 5298 [b]	PG	Adult Cow	157.8 N	2.16 N	79.0 N	31.9 N	**3.90** [d] **D**	49.4 N	0.853 N	0.644 N	43.4 N	Predation
14- 5020 [c]	PG	Adult Cow	192.7 HN	8.38 HN	**32.4 D**	**25.6 M**	**8.30 D**	84.8 N	0.546 N	0.425 N	38.0 N	Not Available
14- 5022 [c]	PG	Adult Cow	124.9 N	4.25 N	2865 HN	**9.85 D**	**1.35 D**	28.9 N	**0.245 M**	0.067 N	1.83 N	Not Available
AN542 [c]	PG	Adult Cow	178.0 N	2.13 N	201.0 HN	28.6 N	**7.23 D**	44.9 N	0.772 N	0.388 N	11.6 N	Not Available
AN555 [c]	PG	Adult Cow	170.1 N	2.29 N	**25.5 D**	23.7 N	4.61 N	34.7 HN	0.547 N	0.581 N	6.62 N	Not Available

[a] To determine trace nutrient status project data was compared against reference ranges for moose liver at Prairie Diagnostic Services Inc. Saskatoon, SK using values from: Puls, R. (1994). Mineral Levels in Animal Health, 2nd Edition. Sherpa International, Clearbrooke, BC (Dr. B. Blakely, Western College of Veterinary Medicine, Toxicology Department, personal communication, 2017). Status designated as: Toxic (T), High Normal (HN), Normal (N), Marginal (M), Deficient (D). NB: Analytical methods and reference ranges used to measure and interpret trace nutrient levels in

moose tissue in other studies may be different. Caution is warranted when comparing and interpreting results generated here with those reported elsewhere.

[b] Radio-collared moose tracked as part of the BC Provincial Moose Research Program.

[c] Moose tissue sample collected opportunistically (e.g. found dead, road kill, other research etc.) in study region but moose not tracked as part of the ongoing BC Provincial Moose Research Program.

[d] Error in classification noted in raw data provided by testing laboratory. Interpretation corrected using other results from the same report and/or reference ranges reported in O'Hara et al. (2001) where marginal to deficient levels in liver and kidney: Cu <10-25 ppm and Fe <30 ppm.

Table C. Comparative magnesium (Mg), manganese (Mn), iron (Fe), cobalt (Co), copper (Cu), zinc (Zn), selenium (Se), and molybdenum (Mo) levels measured in paired, post mortem kidney and liver samples collected from n=3 radio-collared moose and n=3 opportunistically encountered moose in British Columbia, Canada.

			Trace Mineral Concentration and Interpretation [a]									
Moose WHID or ID	**Study Area**	**Age-Sex Class**	**Mg (ppm)**	**Mn (ppm)**	**Fe (ppm)**	**Co (ppb)**	**Cu (ppm)**	**Zn (ppm)**	**Se (ppm)**	**Mo (ppm)**	**Hg (ppb)**	**Cause of Death**
14-5254 [b] Liver	Big Creek	Adult Cow	212.4 HN	3.53 N	230.1 N	202.6 HN	204.9 N	43.4 N	1.16 N	3.45 HN	34.2 N	Gunshot
14-5254 [b] Kidney	Big Creek	Adult Cow	187.2 HN	3.58 N	**42.3 D**	96.9 HN	**4.63 [d] D**	31.1 N	0.886 N	0.739 N	35.2 N	Gunshot
14-5290 [b] Liver	PG	Adult Cow	243.4 HN	**1.86 M**	**87.2 M**	103.5 HN	92.3 N	259.3 HN	1.42 N	2.69 HN	11.8 N	Apparent Starvation
14-5290 [b] Kidney	PG	Adult Cow	176.7 N	**1.51 M**	**28.8 D**	37.6 N	11.8 N	251.3 HN	0.733 N	0.489 N	12.7 N	Apparent Starvation
14-5298 [b] Liver	PG	Adult Cow	183.9 HN	2.92 N	**83.0 M**	78.9 N	80.5 N	28.7 N	1.90 HN	1.59 HN	10.4 N	Predation
14-5298 [b] Kidney	PG	Adult Cow	157.8 N	2.16 N	79.0 N	31.9 N	**3.90 [d] D**	49.4 N	0.853 N	0.644 N	43.4 N	Predation
14-5020 [c] Liver	PG	Adult Cow	296.6 HN	7.71 HN	292.9 N	51.7 N	72.0 N	105.1 HN	0.602 N	1.40 N	19.7 N	Not Available

