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Wildlife Health and a Growing Agriculture Industry in the Northwest Territories: A Report on Active and Passive Northern Wildlife Health Surveillance Activities done through the University of Calgary

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EXECUTIVE SUMMARY

The Northwest Territories Agriculture Strategy: *The Business of Food: A Food Production Plan* established in March 2017 aims to '*Build a relevant and viable agriculture industry'*, including expanding livestock production. While this has great potential to improve livelihoods and food security, expanded livestock production in the Northwest Territories (NWT) has the potential to interact with free-ranging wildlife with possibly negative consequences for domestic and wild animal health, as well as human health. In order to predict and mitigate potential negative interactions, it is important to understand the pathogen diversity and current status of wildlife health in the NWT and to have sensitive and timely surveillance programs in place to detect changes in wildlife health that may pose a risk to, or have originated from, domestic livestock.

Our team has been working on wildlife health in partnership with communities in the Canadian north for over 20 years. Our work includes active wildlife health monitoring and surveillance through community-based monitoring, documentation of local and traditional knowledge, standardized monitoring protocols and targeted research programs as well as passive wildlife surveillance in partnership with the Canadian Wildlife Health Cooperative. Herein we describe these ongoing activities and highlight specific infectious diseases that may be a concern for livestock production.

Our active surveillance programs have focused on ungulates, primarily caribou and muskoxen, but also moose and Dall's sheep. Through hunter-based sampling, testing of animals captured for radio collaring, and targeted scientific studies we have identified several new and previously described viruses, bacteria, and parasites that wild ungulates may share with domestic ungulates such as cattle and sheep. Key pathogens of concern for the livestock industry identified through these programs and passive surveillance include *Erysipelothrix rhusiopathiae*, Orf virus and *Brucella suis* biovar 4. Winter tick (*Dermacentor variabilis), Toxoplasma, Neospora* and several viral and bacterial pathogens may also be shared among wild and domestic ungulates.

Hunter-based sampling of animals harvested for subsistence purposes, together with comprehensive disease testing of animals captured for the purposes of radio collaring, can provide extensive information on pathogen biodiversity, abundance, and geographic distribution. Such information is important to inform the agriculture industry about potential disease risks to livestock, as well as to track any changes in wildlife health. Local and traditional knowledge, paired with hunter-based sampling, provides tremendous insights into past and current animal health conditions and can provide an early detection system for emerging disease syndromes and changes in animal health.

Outside of the active surveillance program, the number of animals and/or tissues with abnormalities submitted for disease investigation (passive surveillance) from the Canadian North is relatively low compared to southern areas of Canada. Hotspots for submissions are often communities with active wildlife health research programs and where personal connections with researchers, or increased community capacity as a result of the research projects, increase the likelihood of abnormalities in wildlife being reported and subsequently submitted. Nevertheless, the overall low rates of submission across this vast landscape could underestimate the presence of other diseases that may be relevant to livestock production. Increased animal health capacity, and a smoother 'pipeline' of submission, diagnosis, and communication of results to communities would improve abilities to detect 'new' diseases, track known diseases, and better understand and prevent risks associated with domestic animal wildlife interactions. An effective wildlife health surveillance system can identify diseases of concern for human health. These efforts provide opportunities for risk-based guidance communication regarding mitigation methods to prevent human disease and recognize the sociocultural importance and nutritional value of traditional foods (typically wild game).

Although not the focus of this report, there is also the potential risk that livestock could introduce diseases to the North. These risks are worthy of consideration as animal agriculture expands in the NWT.

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INTRODUCTION

The Northwest Territories (NWT) Agriculture Strategy: "The Business of Food: A Food Production Plan" was established in March 2017 (GNWT 2016). Its overarching goals are to:

- Build a relevant and viable agriculture industry
- Support the safe, sustainable development of food production systems
- Contribute to the sustainability of NWT communities
- Encourage and support the transfer of food production skills
- Increase the availability of local food for northern residents
- Reduce the cost of food for northern residents

A component of this framework is to promote domestic livestock production in the NWT. Central to the establishment of a healthy and economically viable livestock industry will be the prevention and control of infectious diseases. Threats may come from the livestock industry itself, or from contact with endemic wildlife species. Similarly, the establishment of livestock industry in the NWT may pose risks to endemic wildlife through the introduction of new diseases or amplification of endemic diseases. To mitigate those risks, the Strategy recommends to *"Work in partnership to assess, minimize and respond to health risks associated with the transfer of pathogens and parasites from domestic livestock, wild species, and other native species."* (Recommendation 5.7)

In this report, we summarize some of the active and passive wildlife health surveillance activities done in the Canadian North by the Kutz Research Group, the Faculty of Veterinary Medicine, University of Calgary and the Alberta Regional Centre of the Canadian Wildlife Health Cooperative (CWHC) since 2003. We identify some of the key pathogens that may be of importance to a domestic livestock industry, highlight considerations with respect to how a domestic livestock industry may affect wildlife species, and discuss some mechanisms for ongoing surveillance. We focus on three infectious agents: the parapox virus orf, the bacteria *Erysipelothrix rhusiopathiae* and *Brucella* spp. These three diseases have been selected due to both their relevance as pathogens of livestock and the documented increased detection in wildlife from the Arctic and subarctic regions.

ACTIVE SURVEILLANCE

The Kutz Research Group has worked on various wildlife health issues collaboratively with the governments of NWT, Nunavut, and Yukon since the early 2000s. Herein we will report on the main wildlife health monitoring programs and targeted surveillance activities that have the most relevance to the NWT.

Community-based Monitoring of Wildlife Health in the Sahtú Settlement Area (see Carlsson et al. 2015 and Appendix I)

Initiated in 2003 in response to community concerns about wildlife health, this program aimed at collecting baseline information on parasites and disease exposure in moose and caribou populations of the Sahtú Settlement Area (SSA) (Brook 2009). Hunters were supplied with standardized sampling kits (Kutz 2013) and requested to collect samples and data from animals that they harvested for subsistence purposes.

Figure 1. Samples collected by harvesters in the Sahtú Settlement Area to assess the health status of woodland caribou and moose. The same approach has been used in subsequent monitoring efforts in the Canadian North.

Between 2004-2014 samples from 243 caribou and 88 moose were tested for exposure to the protozoan parasites *Neospora caninum* and *Toxoplasma gondii,* as well as alphaherpesvirus, parainfluenza virus and pestivirus (Carlsson et al., 2015). All these infectious agents are relevant to both wildlife and livestock health as they can cause disease and/or infertility.

The key findings of this project were that all the investigated agents are circulating in most of the investigated caribou and moose populations. In particular, there was a high rate of exposure to pestivirus in moose and woodland caribou. Detailed results can be found in Appendix I. The Sahtú Wildlife Health monitoring program is currently being administered by Sahtú regional office, Environment and Natural Resources, Government of the NWT.

Widespread Serological Survey of Caribou in the Canadian Arctic

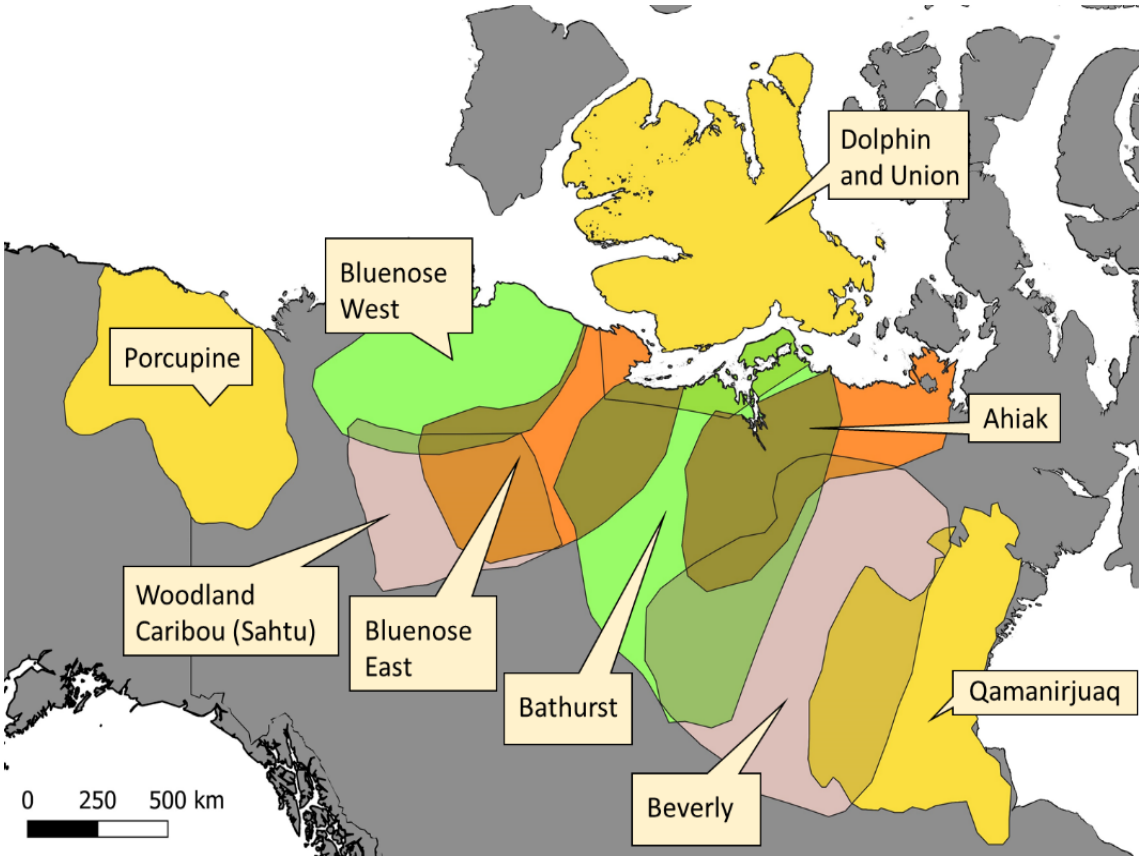
This work was initiated during International Polar Year (IPY) 2007-2009 as a component of the PhD thesis by Patricia Curry (2012) and expanded thereafter with a broader survey done by Post-Doctoral Fellow Anja Carlsson (Carlsson et al. 2019, In Review). It focused on assessing exposure to various infectious agents in seven caribou herds from North-America and Greenland.

For the two studies combined, a total of over 700 caribou blood samples (either serum or filter paper) were collected between 2000 and 2016. Samples were tested for antibodies against: *Neospora caninum*, *Toxoplasma gondii*, alphaherpesvirus, parainfluenza virus (PI3) and pestivirus, *Brucella suis* biovar 4, bovine respiratory syncytial virus (BRSV) and West Nile virus (WNV). All these diseases are of economic importance for livestock. In addition, some of them (*Toxoplasma, Brucella*, West Nile virus) can be transmitted to people.

Most of the investigated agents were widespread in caribou populations, with considerable variation among pathogens and populations. PI3 and BRSV were rare and WNV was not detected, likely as a result of climate factors limiting its spread from southern locations. Seropositivity to alphaherpes virus (presumably Cervid herpes virus, das Neves et al., 2010) and pestivirus (species identity unknown) was common. *Neospora* varied considerably among herds, and *Toxoplasma* and *Brucella* were present, but less common (Figure 2).

Most of the pathogens above (e.g. *Neospora, Toxoplasma, Brucella)* may be shared among wild and domestic ruminants. However, the serological assays used were not specific to pathogens of wildlife, rather they were designed for domestic animal species.

Thus, while we detected seropositivity against alphaherpes viruses and pestiviruses, it is unlikely that these are the same as those found in domestic livestock (for example Bovine Herpes Virus-1 or Bovine Viral Diarrhea virus). The potential for viruses specific to caribou to transmit to livestock, and vice versa, remains unknown.



	Pathogen	Main impact on livestock	Transmissible to human ?	Porcupine	Woodland Caribou (Sahtu)	Blue Nose West	Blue Nose East	Dolphin and Union	Bathrust	Ahiak-Beverly	Qamanirjuaq
Bacteria	*Brucella suis* biovar 4	Abortion, weak calf, joint disease	Yes	Negative	NA	<50%	<50%	<50%	<50%	Negative	Negative
	Erysipelothrix rhusiopathiae	Joint disease, skin lesions, acute death, abortion	Yes	<50%	NA	<50%	<50%	<50%	<50%	<50%	<50%
Parasites	*Neospora caninum*	Abortion	No	Negative	<50%	Negative	NA	<50%	<50%	<50%	>50%
	Toxoplasma gondii	Abortion	Yes	Negative	NA	Negative	<50%	<50%	<50%	Negative	<50%
Virus	Alphaherpesherpesvirus	Respiratory problems, abortion	No	<50%	<50%	>50%	>50%	>50%	<50%	>50%	>50%
	Bovine Respiratory Syncitial virus	Respiratory problems	No	Negative	NA	Negative	NA	Negative	Negative	NA	Negative
	Para-influenza virus (PI3)	Respiratory problems	No	<50%	Negative	<50%	<50%	Negative	<50%	<50%	<50%
	Pestivrus	Abortion, weak calf/lamb, respiratory problems	No	>50%	>50%	>50%	<50%	<50%	>50%	>50%	<50%
	West Nile Virus	Neurologic disease (reindeer)	Yes	Negative	NA	Negative	NA	Negative	Negative	NA	Negative

Figure 2. Summary of serological analysis results and origin of over 700 caribou blood samples collected in 2000-2016 (derived from Curry 2012; Carlsson et al. 2015; Carlsson et al. 2019 In review; Kutz et al. unpublished data). Serological tests indicate whether the animal has been previously exposed to the infectious agent. A description of the effect of the different pathogens on livestock and whether they can infect human are also provided.

Winter Ticks in the NWT (see Appendix II)

Dermacentor albipictus, also known as the winter tick, is an important parasite of moose and other ungulate species. In 1989, the northern range limit of winter ticks was thought to be approximately 62° N in the Yukon, and near the Alberta border for the NWT, but in the early 2000s, anecdotal reports of clinically affected moose in the SSA, NWT suggested a significant range expansion (Kutz et al. 2009). In response to increasing reports of hair loss in moose in the Sahtú, master's student, Cyntia Kashikavura, investigated the occurrence of *D. albipictus* on moose and caribou hides from the Sahtú submitted by local hunters. She also investigated growth and development of winter ticks on captive reindeer and attempted to develop a serological assay to detect antibodies to ticks using cattle as a model (Kashivakura 2013). Winter ticks were confirmed in five out of 30 moose at 66° N (Figure 3). The development of ticks on captive reindeer was similar to that reported in moose. There was no consistent pattern in antibody response after exposure to ticks, which made a serological assay unreliable as a diagnostic test. Additional work by Dr. Alessandro Massolo, based at the University of Calgary at the time, suggested that the climate envelope for winter ticks (the climate where a species currently lives) would extend up the Mackenzie Valley (unpublished data).

Winter ticks can infest domestic livestock and under certain husbandry conditions, the infestation levels can amplify over time. Livestock movement also poses a significant risk of importing and translocating tick species into and throughout the NWT as has been documented in other countries (Liebisch et al. 2010). Given the current and predicted climate suitability, and the presence of several suitable wild host species (moose, caribou, white-tailed deer, etc.), winter ticks are likely to establish throughout the boreal regions of the NWT and possibly also into the tundra ecozone.

Moose are highly susceptible to winter tick infestations and can cause population declines (Jones et al. 2019). Effects include extensive hair loss, anemia and skin disease that can have severe outcomes, including mortality, associated with altered thermoregulation and distraction from foraging. Less extensive, but still substantial, degrees of hair loss have been associated with winter tick infestations on caribou. Although not as studied in caribou as in moose, winter ticks are likely to have negative impacts on this species as well (Bondo et al. 2018). Domestic dogs, and most other mammalian species also can serve as hosts for winter ticks, and although they may not be as severely affected, they can act as transport hosts, bringing ticks into new areas, and under suitable climatic conditions, extending the geographic range of these ticks.

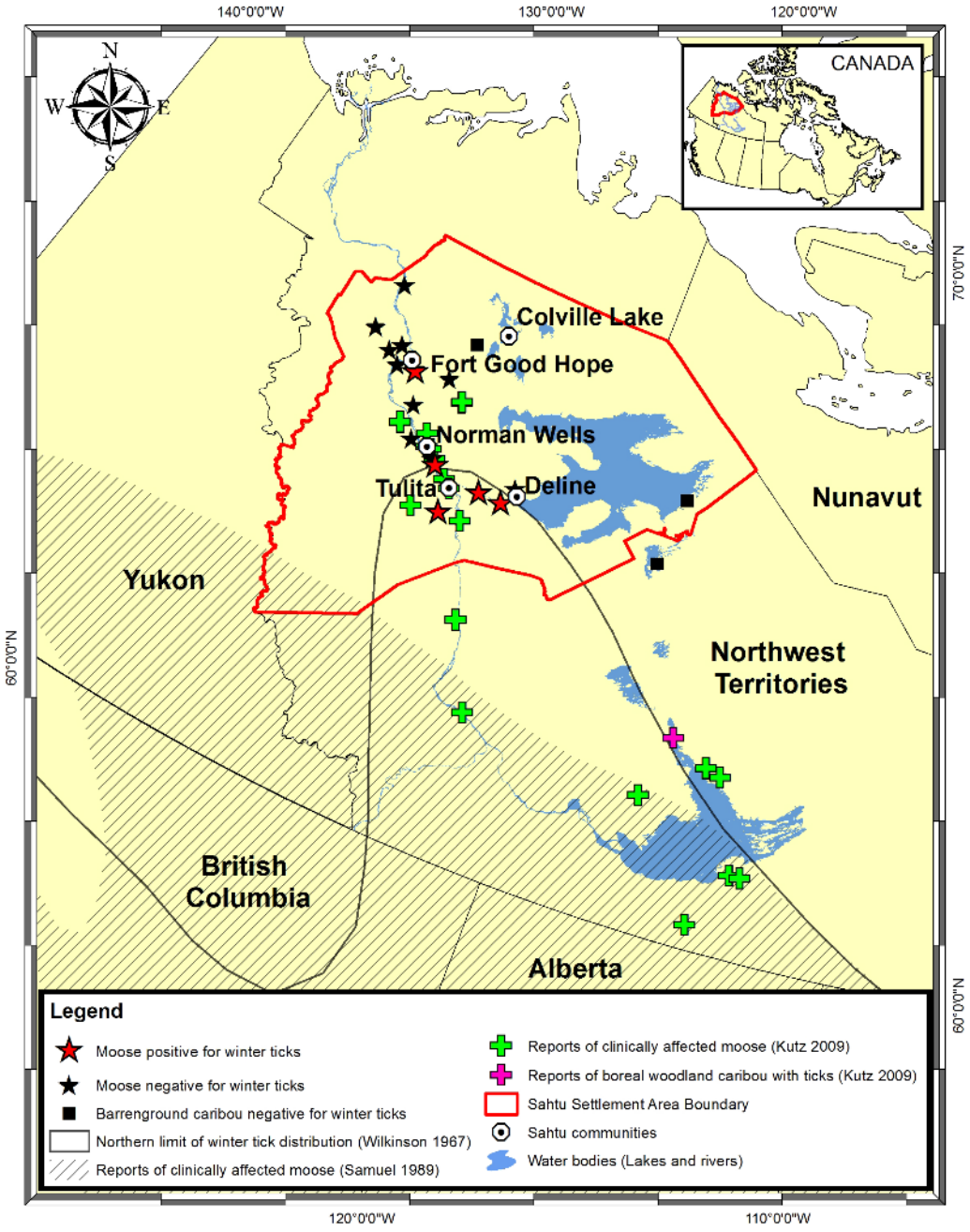


Figure 3. Map with historical observations of clinically affected moose or caribou in the NWT (Wilkinson 1967, Samuel 1989, and Kutz 2009), and more recent geographic locations where winter ticks were found in moose in the SSA (Kashivakura 2013).

The Muskox and Caribou Health Research Program

This program was initiated in 2008 in response to the changing health status of muskoxen on Victoria Island. It is lead by the Kutz Research Group, University of Calgary and is a collaborative program among universities, communities, industry, and territorial and federal government agencies. It is based primarily in the Inuvialuit Region of the NWT, and settlement Kitikmeot region of Nunavut in three communities (Cambridge Bay, Kugluktuk, Ulukhaktok), and has spanned multiple collaborations with other research groups in Canada and abroad.

The program uses three complementary approaches to investigate health of muskoxen and caribou:

i. Hunter-based sampling and examination of archival samples: Samples are collected by hunters or through other research/monitoring projects done by scientists or governmental agencies. Through standardized sampling approaches of animals that are harvested for subsistence, outfitted hunting, or through collaring activities, we can measure specific health traits of the animals and test for targeted infectious agents.

ii. Local ecological knowledge: We collaborate closely with community harvesters to document local ecological knowledge of wildlife health. This provides information on current and historical animal health status and trends in population, disease, behaviour, and threats.

iii. Disease investigation: Pathological examination of dead animals is a unique opportunity for our team to collect extensive information on health status of the animal in addition to our standardized sample collection and testing. We work with wildlife pathologists (currently Dr. Jamie Rothenburger) at the University of Calgary to investigate mortality events in the field or samples submitted through the hunter-based sampling program.

The three approaches are not exclusive and constantly inform each other to ensure an efficient and adequate health monitoring program.

Since 2014, we have collected samples from 323 muskoxen and 161 caribou harvested or captured near Ulukhaktok, Kugluktuk, and Cambridge Bay. The samples were tested for a wide range of pathogens and health indicators, such as stress hormone levels in hair or parasites in feces. Appendix III is a summary of our monitoring activities distributed in the communities.