Moose WHID or ID	Study Area	Age-Sex Class	Mg (ppm)	Mn (ppm)	Fe (ppm)	Co (ppb)	Cu (ppm)	Zn (ppm)	Se (ppm)	Mo (ppm)	Hg (ppb)	Cause of Death
14-5020 [c] Kidney	PG	Adult Cow	192.7 HN	8.38 HN	**32.4 D**	**25.6 M**	**8.30 D**	84.8 N	0.546 N	0.425 N	38.0 N	Not Available
14-5022 [c] Liver	PG	Adult Cow	149.5 N	5.85 N	**57.2 D**	37.5 N	**5.76 D**	57.4 N	0.617 N	0.355 N	14.1 N	Not Available
14-5022 [c] Kidney	PG	Adult Cow	124.9 N	4.25 N	2865 HN	**9.85 D**	**1.35 D**	28.9 N	**0.245 M**	0.067 N	1.83 N	Not Available
AN542 [c] Liver	PG	Adult Cow	170.9 N	2.48 N	359.5 N	48.8 N	73.3 N	94.2 HN	0.719 N	1.21 N	5.61 N	Not Available
AN542 [c] Kidney	PG	Adult Cow	178.0 N	2.13 N	201.0 HN	28.6 N	**7.23 D**	44.9 N	0.772 N	0.388 N	11.6 N	Not Available

[a] Toxic (T), High Normal (HN), Normal (N), Marginal (M), Deficient (D).

[b] Radio-collared moose tracked as part of the BC Provincial Moose Research Program.

[c] Moose tissue sample collected opportunistically (e.g. found dead, road kill, other research etc.) in study region but moose not tracked as part of the ongoing BC Provincial Moose Research Program.

[d] Error in classification noted in raw data provided by testing laboratory. Interpretation corrected using other results from the same report and/or reference ranges reported in O'Hara et al. (2001) where marginal to deficient levels in liver and kidney: Cu <10-25 ppm and Fe <30 ppm.

Table D. Trace nutrient levels measured in post mortem liver samples collected from adult female moose (n=5) in the PG South study area of British Columbia, Canada compared to levels determined in free-ranging moose populations in Alaska and Minnesota, USA where moose health status is considered suboptimal [a, b].

Trace Nutrient	British Columbia Moose PG Study Area (Mean ± SE)	O'Hara et al. (2001) Moose Colville River Alaska, USA (Mean ± SE) [a]	P value Unpaired t-test (α=0.05)	Murray et al. (2006) Moose Minnesota USA (Mean ± SE) [b]	P value Unpaired t-test (α=0.05)
Magnesium (Mg) ppm	208.86±26.91 n=5	-	-	580.2±14.2 n=106	P=0.0001
Manganese (Mn) ppm	4.16±1.12 n=5	-	-	8.4±0.4 n=106	P=0.0258
Iron (Fe) ppm	175.96±62.44 n=5	120.53±17.27 n=9	P=0.651 NSD	1335.7±120.1 n=106	P=0.0394
Copper (Cu) ppm	64.77±15.19 n=5	9.80±4.22 n=9	P=0.0008	92.9±9.37 n=106	P=0.519 NSD
Zinc (Zn) ppm	108.94±39.95 n=5	66.53±19.37 n=9	P=0.299 NSD	331.3±3.9 n=106	P=0.0001
Selenium (Se) ppm	1.05±0.26 n=5	-	-	3.2±0.3 n=106	P=0.124 NSD
Molybdenum (Mo) ppm	1.45±0.38 n=5	1.54±0.03 n=9	P=0.751 NSD	3.31±0.13 n=106	P=0.0027

[a] O'Hara, T.M. et al. (2001). Mineral and heavy metal status as related to a mortality event and poor recruitment in a moose population in Alaska. *Journal of Wildlife Diseases*, 37:509-522.