This program is ongoing and very extensive. For the purposes of this report, we focus on the key targeted studies on:

A. *Erysipelothrix rhusiopathiae* disease investigations and serological survey
B. Traditional ecological knowledge on muskox and caribou health
C. Orf investigations
D. *Brucella suis* biovar 4 investigations

A. Erysipelothrix rhusiopathiae

This bacterium was first identified in muskoxen during multiple widespread mortality events in 2009-2012 on Banks and Victoria Island (Kutz et al. 2015). Using archived blood samples from previous research and monitoring projects, as well as our hunter-based sampling, we have investigated exposure to *E. rhusiopathiae* in both muskoxen (892 samples) and caribou (3,190 samples) over the last 50 years across North America. Our results indicate that although the bacterium has been circulating in muskox and caribou populations since at least the 1970s, exposure to *E. rhusiopathiae* has increased in recent years and might be associated with population declines in some muskox populations (Mavrot et al. In Prep). We documented a single outbreak strain of the bacterium in muskox mortalities on Victoria and Banks Islands in 2009-2013 (Forde et al. 2016b). The same strain was found in dead muskoxen, Peary caribou and Arctic fox carcasses on Prince Patrick Island in 2017, and in a seal found dead on Victoria Island (Mavrot et al. unpublished data). This suggests that a pathogenic strain of *E. rhusiopathiae* (maybe) is circulating among different host species on the Arctic Archipelago. Cloacal swabs from over 600 snow geese were negative on culture for *E. rhusiopathiae* (Forde et al. 2016b, Reid and Kutz unpublished data). None of the 115 wolves or 75 fish obtained from Banks and Victoria Islands in 2011-2013 were culture or polymerase chain reaction (PCR) positive for the bacterium (Forde et al. 2016b).

Although *E. rhusiopathiae* has been documented in a large spectrum of host species, no survey data exist on exposure in other species of the Arctic (rodents, birds, seal, fish) and on their possible role in the epidemiology of the disease.

A human case of *E. rhusiopathiae* infection was recently documented in the Inuvialuit region (Groeschel et al. 2019). The source of infection is unclear and the strain was different than the main strain that we have detected circulating in muskoxen and caribou. This case highlights the importance of awareness and early recognition of pathogens circulating in wildlife or the environment and the need for good communication between different partners involved in the health of humans and animals in the North.

Further study is necessary to better understand the risk that wildlife-associated *E.* rhusiopathiae infections may pose to domestic animals, but *E. rhusiopathiae* is a known

cause of serious disease to domestic swine and poultry (the disease in these species is called erysipelas). The wildlife exposure that we have documented can highlight geographical areas that may be of particularly high risk for domestic animals and be included in recommendations for vaccination protocols. It would be useful to compare Arctic strains to those found in domestic animals to better understand the transmission dynamics of this pathogen (i.e., are the bacteria ubiquitous in the northern environment or were they recently introduced?).

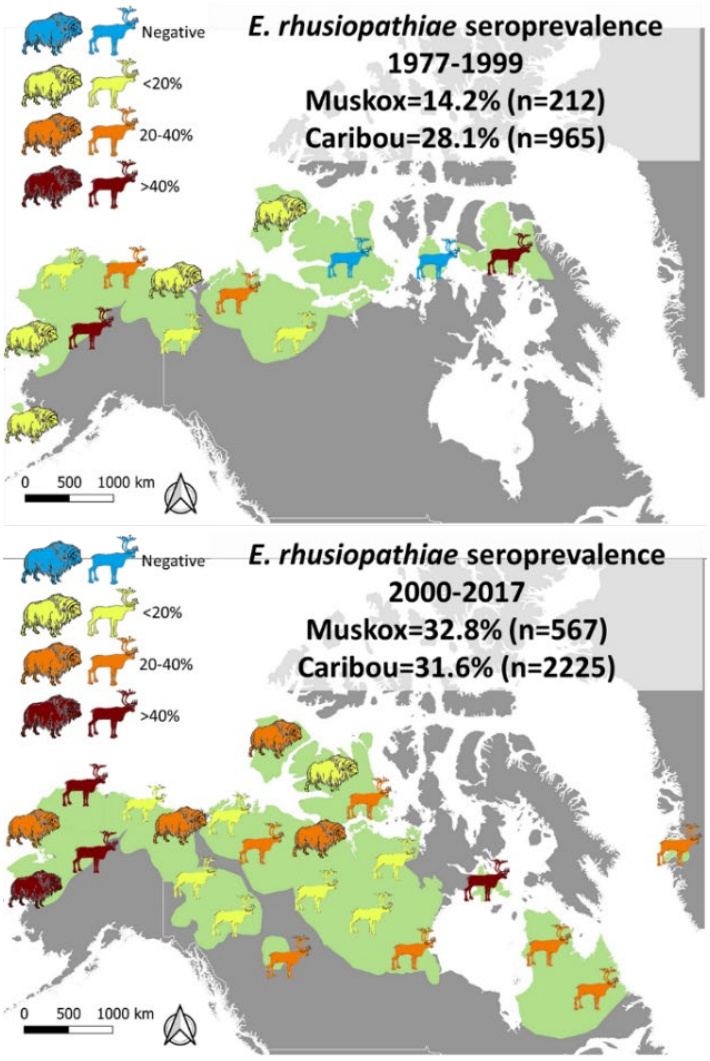


Figure 4. Summary of serological investigation for exposure to *Erysipelothrix rhusiopathiae* in muskox and caribou populations across North America (Kutz et al. unpublished data; Mavrot et al. submitted.)

B. Traditional Ecological Knowledge on Muskox and Caribou Health

The thesis research by Dr. Matilde Tomaselli demonstrated the value of local and traditional knowledge in informing wildlife health status and trends

(Tomaselli 2018). Dr. Tomaselli's work demonstrated that local and traditional knowledge could be used for early disease detection, spatial and temporal description of disease outbreaks, to monitor trends in diseases, and to guide further scientific investigations (Tomaselli et al. 2018). Key findings from Tomaselli's work lead to further scientific investigations on Parapox virus (Dalton, van der Meer et al. In prep) and *Brucella suis* biovar 4 (Tomaselli et al., In Review) in muskoxen.

C. Parapox Virus 'Orf'

Orf is a virus that causes skin lesions (scabs). We documented orf virus for the first time in muskoxen on Victoria Island in 2016 (Tomaselli et al. 2016). As a component of the PhD thesis by Chimone Dalton, we subsequently did gross, histological, and molecular examination of skin samples from 60 muskoxen. Dalton detected the virus in muskoxen with and without orf-like lesions in all the investigated locations on Victoria Island and the adjacent mainland. All muskoxen were infected with a common strain, which is different from the strains isolated in Alaska in muskoxen and other wild ungulates (Tryland et al. 2018; Chimone et al. unpublished data).

Although only recently discovered in Canadian muskoxen, our data indicate that orf virus is distributed widely on both Victoria Island and mainland muskoxen and can cause disease in affected animals. Orf has likely been present but undetected for a long time in muskox populations, however, it is not clear why the disease has emerged at this point. The risk of orf transmission between livestock and wildlife remains unknown but is likely possible given that this virus tends to be a generalist. No data currently exist on the occurrence of orf in caribou, Dall's sheep and mountain goats in Canada, although all three species are susceptible to the virus. Collectively, these knowledge gaps emphasize the need for further investigation and a role for an efficient animal disease surveillance system in Arctic wildlife.

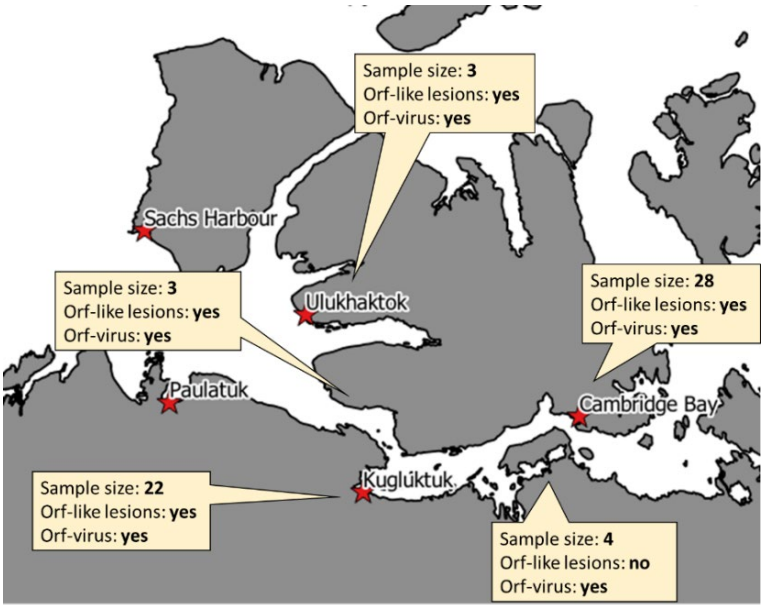


Figure 5. Summary of the examination and analysis of 60 muskox skin samples collected on Victoria Island and the adjacent mainland in 2016 for each location shown: the number of samples examined, the presence of orf-like lesions and the detection of the orf virus using biomolecular techniques (PCR). (Dalton et al. In Prep)

D. *Brucella suis* Biovar 4

Rangiferine Brucellosis (*B. suis* biovar 4) has been known to occur in caribou herds from the North American Arctic since the 1960s and is considered endemic in this species. In contrast, infection in muskoxen is rarely reported (Tomaselli 2016). Our monitoring between 2000 and 2016 confirmed exposure ranging from 0-5% in caribou herds on the Canadian mainland (Bluenose West, Bluenose East, Bathurst, Beverly, and Ahiak) and a seroprevalence of 15% in the Dolphin and Union caribou herd. Seroprevalence appears to vary across herds and time (Figure 2).

In muskoxen, seropositivity is rare, however, near Cambridge Bay (Victoria Island) seroprevalence appears to have increased from 0.8% in 1989-2001 to approximately 5% in 2010-2016 (Tomaselli et al. in review). Our more recent testing from 2017-2018 confirms the higher exposure rate in the Cambridge Bay muskox population and suggests an expansion of the bacterium to muskoxen on the adjacent mainland (Kent Peninsula) and around Ulukhaktok (Victoria Island) (Kutz et al. unpublished data).

PASSIVE SURVEILLANCE

Overview

The University of Calgary Faculty of Veterinary Medicine and Canadian Wildlife Health Cooperative (CWHC) Alberta have received submissions of northern wildlife over the past eight years. Many of these submissions were the result of ongoing research in these northern communities which has fostered relationships, built capacity in disease surveillance, and facilitated the submission of cases. In particular, collaborations with hunters and hunter-based sampling programs have raised awareness of wildlife disease. The availability of diagnostic support through University of Calgary and the Wildlife Veterinarian of the Government of the NWT have supported increased submissions and contributed to enhanced disease surveillance in northern wildlife.

To understand the presence/absence of wildlife diseases of relevance to agriculture in the North, we searched the CWHC database for all submissions from the Yukon, NWT and Nunavut. We also assessed all wildlife cases submitted from the North to the Alberta Region of the CWHC in our University of Calgary Diagnostic Services Unit database.

Example Case: Brucellosis in a Muskox

In the spring of 2016, a mature female muskox from Cambridge Bay, Nunavut was euthanized after being observed on the land unmoving and unafraid of people. Samples of diseased tissues were submitted for diagnostic investigation to the Alberta Region of the CWHC at the University of Calgary. This animal had inflammation of the mammary gland (mastitis), lymph nodes (lymphadenitis) and uterus (endometritis). The bacteria, *Brucella suis* biovar 4 was isolated from a variety of tissues, including inflamed tissues, providing strong evidence that the bacteria was the cause of disease in this individual.

This case demonstrates the importance of reporting and submitting tissues and whole carcasses from animals that appear sick. Only through ongoing reporting and accurate diagnoses will we be able to accurately track wildlife disease, appropriately inform harvesters of zoonotic risks, and understand what the risks are for domestic livestock.

Results

In total, 243 cases from the North were submitted to the Alberta Region of the CWHC. Only a portion of these (81 cases) were for diagnostic investigation of abnormalities. The rest were research-related submissions and health assessments (described above). Submissions of abnormal animals and/or tissues were examined by

board-certified veterinary pathologists. The vast majority (91%) were mammals. Only 9% of submissions were birds.

Starvation was the most common cause of death. Infectious and inflammatory diseases were the second most common category of diagnosis and have the most relevance for livestock production in the North. The three major infectious diseases of potential importance to agriculture in NWT include: orf virus, erysipelas, and brucellosis, as well as secondary bacterial and parasitic infections, which are described below.

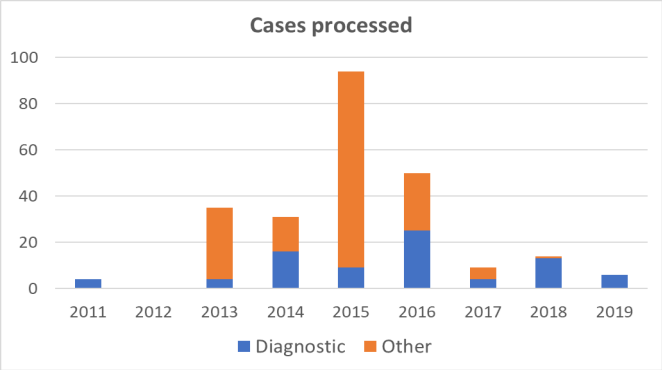


Figure 6. Northern cases submitted each year to the University of Calgary for diagnostic investigation or for other reason (research, health assessment, specific disease testing, parasite identification).

Our surveillance activities identified cases of trauma. In some instances, samples were submitted based on suspicion of an infectious cause. Laboratory analyses including autopsy examination, histopathology and other tests were essential to determining the nature of these lesions and to rule out important infectious causes of morbidity, such as brucellosis. For instance, in 2018, a hunter noticed large swellings on the ribs of a muskoxen and was concerned about consuming the meat out of fear that these abnormalities were signs of tuberculosis or brucellosis. Examination of the tissues revealed that the swellings were healing rib fractures, probably caused by trauma from fighting. Furthermore, there was no inflammation, as would be expected in cases of infection.

Degenerative causes of disease were relatively rare. This likely reflects that animals with chronic, degenerative illnesses are unlikely to survive in harsh environments. In two animals, (seal and caribou), there was scar tissue on the liver and a third had arthritis of a forelimb joint.

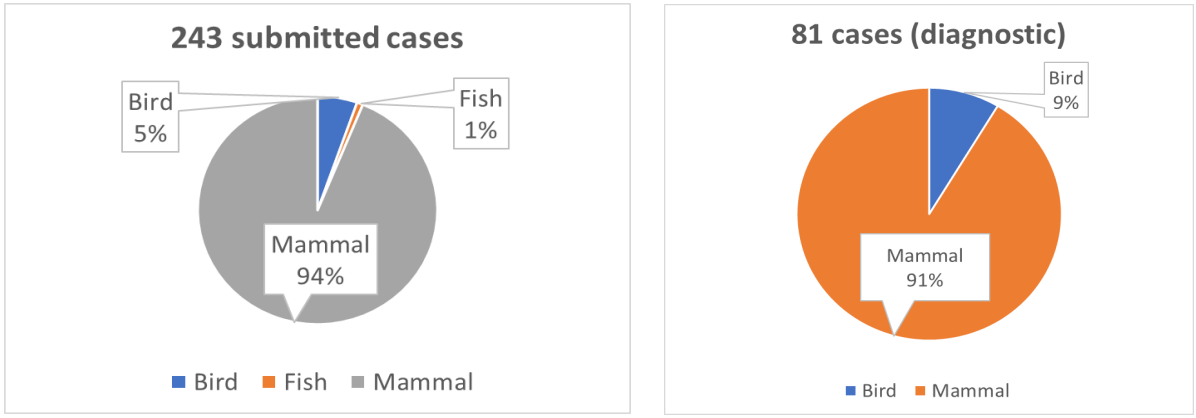


Figure 7. Distribution of (a) all submitted cases and (b) those submitted for diagnostic investigation by class.

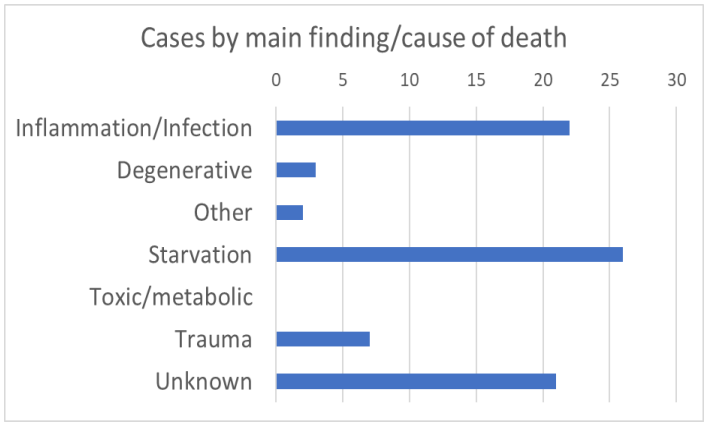


Figure 8. Main findings of 81 cases submitted to the University of Calgary for diagnostic investigation.

Starvation is a frequent cause of death and requires that other potential causes of death are ruled out through laboratory testing. A variety of animal species died of starvation including wolf, wolverine, fox, caribou, Canada goose and snow goose. Among rare causes of death, there were two cases of strychnine poisoning (a grey wolf and a grizzly bear) in the Yukon. There were also rare cases of cancer/tumors. For instance, a grey wolf from Norman Wells and a moose from Wekweeti both had skin tumors.

When the cause of death is unknown, there is still useful information to be gained from these cases. The laboratory assessment often rules out common causes of death and disease, such as trauma and infections. Additional samples and measurements can contribute to ongoing wildlife monitoring programs. For example, the University of Calgary has supported the Government of the NWT's (GNWT) efforts to document nutritional (body) condition of caribou by performing bone marrow fat analysis on long bone samples.

This information is valuable to establish baselines of nutritional body condition for important species and monitor for changes over time.

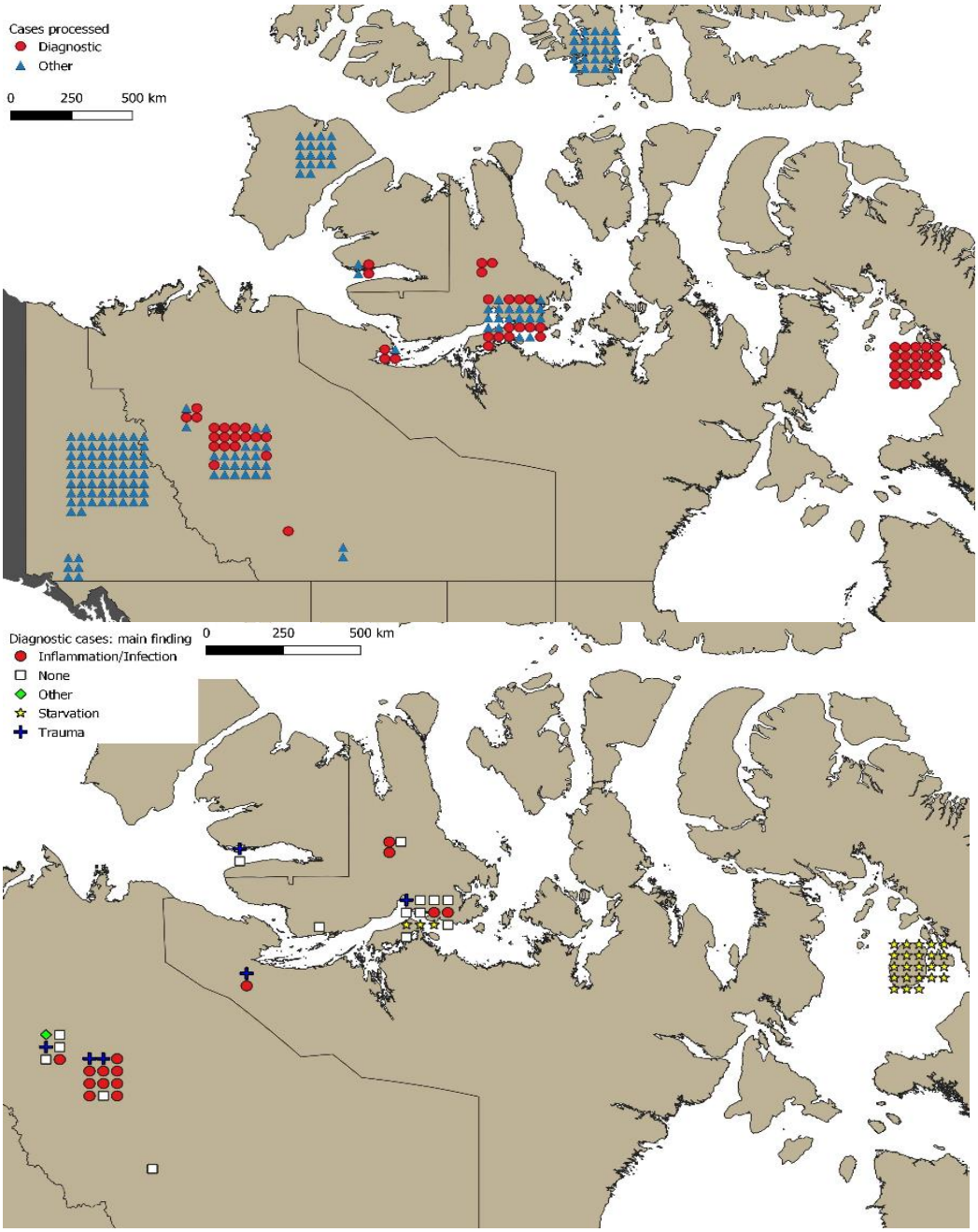


Figure 9. Above, location of cases submitted to the University of Calgary. Cases are classified as sent for diagnostic investigation and other testing (research, health assessment, specific disease testing, parasite identification). Below: Location of cases submitted for diagnostic investigation classified following the main finding of the pathological examination.

INFECTIOUS DISEASES OF NOTE

Orf Virus

Scabby mouth (also called contagious ecthyma or contagious pustular dermatitis) is a highly contagious disease caused by the orf virus. Affected animals develop crusting skin lesions on the mouth, legs, and, sometimes, the udder. The disease is typically found in sheep and goat species (wild and domestic) but has been reported in other ungulate species such as caribou, reindeer or black-tailed deer (Tryland et al. 2018).

Orf typically infects young or weakened sheep and goats, although muskoxen of all ages appear to also be susceptible to infection. The virus itself is not life-threatening and the affected animals usually recover spontaneously within weeks. However, the skin damage caused by the virus can serve as an entry point for bacteria and lead to systemic infections. In addition, the lesions are painful, which reduces the ability to eat and could further compromise an infected animal.

In domestic animals, treatment of orf is typically supportive and includes food and fluid therapy for poor body condition and antibiotics for bacterial infections. A vaccine against orf virus exists but is currently not authorized in Canada. There is currently no preventative vaccine or treatment options for affected wildlife.