[b] Murray, D.L. et al. (2006). Pathogens, nutritional deficiency, and climatic influences on a declining moose population. *Wildlife Monographs*, 166:1-30.

Table E. Trace nutrient levels measured post mortem liver samples collected from adult female moose (n=2) and moose calves of the year (n=4) in the Bonaparte study area of British Columbia, Canada compared to levels determined in free-ranging moose populations in Alaska and Minnesota, USA where moose health status is considered suboptimal [a, b].

Trace Nutrient	British Columbia Moose PG Study Area (Mean ± SE)	O'Hara et al. (2001) Moose Colville River Alaska, USA (Mean ± SE)	P value Unpaired t-test (α=0.05)	Murray et al. (2006) Moose Minnesota USA (Mean ± SE)	P value Unpaired t-test (α=0.05)
Magnesium (Mg) ppm	206.28±12.77 n=6	-	-	580.2±14.2 n=106	P=0.0001
Manganese (Mn) ppm	3.52±0.45 n=6	-	-	8.4±0.4 n=106	P=0.0047
Iron (Fe) ppm	243.8±79.17 n=6	120.53±17.27 n=9	P=0.0883 NSD	1335.7±120.1 n=106	P=0.0335
Copper (Cu) ppm	136.41±22.39 n=6	9.80±4.22 n=9	P=0.0001	92.9±9.37 n=106	P=0.277 NSD
Zinc (Zn) ppm	71.85±10.60 n=6	66.53±19.37 n=9	P=0.838 NSD	331.3±3.9 n=106	P=0.0001
Selenium (Se) ppm	0.79±0.20 n=6	-	-	3.2±0.3 n=106	P=0.0598 NSD
Molybdenum (Mo) ppm	1.78±0.17 n=6	1.54±0.03 n=9	P=0.113 NSD	3.31±0.13 n=106	P=0.0064

[a] O'Hara, T.M. et al. (2001). Mineral and heavy metal status as related to a mortality event and poor recruitment in a moose population in Alaska. *Journal of Wildlife Diseases*, 37:509-522.

[b] Murray, D.L. et al. (2006). Pathogens, nutritional deficiency, and climatic influences on a declining moose population. *Wildlife Monographs*, 166:1-30.

Table F. Trace nutrient levels measured in post mortem kidney samples collected from adult female moose (n=7) in the PG South study area of British Columbia, Canada compared to levels determined in Alaskan moose found dead during a mortality event in a population with poor recruitment [a].

Trace Nutrient	British Columbia Moose PG Study Area (Mean ± SD)	O'Hara et al. (2001) Moose Colville River Alaska, USA (Mean ± SD)	P value Unpaired t-test (α=0.05)
Iron (Fe) ppm	466.51±1059.5 n=7	37.9±7.59 n=9	P=0.240 NSD
Copper (Cu) ppm	6.19±3.69 [b] n=6	3.72±0.60 n=9	P=0.066 NSD
Zinc (Zn) ppm	88.60±79.38 n=7	43.0±26.5 n=9	P=0.127 NSD

[a] O'Hara, T.M. et al. (2001). Mineral and heavy metal status as related to a mortality event and poor recruitment in a moose population in Alaska. *Journal of Wildlife Diseases*, 37:509-522.
[b] Outlier: 66.8 ppm (toxic level) removed from analysis.