Transmission from one animal to the other occurs from direct contact with skin lesions, but also through contaminated environment or other

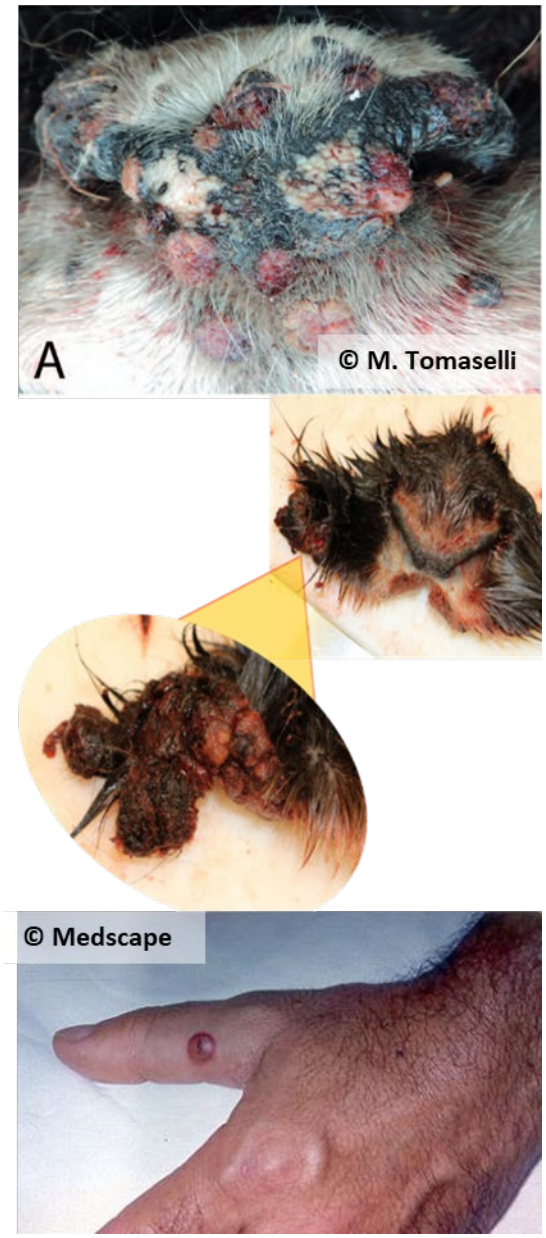


Figure 10. Nostrils from muskoxen affected by orf. Note the nodules of skin that lack hair and are bright pink-red. The muskoxen were from Cambridge Bay (Nunavut 2016) and Ulukhaktok (NWT 2017). The bottom picture shows a typical orf-induced lesion in human.

objects. Orf virus is able to survive for weeks or months in a warm and dry environment. Although the virus survival is shorter in cold and wet environment, no data exist on its ability to persist on the Arctic landscape.

Orf is a zoonotic pathogen, meaning that people can get infected. Infection in people typically occurs through breaks in the skin barrier, the disease is rarely severe and lesions generally heal within a few weeks.

Orf virus has been known to cause disease in wild goats and sheep around the world for decades (Nandi et al. 2011). It has also been documented in reindeer and muskoxen in Northern Europe (Tryland et al., 2005) and mountain goats, caribou and muskoxen in Alaska (Tryland et al. 2018).

Erysipelothrix rhusiopathiae

Erysipelothrix rhusiopathiae is a bacterial pathogen that causes the disease erysipelas. In domestic animals, it is an important pathogen in the swine production industry and vaccination is routinely used in production facilities to prevent infection. In poultry, it has emerged in recent years as an increasingly frequent cause of mortality in non-caged poultry and fowl farming (Forde et al. 2016a; Jansson 2018).

This bacterium is an opportunistic pathogen, meaning that it tends to cause disease in animals that are potentially compromised for another reason. It is also a generalist pathogen that can infect and cause disease in a wide range of host species (e.g. mammals, birds, fish, and insects). It can survive in the environment, although no data exist on its persistence in Arctic and subarctic landscapes. The bacterium is spread via direct contact with contaminated body fluids (e.g. saliva and excretions), contaminated soil or water, and possibly by insects.

In domestic animals, the disease can cause death very quickly through blood poisoning (septicemia). Milder forms of the disease are typically associated with skin-lesions, inflammation of the joints or of the heart. In sheep, *Erysipelothrix* has been associated with pregnancy loss (abortion) (Fthenakis et al. 2006).

Erysipelothrix can also be transmitted to people, usually causing skin lesions, but infection can also progress to severe heart and joint infections. Human infection typically occurs through direct contact with infected animals or animal parts, making animal care workers, farmers, and hunters at risk. If detected in time, the disease in people can easily be treated with antibiotics.

In North American wildlife, *E. rhusiopathiae* has been isolated in several Arctic species such as muskox, caribou, ringed seal and Arctic fox. The bacterium has been associated with multiple die-offs in caribou and muskoxen in Canada and Alaska (Kutz et al. 2015; Forde et al. 2016b; Bondo et al. 2018). Investigation of muskox and caribou carcasses indicates that infection with *E. rhusiopathiae* probably causes a quick death of the animal. Information on the mechanisms of disease caused by this bacterium in wildlife is limited. In Arctic fox, *E. rhusiopathiae* has been associated with poor body condition, shaggy haircoat and joint diseases.

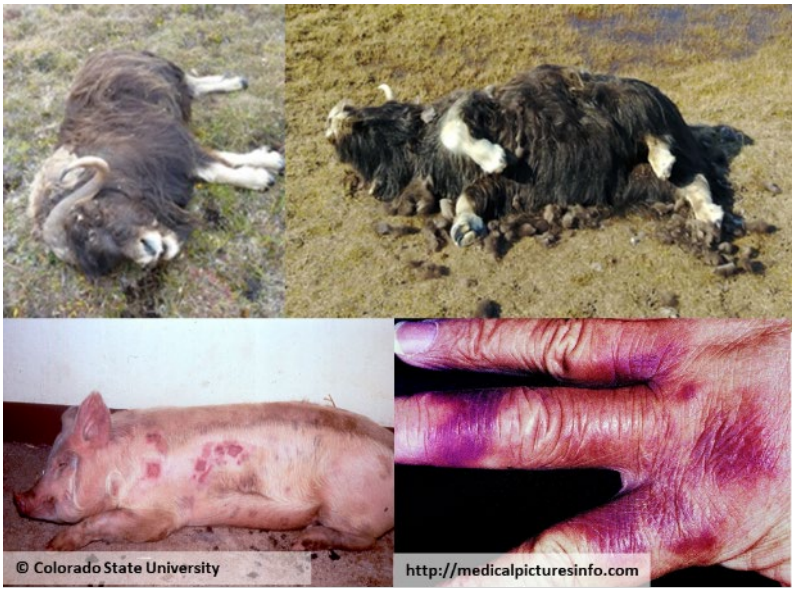


Figure 11. Upper row: muskox carcasses found on Victoria Island (2011) and Prince Patrick Island (2017). In both cases, the same strain of *Erysipelothrix rhusiopathiae* was isolated. Lower row: Lesions caused by the bacterium in a pig and a person. Note the typical diamond-shaped appearance of the skin lesions on the pig.

Brucella spp.

Brucellosis is a zoonotic (can infect people) disease caused by several species of the bacterium *Brucella*. While many species of *Brucella* exist, the most relevant for the Canadian North is *Brucella suis* biovar 4, which is present in caribou and muskox populations (Rangiferine brucellosis, Godfroid et al. 2013) and *Brucella abortus* in bison. In recent years, a substantial increase in the occurrence of brucellosis has been documented in muskoxen from Victoria Island and in caribou from the Dolphin and Union herd (Tomaselli et al. 2019, Fernandez-Aguilar and Kutz unpublished data). These bacteria can infect other species such as dogs, marine mammals and humans.

Brucellosis is a slowly progressive disease from which many infected individuals do not recover (Ficht, 2003). Typically, *Brucella* infection will affect joints, mammary glands

and reproductive organs, causing abortion, infertility, mastitis, and limping. However, the bacteria can also affect other organs such as lymph nodes or bones (Megid et al. 2010).

Animals can get infected through contact or ingestion of body fluids (semen, urine, and milk) or material (for example eating afterbirth). Young animals can also become infected by consuming the milk of females with mammary gland infections. *Brucella* can survive well in cold and wet conditions, but no data exist about its ability to persist in the Arctic environment.

In Canada, brucellosis has been eradicated from the domestic livestock herd and the greatest risk of transmission to domestic animals and humans is from infected wildlife. Brucellosis is a federally reportable disease that is managed by culling all infected and in-contact domestic livestock. Antibiotics do not cure the disease and there is no vaccine available in Canada (Government of Canada 2011). With the expansion of domestic livestock into the North, brucellosis is a concern, particularly for cattle, sheep, goats, and pigs.

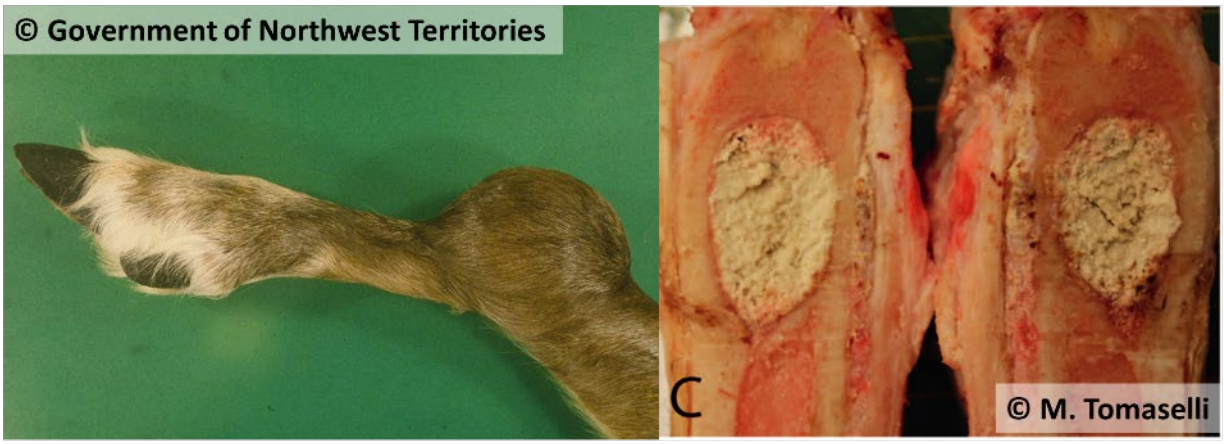


Figure 12. Left: typical joint swelling (bursitis) caused by *Brucella suis* biovar 4 in a caribou. Right: Bone infection (osteomyelitis) caused by Brucella in the bone of a muskox harvested in 2016 on Victoria Island.

People can get infected with brucellosis. Typically, infected people experience high fevers and other flu-like symptoms, with intermittent relapses (the disease in people is sometimes referred to as “undulant fever”). If discovered early, the infection can be treated with antibiotics. If left untreated, the bacteria can establish in organs and joints and become a debilitating disease that is difficult to treat, even with antibiotics (Dean et al. 2012).

Other Infectious Diseases

Submissions to the CWHC (Alberta) identified a range of secondary/opportunistic pathogens similar to what is expected to occur in domestic animals. Isolated bacteria from infections of the pleura, lungs, muscle and lymph nodes include opportunistic pathogens such as: *Escherichia coli, Pseudomonas sp., Trueperella pyogenes, Staphylococcus* sp., *Streptococcus, Pasteurella multocida, Carnobacterium* sp. and *Fusobacterium necrophorum*. There is no real threat of transmission between wildlife and domestic animals for these pathogens as they are widespread in both populations as part of their normal microbial communities and tend to only cause disease if the host is compromised (e.g. wounds or immune system deficiencies).

As is typical of wildlife, parasitic infections were common among submissions. Animals were infected with the following parasites: *Sarcocystis, Echinococcus granulosus* and *Umingmakstrongylus pallikuukensis.* Of these, *Sarcocystis* and *Echinococcus granulosus* have the known potential to infect domestic animals. *Sarcocystis* is a protozoan parasite that often establishes parasitic cysts in muscle tissue. Infection is common in both wildlife and domestic livestock and known *Sarcocystis* species in northern wildlife are not thought to be a human health risk.

Echinococcus canadensis is a tapeworm parasite carried in the intestines of dogs and related canids, such as wolves and foxes. Eggs are shed in the feces and can survive for extended periods in the environment. Herbivores ingest eggs in the environment and the parasite creates hydatid cysts in the lungs and liver. When organs containing the hydatid cysts are consumed by canids, the lifecycle is completed when the adult tapeworm develops in the intestine of the canid. Livestock, including sheep, goats and cattle, are susceptible to *E. canadensis* infection and develop typical liver and lung cysts. The risk of infection with this parasite in livestock in NWT is likely similar to other areas where wild canids and livestock coexist. In such regions, regular deworming programs for domestic dogs are included in herd health protocols. The parasite is capable of infecting people, although the risk arises from exposure to parasite eggs in canid feces that are deposited in the environment. There is no human health risk from consuming meat from affected animals or contacting the cysts. However, people can get infected through contact with feces from infected dogs or wild canids (Oksanen et al. 2015). Individuals may also express concern about consuming meat from infected animals due to the presence of the cysts.

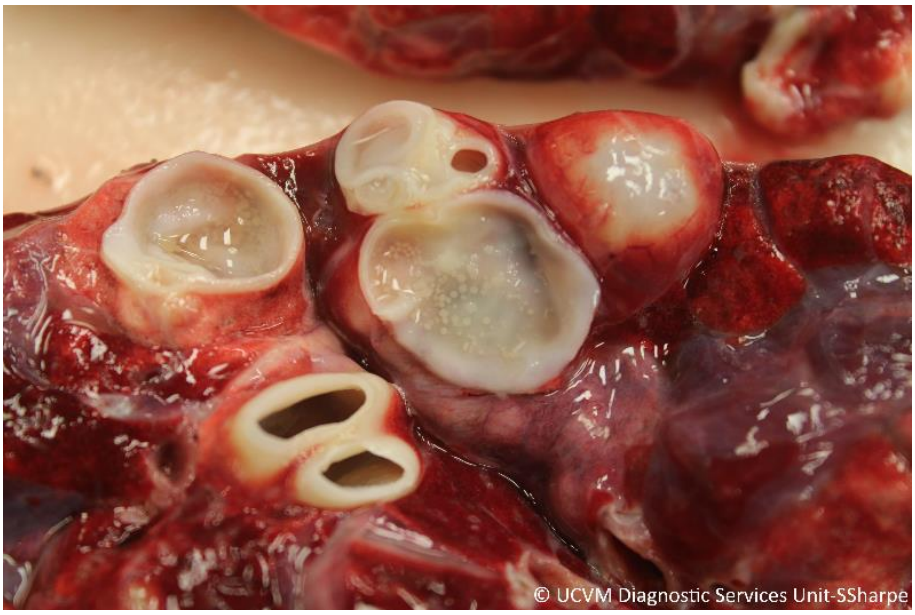


Figure 13. Lung tissue from a moose with multiple tan hydatid cysts typical of Echinococcus canadensis infection

DISCUSSION

Wildlife health surveillance, passive and targeted, is critical for tracking known and emerging diseases in wildlife and assessing the potential impact on people, domestic animals and associated industries, and wildlife sustainability. Enhancing surveillance of all types is critical to safeguard a growing northern agriculture industry.

Disease detection activities in the North benefit from strong local knowledge about the normal appearance of wildlife tissues and thus the ability to detect abnormal, which may indicate disease. Pairing this knowledge with access to diagnostic laboratory support and wildlife disease expertise is a powerful way to understand the baseline disease rates in the North and thus the potential risks to domestic livestock production and human health. Surveillance activities are also crucial to ruling out infectious diseases in cases where the cause is uncertain without supportive laboratory tests (e.g. trauma). Ongoing monitoring and further research into the characteristics of major infectious diseases (brucellosis, erysipelas and orf) is critical to better understand disease dynamics. Further, producers should be aware of the endemic diseases present in wildlife in the NWT and methods to mitigate livestock disease (e.g. separation from wildlife and vaccination protocols).

There is opportunity to enhance passive surveillance, that is submission of entire carcasses or abnormalities, in the NWT. For example, only 81 diagnostic cases were submitted from the North to CWHC Alberta between 2011-2019. Though, additional cases were submitted through other CWHC regional offices, and several cases would have been assessed in-house with the GNWT wildlife veterinarian and further diagnostics not required. Nevertheless, increasing ease and specimen flow for submission of abnormal animals is important to ensure detection of unusual/emerging pathogens and syndromes. Although submission of cases are a cornerstone of passive surveillance and early detection of health concerns for animals and people, other approaches, including targeted surveillance, collection of local ecological knowledge or photo-documentation on web-based platforms (e.g. LEO network) could complement classical passive health surveillance in remote regions.

There are a number of important diseases that could be included in passive surveillance activities. Avian influenza (so-called “bird flu) is one of these. This virus is carried and shed by wild birds and poses a risk to domestic poultry (e.g. chickens, turkeys, ducks, and geese) and human health. Birds submitted to CWHC Alberta from NWT could be tested for the presence of this virus and results could inform local authorities about the potential risk of this pathogen in that area. Also, these data could feed into Canada-wide Avian Influenza surveillance, which is highly relevant since the North includes the northern-most reaches of many birds’ migratory pathways and hosts overlapping ranges

with the East-Asian Australasian Flyway. Asia is considered a hotbed of avian influenza so birds in the North might be the first place in North America where new strains could be detected.

Another important disease that could be included in disease surveillance activities is chronic wasting disease (CWD). This is a fatal condition of wild cervids including deer, elk, moose and caribou that is caused by a misfolded protein called a prion. The disease is similar to bovine spongiform encephalopathy (BSE; also known as "mad cow disease"). Affected cervids develop abnormal behaviour, lose condition and eventually die from brain lesions and associated conditions that may include aspiration pneumonia. Animals become infected through contact with infected animals or contaminated soils. There is no treatment or preventative vaccine available. The disease has spread rapidly from initial locations in southern portions of Saskatchewan and is now established in southern Alberta. There is also likely a high risk of introducing CWD into wild cervids if farmed cervid production is permitted in the North as this agricultural practice was associated with the establishment and spread of the disease in Western Canada.

Many of the challenges of passive wildlife disease surveillance (disease detection in submitted animals), are amplified in the North. These challenges include limited and biased sampling (we only know about the samples and diseases that are submitted to the laboratory for diagnostic testing). The diseases detected may not be representative of the situation in nature. Other barriers include large geographical distances, remote locations, cost of transportation of samples, lack of access to laboratory facilities and limited training of personnel in sample collection. Access to reliable and long-term funding sources remains critical to maintaining and expanding our ability to conduct wildlife disease surveillance activities at the CWHC Alberta Region.

Provision of veterinary diagnostic support for both wildlife and domestic livestock will be critical for a healthy agricultural industry. Understanding why animals become sick or die is an important piece to managing health in any animal population. Since similar challenges that face wildlife disease monitoring are expected to occur in agriculture, there is the potential for synergy between these two sectors to maximize access to diagnostic laboratories and professional networks.

It should be kept in mind that the transmission of diseases between wild and domestic animals can occur in both directions. The predicted increase in farmed animals in the Arctic/subarctic also means a risk of introducing new diseases/strains in naïve native free-ranging populations. Close contact is not always needed between species. For example, consumption of wildlife or livestock carcasses and offal by mobile wild scavengers and

carnivores provides an indirect mechanism to spread diseases among wildlife and domestic animals.

A growing domestic livestock industry offers many potential benefits, but it also introduces new risks to the native wildlife species that are also critical for food security, the economy, and central to the cultures of Indigenous peoples. Health surveillance, of both wildlife and domestic species, prevention of introduction of new diseases, and early detection and rapid response to any animal disease issues, are critical to protect the health, welfare and sustainability of both wildlife and domestic species. There is growing community interest for wildlife health sampling, monitoring and surveillance in the North, including systematic documentation of local knowledge of wildlife health. Support for such community-based monitoring programs, together with ongoing targeted research, and enhanced animal health capacity across the North will help to protect both wildlife and domestic animal health, which will be reflected in human health and wellbeing, as well as sustainable livelihoods.

RECOMMENDATIONS

The passive monitoring of a variety of wildlife species and active monitoring of muskox and caribou in the Canadian Arctic has produced a large amount of information that serves to further guide disease surveillance in the region, inform management and support NWT communities. However, the results also show areas that could be improved and important gaps in knowledge:

- Improving passive surveillance: supporting the submission of abnormalities identified by local citizens to diagnostic labs and building the capacity to respond to and investigate mortality events/die-offs.
- Fostering good collaboration and communication between different partners (researchers, wildlife managers, members of local harvesting committees, and public health service providers) to allow for early detection of health issue in livestock, wildlife and humans. Collection of local knowledge in communities often helps foster collaboration between scientists and hunters/farmers, raise awareness of disease relevant to human, livestock and wildlife and improve reporting of diseases.
- Improving the geographical coverage of monitoring of the main infectious diseases.
- Extend active surveillance to other species to better understand the epidemiology of diseases known to have a broad spectrum of host species (for instance mountain goats and Dall's Sheep for orf, birds and rodents for *Erysipelothrix*, and canids for *Brucella*).
- Although well-documented for lower latitudes, knowledge on the survival of infectious agents in the Arctic environment is often missing.
- Global warming is changing the Arctic landscape at an unprecedented speed. It is important to document and predict how those changes will influence host-pathogen interaction in the coming years.

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Wildlife health and a growing Agriculture Industry in the Northwest Territories:
A report on active and passive northern wildlife health surveillance activities done through the University of Calgary

APPENDICES

Supplement to MR291

APPENDIX I

The Sahtu Wildlife Health Monitoring Program

Summary report of results 2002-2014

Prepared for:
Department of Environment and Natural Resources, Government of the Northwest Territories, and the
Sahtu Renewable Resources Board, Northwest Territories

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Table of Contents

Executive Summary

Background

The Wildlife Health Monitoring (WHM) Program began in 2003 in response to community concerns about wildlife health under a regime of rapid environmental change in the Sahtu Settlement Area, Northwest Territories. To address these concerns, the University of Saskatchewan (initially) and then the University of Calgary, together with the Department of Environmental and Natural Resources (ENR), Government of the Northwest Territories, the Sahtu Renewable Resources Board (SRRB) and the Sahtu Renewable Resources Councils (SRRC), established the Wildlife Health Monitoring Program.