Table G. Manganese (Mn), iron (Fe), cobalt (Co), copper (Cu), zinc (Zn), selenium (Se), and molybdenum (Mo) levels measured in blood (serum) samples collected from live-captured moose in the Bonaparte (n=20 adult cows, n= 19 calves of year), Big Creek (n=4 adult cows), PG South (n=20 adult cows), and JPRF (n=7 adult cows) study areas of British Columbia, Canada in winter 2017.

Study Area and Moose Age-Sex Class	Manganese (Mn) ng/ml	Iron (Fe) ug/ml	Cobalt (Co) ng/ml	Copper (Cu) ug/ml	Zinc (Zn) ug/ml	Selenium (Se) ug/ml	Molybdenum (Mo) ng/ml
			Mean ± SD (95% Confidence Interval) [a]				
Bonaparte Adult Cow	1.75±0.69 (1.40-2.11) n=17 [a]	14.73±14.67 (7.87-21.59) n=20	0.34±0.13 (0.27-0.41) n=16 [a]	0.26±0.04 (0.24-0.29) n=20	0.45±0.09 (0.41-0.49) n=20	0.043±0.010 (0.038-0.048) n=19 [a]	4.71±2.53 (3.49-5.93) n=19 [a]
Bonaparte Calf of Year	1.66±0.52 (1.37-1.95) n=15 [a]	13.27±7.65 (9.58-16.96) n=19	0.30±0.13 (0.23-0.37) n=15 [a]	0.27±0.06 (0.24-0.30) n=19	0.43±0.09 (0.39-0.47) n=19	0.032±0.010 (0.027-0.037) n=18 [a]	4.82±3.17 (3.29-6.34) n=19
Big Creek Adult Cow	2.03±1.54 (0-5.85) n=3 [a]	4.58±1.94 (1.49-7.66) n=4	0.38±0.09 (0.21-0.50) n=4	0.32±0.04 (0.25-0.38) n=4	0.39±0.05 (0.31-0.47) n=4	0.043±0.005 (0.035-0.051) n=4	5.08±3.65 (0-10.88) n=4
PG Adult Cow	1.85±0.23 (1.72-1.98) n=14	1.71±0.27 (1.56-1.87) n=14	0.28±0.04 (0.26-0.30) n=14	0.38±0.12 (0.31-0.45) n=14	0.51±0.04 (0.50-0.56) n=14	0.045±0.017 (0.044-0.075) n=14	4.88±3.106 (3.09-6.67) n=14
John Prince Adult Cow	1.79±0.44 (1.38-2.19) n=7	2.36±0.77 (1.64-3.07) n=7	0.43±0.10 (0.34-0.53) n=7	0.39±0.06 (0.33-0.44) n=7	0.52±0.07 (0.46-0.59) n=7	0.055±0.006 (0.050-0.060) n=7	5.00±5.46 (0-10.05) n=7

[a] Trace nutrient levels below assay detection limits (BDL) **not included** in analysis. BDL samples occurred only for selected nutrients in the Bonaparte [Mn (n=5), Co (n=8), Se (n=2), and Mo (n=1)] and Big Creek [Mn (n=1)] study areas. Method detection limits: Mo (0.9 ng/ml), Co (0.2 ng/ml), Mn (1.0 ng/ml), and Se (0.015 ug/ml).

Table H. Trace nutrient levels measured in serum collected from live-captured adult female moose (n=45) and moose calves of the year (n=19) in British Columbia, Canada in winter, 2017 compared to laboratory reference ranges for domestic cattle, sheep, and cervids [a].