Objectives

(i) To establish baselines of body condition, body size, and pathogen occurrence in caribou and moose,
(ii) To establish protocols and methods that could be used for community based monitoring,
(iii) To share knowledge about wildlife health and wildlife disease and
(iv) To build community capacity for wildlife health monitoring.

This report outlines the progress the WHM program has made since its initiation in 2003, briefly summarizes the main results, and discusses recommendations for moving forward.

Methods

Local subsistence hunters were identified by the communities to be Wildlife Health Monitors. They were trained to collect data using standardized sampling protocols and pre-prepared sampling kits. Samples collected included the lower jaw, the left metatarsal, fecal sample, the left kidney with fat, a piece of the liver, blood on filter papers and a piece of hide. Hunter observations of the animals' condition and any unusual findings were also recorded. Samples were collected and processed according to standardized protocols that were later used as the basis for those developed by the CircumArctic Rangifer Monitoring and Assessment Network. Initial sample processing was completed at the ENR office in Norman Wells, whilst further analysis for fecal parasitology, serology and abnormalities was conducted at collaborating institutions and diagnostic laboratories in Canada, Norway and the United States. Blood samples were tested for exposure to the protozoan parasites *Neospora caninum* and *Toxoplasma gondii* as well as alphaherpes virus, parainfluenza virus and pestivirus, using enzyme-linked immunosorbent assays (ELISA) or virus neutralization assays. Workshops and community meetings were held to disseminate results and receive feedback on methods and interpretations. In 2005, focus group interviews, documenting knowledge of past and current observable abnormalities and diseases in wildlife were, held with experienced

harvesters and elders from all communities of the Sahtu.

Results and discussion

Sample sizes

From 2004 to 2013, Wildlife Health Monitors in the Sahtu collected a total of 327 samples from moose and caribou. Of these samples 133 were from the Bluenose East caribou herd (BNE), 88 from the Bluenose West caribou herd (BNE), 26 from woodland caribou (WC) and 84 from moose. The samples/data collected most often were recordings of location of harvest (297/327), the sex of the animal (259/327), the metatarsal (254/327) and blood samples on filter paper (246/327). In general, sample sizes were too limited to analyse population trends in body condition and body size, however, measured body condition indices generally corresponded with hunter assessments, and trends for BNE were concordant with those reported elsewhere.

Parasites

The program lead to the identification of new parasites previously unknown in the Sahtu, including the leg worm, *Onchocerca cervipedis,* and the winter tick, *Dermacentor albipictus* in moose*,* as well a previously uncharacterized new species, of protostrongylid nematode, *Varestrongylus elegunenienses,* in caribou, moose, and muskoxen. The overall prevalence of parasites revealed by fecal parasitology (i.e. eggs/ oocysts/ larvae from helminths and protozoans) was low, with strongyle eggs being the most prevalent in caribou and Nematodirinae eggs the most prevalent in in moose. Visual gross analysis of hide samples from the metatarsal revealed the presence of *Besnoitia tarandi,* a protozoan tissue dwelling parasite, with 56.7% (38/67) prevalence in BNE and 53.2% (33/62) in BNW.

Serology

Samples collected as part of the WHM program contributed to the development and validation of blood collected on filter paper for the use in wildlife serology.

The highest seroprevalence of *N. caninum* was found in moose at 16.1% (15/31), then WC at 13.6%, (3/22) and BNE at 2.1% (2/95), whilst none (0/47) of the BNW samples were seropositive. Only BNW samples were tested for *T. gondii* and none were seropositive (0/34). More than half the BNE (66.7% (14/21)) and WC (57.1% (4/7)) samples tested for exposure to pestivirus were positive, whilst only one of six (16.7%) of the BNW samples and none of the moose samples (0/5) were positive. For alphaherpesherpesvirus, the highest seroprevalence was 60.8% (28/46) for BNW, then WC at 45.5% (10/22), whilst the lowest was for BNE at 18.5% (10/54). Two out of nine (22.2%) moose samples tested positive for exposure alphaherpesvirus. Only male BNW tested positive for antibodies to parainfluenza virus, with four out of 34 samples testing positive (5.5%).

Continued monitoring of all these pathogens is important and relevant to management since they have the potential to significantly impact individual and

herd health. *Neospora caninum* and *T. gondii* have the potential to cause neurological disease, infertility, and weak calves in caribou and moose. In livestock, bovine alphaherpes-, pesti-, and paramyxo-viruses contribute to the bovine respiratory disease complex. During severe infections effects can range from diarrhoea, to abortion and lethal disease. Here, we used tests originally developed for bovine serology and results may indicate an immunological cross-reaction against viral counterparts to known bovine (or other ruminant) viruses, and, therefore, specific viruses are often not known. However, for alphaherpesvirus the reactor is very likely the Cervid Herpes Virus 2 (CvHV2), and we suspect that others are also *Rangifer* specific. CvHV2 was identified as the primary agent in an outbreak of keratoconjunctivitis in Norwegian reindeer and experimental infections in reindeer have been linked to neonatal death and abortion. The impacts of the other viruses (or similar cervid-specific viruses) are not well studied, although BVDV viremia in reindeer has been demonstrated by experimental infection. Monitoring pathogens is also important because they can be sensitive to climate conditions. In light of the rapid changes that are occurring in the Arctic, continued monitoring of these parasites should be a priority.

Stress

Physiological stress was assessed by dental enamel hypoplasia (tooth defects) and glucocorticoids (stress hormone) levels. Analysis of linear enamel hypoplasias could be a promising tool for measuring stress occurring in the early lives of caribou (methodology developed through this program, see Wu et al. 2012). Further analysis is needed to explore the relationship between stress, disease and body condition in order determine if glucocorticoids can be used as biomarkers for overall health, but the data provided so far in the program form a baseline that can be used for comparative work in the future to monitor trends.

Concluding remarks

Monitoring biological indicators of health is important because they can provide complementary and predictive data of population health and population trends. Community-based monitoring studies are often less costly than population censuses and can be conducted more often and, therefore, track animal health changes as they occur. Data obtained from such monitoring programs can provide crucial wildlife health information for managers in years when census data are not available. Importantly, community-based observations have the potential to be predictive – identifying changes in animal health well before the effects of these changes are measurable through population censuses. A community-based monitoring approach also has the additional advantage of involving local communities and hunters who depend on caribou for their livelihood. Hunters are the eyes on the land and are in a unique position to observe changes when they happen and hold traditional knowledge that can contribute to an integrated view of individual and population health.

The Sahtu WHM Program has lead to extensive interaction between researchers, wildlife managers, harvesters and other stakeholders. The Program has provided

basic knowledge of health for caribou and moose, documented 'new to us' pathogens, and detected range expansion of others. For some health indicators, the sample sizes are adequate for evaluating trends. The WHM program has provided the essential network that has also allowed focused and intensive sampling to address specific questions (e.g., increased collections of jaws in 2005-2006 to establish the dental enamel hypoplasia technique, increased moose hide submissions in 2010-2012 to investigate tick range expansion). All data have been provided to wildlife management agencies to be used in policy and wildlife management. The program has also contributed to unique research projects and generated peer-reviewed publications. Overall, data presented here demonstrate that we have been somewhat successful in recording baselines of health of caribou and moose. However, the Program is constrained by small sample sizes.

The program began as a partnership among Government, Co-Management Board, and University and is an example of a successful multi-agency collaboration that has evolved over time. For the program to continue in a meaningful capacity it is critical that primary responsibility for program administration sits with a local agency (GNWT or SRRB). This will ensure ongoing community engagement, efficient program management, accurate sampling and data collection and storage, timely and appropriate communication of results, and improved speed of decision making in matters that concern the monitoring objectives. University-based researchers and wildlife health organizations (such as the Canadian Wildlife Health Cooperative) remain as essential team members that can provide the diagnostic expertise, contribute to interpretation of results and trends, and follow-up on novel discoveries and emerging issues.

Recommendations

In addition to the above considerations, several key points that can improve data quality and long-term success of the program are outlined below:

- Managers, researchers and communities should work together regularly to evaluate progress, identify priority research and monitoring areas, and adapt the program activities as needed
- Sampling and methodology should be standardized across years and regions.
- Wildlife Health Monitors and laboratory personnel should receive structured training and 'refresher' training regularly
- Samples should be collected during targeted time periods to ensure consistency over-time and comparisons between years
- To increase quality and quantity of samples we suggest collecting a reduced kit on an annual basis, complemented with full biological collections every 3-5 years.
    - For reduced kits we recommend: the right metatarsal, blood samples, fecal sample, jaw, a piece of hide from the rump, back fat measurement, hunter assessment of body condition, abnormalities,

information on sex, age, pregnancy status, location and date of the kill and the name of the hunter.

- A dedicated person should distribute kits, receive samples and be responsible for recording metadata on standardised data sheets and ensure quality of samples.
- Initial sample processing can occur in local laboratories. As much as possible this should occur to enhance local capacity and ownership.
- Data should be stored in one place in a master data file with one person/agency responsible for regular updating and maintenance.
- For long-term success, the program should be 'owned' and administered locally or regionally, with local capacity for training and sample processing
- Data should be shared and integrated with other similar programs across the herd range to boost sample sizes and contribute to the bigger picture
- Results should be disseminated to the communities on an annual basis

1. Introduction

The Sahtu Settlement Area (SSA) was established in the Northwest Territories in 1993 through a land claim agreement between the Sahtu Dene, Sahtu Métis and the Governments of Canada and the Northwest Territories. The settlement area lies just south of the treeline and encompasses 41,437 km^2 of land. It includes the communities of Déline, Tulita, Norman Wells, Fort Good Hope and Colville Lake[1]. In a 2012 census, 74% of the 2680 Sahtu residents were registered as aboriginal. In the Sahtu, people rely heavily on subsistence hunting and country foods dominate the diet for more than 60% of Sahtu residents[2]. The SSA is home to a wide variety of important wildlife species, including barren-ground and woodland caribou (*Rangifer tarandus* ssp.), moose (*Alces alces gigas* and *A. a. andersoni)* muskoxen (*Ovibos moschatus moschatus*) and Dall's sheep (*Ovis dalli dalli*). Sahtu wildlife are important sources of food through subsistence hunting, generate economic activity through hunting and tourism, and are critical for the maintenance of many cultural traditions[1].

In 2002, a workshop co-hosted by the Department of Environmental and Natural Resources (ENR), Government of the NWT (GNWT) and the Sahtu Renewable Resource Board (SRRB) brought together government and academic scientists and representatives from all five communities in the Sahtu with the aim of determining research and monitoring needs. Wildlife health was identified as a priority, with local subsistence hunters expressing a keen interest in having a more active role in wildlife health monitoring and research[3]. Today, northern Canada is undergoing significant changes due to accelerated climate warming and anthropogenic disturbances, which threaten the persistence of animal populations and traditional ways of life[4]. The Sahtu is currently experiencing unprecedented landscape changes associated with increasing exploration and development of shale oil reserves. In both the initial workshop in 2002, and in interviews and meetings since then, the Sahtu Dene and Métis have clearly voiced their concerns regarding the impacts of these changes on wildlife health and food safety and security.