Trace Nutrient	British Columbia All Moose Mean and Range	Laboratory Reference Range Adult and Growing Cattle [b]	% British Columbia Moose Less Than Range	Laboratory Reference Range Adult and Growing Sheep [b]	% British Columbia Moose Less Than Range	Laboratory Reference Range Adult Cervids [c]	% British Columbia Moose Less Than Range
Manganese (Mn) ng/ml	1.77 (1.00-3.80) n=56 n=8 BDL[d]	0.9-6.0	12.5% n=8/64	1.0-6.0	12.5 n=8/64	-	-
Iron (Fe) ug/ml	9.46 (1.10-72.0) n=64	1.1-2.5	0.0 n=0/64	0.9-2.7	0.0 n=0/64	-	-
Cobalt (Co) ng/ml	0.33 (0.21-0.74) n=56 n=8 BDL[d]	0.17-2.0	12.5% n=8/64	0.18-2.0	12.5% n=8/64	-	-
Copper (Cu) ug/ml	0.31 (0.18-0.57) n=64	0.6-1.1	100% n=64/64	0.75-1.7	100% n=64/64	0.70-1.8	100% n=64/64
Zinc (Zn) ug/ml	0.46 (0.32-0.76) n=64	0.60-1.90	93.8% n=60/64	0.55-1.20	84.4% n=54/64	1.1-2.5	100% n=64/64
Selenium (Se) ug/ml	0.041 (0.016-0.075) n=62 n=2 BDL[d]	0.07-0.14	98.4% n=63/64	0.06-0.20	92.2% n=59/64	0.05-0.14	81.3% n=52/64
Molybdenum (Mo) ng/ml	4.84 (0.92-17.0) n=63 n=1 BDL[d]	2.0-35	12.5% n=8/64	1.0-50	0.03% n=2/64	-	-

[a] Trace nutrient reference ranges are not available for moose serum.

[b] Herdt, T.H. and Hoff, B. (2011). The use of blood analysis to evaluate trace mineral status in ruminant livestock. *Veterinary Clinics: Food Animal*, 27:255-283.

[c] University of Guelph Animal Health Laboratory.

[d] Trace nutrient levels below assay detection limits (BDL) **included** in analysis. BDL samples occurred only for selected nutrients in the Bonaparte [Mn (n=5), Co (n=8), Se (n=2), and Mo (n=1)] and Big Creek [Mn (n=1)] study areas. Method detection limits: Mo (0.9 ng/ml), Co (0.2 ng/ml), Mn (1.0 ng/ml), and Se (0.015 ug/ml).

Table I. Serum iron (Fe), copper (Cu), and zinc (Zn) levels measured in adult female moose (n=45) and moose calves of the year (n=19) live-captured in British Columbia, Canada in winter 2017 compared to levels determined in live-captured, free-ranging moose from American populations where health status is considered suboptimal [a, b, c].

Trace Nutrient	British Columbia Moose All Study Areas (Mean ± SD)	Flynn et al. (1977) Moose Kenai Peninsula, Alaska, USA (Mean ± SD)	P value Unpaired t-test (α=0.05)	O'Hara et al. (2001) Moose Colville River Alaska, USA (Mean ± SD) [e]	P value Unpaired t-test (α=0.05)	Becker et al. (2010) Moose Northwestern Wyoming, USA (Mean ± SD)	P value Unpaired t-test (α=0.05)
Iron (Fe) ug/ml	9.46±10.75 n=64	-	-	-	-	2.32±0.46 n=16	P=0.01
Copper (Cu) ug/ml	0.31±0.08 n=64	0.61±0.07, n=13	P=0.0001	0.29±0.12 n=43	P=0.303 [NSD]	0.45±0.10 n=16	P=0.0001
Zinc (Zn) ug/ml	0.46±0.08 n=64	0.83±0.06, n=13	P=0.0001	0.89±0.18 n=49	P=0.0001	0.71±0.09 n=16	P=0.0001

[a] Flynn et al. (1977). Indications of copper deficiency in a subpopulation of Alaskan moose. *Journal of Nutrition*, 107:1182-1189.

[b] O'Hara et al. (2001). Mineral and heavy metal status as related to a mortality event and poor recruitment in a moose population in Alaska. *Journal of Wildlife Diseases*, 37:509-522.

[c] Becker et al. (2010). Nutritional condition of adult female Shiras moose in northwest Wyoming. *Alces*, 46:151-166.

Statistical analysis of comparisons of serum trace mineral levels in adult moose cows sampled in winter 2017

- Variation in serum selenium levels was not explained by study area (One-way ANOVA, F3,40=1.865, P=0.151, n=19 Bonaparte, n=14 PG South, n= 4 Big Creek, n=7 JPRF).
- Variation in serum manganese levels was not explained by study area (One-way ANOVA, F3,37=0.1999, P=0.896, n=17 Bonaparte, n=14 PG South, n= 4 Big Creek, n=7 JPRF).
- Variation in serum molybdenum levels was not explained by study area (One-way ANOVA, F3,40=0.0215, P=0.996, n=19 Bonaparte, n=14 PG South, n= 4 Big Creek, n=7 JPRF).
- Variation in serum zinc levels was explained by study area (One-way ANOVA, F3,41=4.308, P=0.009, n=20 Bonaparte, n=14 PG South, n= 4 Big Creek, n=7 JPRF). Zinc levels measured in moose from the Big Creek study area were lower than those measured in the PG South study area and in the JPRF (Tukey-Kramer, P<0.05). Zinc levels were similar in moose from the PG South, Bonaparte, and JPRF study areas (Tukey-Kramer, P>0.05). There was no difference in Zinc levels measured in moose from the Bonaparte and Big Creek study areas (Tukey-Kramer, P>0.05).
- Variation in serum copper levels was explained by study area (One-way ANOVA, F3,41=20.341, P<0.0001, n=20 Bonaparte, n=14 PG South, n= 4 Big Creek, n=7 JPRF). Copper levels measured in moose from the Bonaparte study area were lower than those measured in the PG South study area and in the JPRF (Tukey-Kramer, P<0.05). Copper levels in moose from the Big Creek study area were also lower than those recorded in moose from the PG South study area (Tukey-Kramer, P<0.05) but similar to those recorded in the JPRF (Tukey-Kramer, P>0.05). There was no difference in Copper levels measured in moose from the Bonaparte and Big Creek study areas (Tukey-Kramer, P>0.05) or in Copper levels measured in the PG South study area and the JPRF (Tukey-Kramer, P>0.05).
- Variation in serum cobalt levels was explained by study area (One-way ANOVA, F3, 37=3.720, P=0.0196, n=16 Bonaparte, n=14 PG South, n= 4 Big Creek, n=7 JPRF). Cobalt levels measured in moose from the PG South study area were lower than those measured in the JPRF (Tukey-Kramer, P<0.05). Cobalt levels were similar in moose from the Bonaparte, Big Creek, and JPRF study areas (Tukey-Kramer, P>0.05). There was no difference in Cobalt levels measured in moose from the PG South and the Bonaparte or Big Creek study areas (Tukey-Kramer, P>0.05).
- Variation in serum iron levels was explained by study area (One-way ANOVA, F3,41=5,757, P=0.0022, n=20 Bonaparte, n=14 PG South, n= 4 Big Creek, n=7 JPRF). Iron levels measured in moose from the PG South and JPRF study areas were lower than those measured in Bonaparte study area (Tukey-Kramer, P<0.05). Iron levels were similar in moose from the Bonaparte and Big Creek study areas (Tukey-Kramer, P>0.05) and in the PG South and JPRFs study areas (Tukey-Kramer, P>0.05).

Association of Trace Nutrient Levels in Liver and Kidney Samples Collected from Dead British Columbia Moose

There was no difference in levels of magnesium, manganese, iron, zinc, or selenium measured in the livers of dead moose from the Bonaparte and PG South study areas (Unpaired t-test, P>0.05, n=6 Bonaparte, n= 5 PG South). Liver copper levels were lower in dead moose from the PG South study area than in the Bonaparte study area (Unpaired t-test, t_9=2.531, P=0.0322, n=6 Bonaparte, n= 5 PG South).

- Magnesium (Mg): Pearson Correlation, r^2=0.548, P=0.092, n=6, No Significant Association.
- Manganese (Mn): Pearson Correlation, r^2=0.912, P=0.003, n=6, Significant, Positive Association.
- Iron (Fe): Pearson Correlation, r^2=0.309, P=0.331, n=5 (n=1 outlier removed), No Significant Association.
- Cobalt (Co): Pearson Correlation, r^2=0.955, P=0.001, n=6, Significant, Positive Association.
- Copper (Cu) Pearson Correlation, r^2=0.030, P=0.742, n=6, No Significant Association.
- Zinc (Zn): Pearson Correlation, r^2=0.926, P=0.002, n=6, Significant, Positive Association.
- Selenium (Se): Pearson Correlation, r^2=0.420, P=0.164, n=6, No Significant Association.
- Molybdenum (Mo): Pearson Correlation, r^2=0.683, P=0.042, n=6, Marginally Significant Positive Association.
- Mercury (Hg): Pearson Correlation, r^2=0.180, P=0.402, n=6, No Significant Association.

Appendix P – Case Review of Possible Copper Toxicity

On May 14, 2015 BCPMRP researchers in the PG South study area received a mortality signal from the radio-collar of an adult female moose (WHID 14-4501) captured in winter 2014. A mortality site investigation was initiated early on May 14, 2014 and the dead moose was located in an open, mossy area in a black spruce stand (Figure 14). The carcass was not in rigor mortis. Abundant and engorged winter ticks were noted but tick associated hair loss was limited. The moose was in poor body condition with evidence of moderate and extensive muscle atrophy. Some scavenging (birds and probable lynx) had occurred.

On necropsy, prescapular and femoral lymph nodes were dark (possible evidence of haemorrhage) and enlarged. The pericardium was filled with fluid (pericardial effusion) and edema was noted near the top of the heart. Kidney fat was lacking while the bone marrow contained visible fat reserves (reported as creamy coloured). There were no signs of predation or accident. "Health" (possible infectious agent) was identified as the most likely cause of death and a full suite of tissues were collected.

A review of archived tissues held by the BC Wildlife Health Group in August, 2016 could only locate a kidney sample from this moose (liver and spleen samples listed as collected on the mortality form were not found in the Nanaimo freezer). The kidney sample was submitted for toxicology testing. Mg, Mn, Co, Cu, Zn, Se, and Mo levels were within normal limits. Fe levels were deficient. Cu levels (66.8 ppm, normal 4-7 ppm) were exceptionally high and interpreted as toxic levels by the diagnostic laboratory.

Chronic copper toxicity is common in domestic sheep and occurs after an animal's liver capacity for copper storage has been exceeded. This may result from a prolonged period of consuming a diet high in copper. It may also occur secondary to liver damage (often associated with the consumption of toxic plants) or molybdenum/sulfate deficiencies [45]. Once liver storage capacity is exceeded, clinical signs of copper toxicity usually manifest as an acute disease characterized by intravascular hemolysis caused by a sudden and massive release of copper from the liver into the circulation [45]. Stressful events such as extreme weather conditions, poor body condition, and other energetic challenges (e.g. pregnancy, lactation, old age etc.) can also facilitate an acute copper mediated hemolytic crisis in affected animals. There is a remote possibility of a genetic copper metabolism abnormality, although this is less probable than other causes.

Copper metabolism in moose is poorly understood and copper toxicity has not been previously reported in this species. Unfortunately, we cannot confirm this interpretation at the present time as liver damage must also be recorded histologically to confirm a diagnosis of copper toxicity. Increased liver enzymes may provide an early warning of a pending copper toxicity crisis and a biochemical panel

could be run on archived serum from this moose if any is available. A review of this moose's radio-collar/movement data and features of its home range may also be useful (i.e. to rule out potential anthropogenic point sources of copper). Capturing and more thoroughly evaluating the trace nutrient status (e.g. with liver biopsies, biochemical screening etc.) of other moose in this area could also be considered.

Figure 14. An adult female moose (WHID 14-4501) found dead in the PG South study area on May 14, 2014.