In response to these concerns, the Wildlife Health Monitoring (WHM) program was initiated. The objective of the program was to work with local hunters to maintain an on-going wildlife health monitoring program that was responsive to the changing needs of the community and the changing health issues that emerge in wildlife.

Specific aims included:

- Establishing baselines of body condition and pathogen prevalence in caribou and moose.
- Establishing protocols and methods that could be used for community based monitoring.
- Sharing knowledge about wildlife health and wildlife disease.
- Building community capacity for wildlife health monitoring.
- Providing a manageable, sustainable, and cost-effective means of monitoring health and condition of key wildlife species in the long-term.

For more detailed information on the initial evolution and challenges of the Sahtu Wildlife Health Outreach and Monitoring Program see Brook et al (2009).

This report will outline the progress the WHM program has made since its initiation in 2002, briefly summarize the main results, and discuss recommendations for moving forward. The purpose of this report was not to provide a quantitative assessment of what is needed or provide in-depth analysis of trends as this is being done elsewhere.

2. Materials and methods

2.1 Community selection

During the early years of the program the main focus was on the health of barren-ground caribou, specifically concerning the Bluenose East (BNE) and Bluenose West (BNW) herds. For this reason, the communities of Colville Lake and Déline, and to a lesser extent Fort Good Hope, were initially targeted. Fort Good Hope expressed an interest to be involved in moose health research and in 2009 a targeted moose-monitoring project was added to the program. Fort Good Hope played a strong role in some of the more targeted research on the winter tick of moose conducted in 2010-2011.

Since 2012 the main research focus concerned the impact of industrial development on wildlife health. Since then workshops and meetings have, therefore, targeted communities along the Mackenzie river (Tulita, Norman Wells and Fort Good Hope) to ensure renewed and continued participation in the program. Notably, several meetings have taken place in Tulita, a community that has had limited involvement in the WHM program in the past. Since its initiation, the wildlife health-monitoring program has collected a solid baseline on health indexes and disease prevalence on barren-ground caribou (Bluenose East and West herds) but very little data exist on woodland caribou. To address this gap, the project was extended to include woodland caribou in 2013.

2.2 Community workshops and interviews

Between 2003-2005, annual workshops were held in each of the communities and in subsequent years workshops were offered as requested by the community. During the workshops updates on research methods, study findings, overview of common diseases of local wildlife and food safety were presented, and feedback on the findings and local perceptions was sought. In 2005, a formal documentation of local ecological knowledge was undertaken through focus group interviews in which thirty-one experienced harvesters and elders from all communities of the Sahtu participated. Interviews documented knowledge of past and current distribution of diseases in caribou, moose, muskoxen and Dall's sheep[3].

It was through these workshops and direct interactions with hunters that feedback on study design and data interpretation was acquired and local priorities and concerns were brought to light. The information gained helped the program to evolve, and also lead to targeted studies and changes in methodology. In 2009 and 2010 more targeted consultations again occurred with communities, with the specific objective to discuss naming of a new lungworm from caribou and muskoxen that was first discovered in the Sahtu[5]. Moose health was also becoming a priority, especially concerns about the apparent emergence of winter tick in the Sahtu. For more information on the structure and results from these workshops see Brook et al. (2009).

2.3 Hunter training

To evaluate the feasibility of sample and data collection, a pilot project was set up in Déline in 2004. Two harvesters from Déline, recommended by the local Renewable Resource Council (RRC), were recruited and trained by a wildlife veterinarian. Training took place through classroom and hands-on sessions. Harvesters were shown pictures of visibly recognizable caribou diseases and were given an overview of the project. They were then trained to collect data and samples from a freshly killed caribou. Data recorded included sex and approximate age of the animal, pregnancy and lactation status, presence of a calf, an overall assessment of body condition and a back fat measurement (Appendix 1). Harvesters were also asked to examine various organs for specific visible abnormalities.

In 2005, the program was expanded and two additional caribou wildlife health monitors (WHMs) were trained in Colville Lake. Caribou were not in the near vicinity at that time so the training was done using a power point presentation and props to illustrate sampling methods. Additionally, in 2005 and 2006, four WHMs from Fort Good Hope were trained to collect moose samples, again by using a presentation. Update sessions with the WHMs, that provided feedback on the results and addressed any concerns or changes in the collection protocols, were held each year during the annual school outreach tour.

In 2007 and 2008, researchers from the program participated in the annual Horton Lake community caribou harvests, arranged by the community of Colville Lake. This provided a great opportunity to work together with local hunters on-the-land, train them in sample collection, and share knowledge. The community harvest also provided the opportunity to work together with a young local videographer and culminated in production of a caribou sampling and disease video that was subsequently distributed to communities and used in hunter training sessions[3] (http://www.carmanetwork.com/display/public/Hunter+Training+Video+%28Summary%29).

In 2013, efforts to expand the program and increase sample sizes were made. The aim was to establish baselines of health indicators from caribou and moose in the

Mackenzie valley to facilitate the detection of changes in health status of these animals in relation to industrial development over time. To share knowledge and train hunters in sample collection the program participated in the 2013 Tulita community fall caribou harvest, at Caribou Flats by the Keele river in the Mackenzie Mountains.

In a move towards a more locally run program, the main responsibility for the BNW sample collection, including coordination of sample collection, distribution of payments and sample analysis, has now been shifted to the Department of Environment of Natural Resources (ENR), GNWT in Norman Wells.

Throughout the years, as protocols, monitoring needs and research focus shifted, there were continual training sessions and meetings to recruit new Wildlife Health Monitors and renew contacts with existing Monitors. This was achieved through formal and informal meetings, by joining individual harvesters on the land and/or by joining community harvests. The annual school outreach tours in winter and public and/or RRC meetings were used to report on findings, explain the project and the sampling process and to inform interested harvesters. Specific harvesters or communities were targeted for training when specific samples were needed for more focused research projects.

2.4 Sampling kits and data sheets

Caribou and moose sampling involved recording data on individual animals as well as collection of specific organs and body parts. A collection kit originally consisted of a small field clipboard with data sheets, sampling diagrams and pre-labelled sample collection bags. The sampling protocol was designed to be comprehensive and to maximize the amount of information gained whilst minimizing time and effort for harvesters. The samples were selected to provide data on age, disease, physiological condition, short and long-term nutritional status and maternal investment in reproductive fitness. The sampling kits evolved over the years and today consist of two tags, pre-labelled Ziploc bags, a map to indicate the location of the kill and one sheet with simple instructions on how to perform the sampling.

The datasheets, tags and information sheets used throughout the years can be viewed in Appendix 1. A full list of samples collected as part of the WHM program and the information these samples can provide on the health status of an animal is illustrated in Table 1. For more detailed information on sampling protocols and sample processing see CARMA, 2008 and Kutz et al, 2013[6, 7].

When samples were collected, the kits were handed in at the local RRC office or the ENR office in Norman Wells. All samples were kept frozen until further processing. Samples were processed by ENR, University of Calgary staff, or sent out to collaborating institutions for further analysis. For each completed sample kit harvesters were compensated for the time and effort taken to collect the sample, and the loss of meat/hide that would otherwise have been used for food or to make

clothing and tools. Compensation was in the form of a gift card, a cheque or gas credit and was distributed by the local RRC office or ENR. The cash value of the reimbursement varied during the course of the program depending on the size of the sample kit, the time and effort needed to collect the samples, and input from the program participants and RRCs.

Table 1. Samples collected by Wildlife Health Monitors and the information that each sample provides on animal body condition, disease or contamination [7].

Sample collected	Animal health information
Hunter Observations	
Location	Herd origin and range
Age	Estimated age of animal
Sex	Sex of animal
Back fat depth	Body condition
Body condition	Overall body condition
Abnormalities: Specifically look for abnormalities such as white spots or cysts on liver, eye, skin on legs, testicles and joints	Presence of parasites and other diseases
Warble count	Level of warble infection
Mandible (Lower Jaw)	
Morphometrics	Body size
Marrow fat	Body condition
Tooth eruption and tooth wear	Age class
Incisor I cementum	Age
Jaw (molars)	Enamel hypoplasia (stress during enamel development)
Metatarsus (lower left hind leg)	
	Besnoitia tarandi cysts, Filarioidea microfilaria, Foot rot (*Fusobacterium* sp.)
Morphometrics	Bodysize
Marrow	Body condition
Feces	Genetics, stress, macro (helminths) and microparasites (protozoa, bacteria, viruses) that are shed in feces.
Blood on filter paper	Serology for various pathogens, pregnancy
Kidney	Contaminants
Riney kidney fat	Body condition
Liver	Contaminants
Testicles	Confirmation of sex, *Brucella suis, Besnoitia tarandi*
Hide	*Hypoderma tarandi* *Dermacentor albipictus* (winter tick) Cortisol levels (from hair)
Abnormalities	Unusual diseases
Lungs	Lungworms, *Echinococcus*

5. Targeted sampling

During the course of WHM program several targeted scientific studies were done. Conceptualization of these studies was driven by concerns of local hunters, elders, and wildlife managers, and the need for easier and more efficient sampling protocols. Studies included; the evaluation of the effectiveness of blood filters strips for caribou disease surveillance[8], winter tick range expansion in moose and possibly caribou[9], dental enamel hypoplasia as a measure of stress[10], and caribou anatomy (http://www.ucalgary.ca/caribou/CaribouAnatomy.html). For the purpose of these studies, more targeted sampling, with reduced sample kits, was often performed. For example, for the dental enamel hypoplasia study, once 20 caribou had been sampled in full, harvesters were asked to collect jaws only from all additional caribou harvested that year in order to increase the sample size.

2.6 Sample processing

2.6.1 Metatarsus, jaw/incisor, kidney, liver

All samples were stored at -20°C at the ENR office in Norman Wells until processed. Processing of the metatarsus, jaw, kidney and liver was performed according to the standardised CARMA level 1 and 2 protocols[6]. Metatarsal bones were visually inspected for signs of infection with *Besnoitia tarandi*[11] and *Onchocerca cervipedis* (after 2010 only)[12]. Later in the program (2014), a 1x2 cm skin sample from the front mid-third of the metatarsus was collected, fixed in 10% buffered formalin and sent to the University of Calgary or the Canadian Wildlife Health Cooperative for histological examination of *Besnoitia*, as described in Ducroq J et al. (2013). Morphological measurements of the metatarsus and jaw were done in accordance with the CARMA protocols[6]. After morphological measurements were taken the first incisor teeth (I1) were extracted using dental elevators and pliers. Teeth were stored in paper envelopes in a dry and cool place and were sent for cementum age analysis to Matson's laboratory, Milltown, Montana, US. The bone marrow fat index was calculated after breaking the metatarsus bone, extracting the marrow, and weighing the wet and dry marrow as described in the CARMA protocols. The Riney Kidney fat index (KFI) was evaluated using a standardized technique to provide a ratio of the weight of the kidney fat to the weight of the kidney X 100; the KFI was reported as a percentage and can be >100%. The kidneys and the livers from a subset of animals were archived at -20°C for future contaminants testing.

2.6.2 Fecal samples

Fecal parasitology was performed at the University of Saskatchewan, the University of Calgary or the Canadian Wildlife Health Cooperative. Fecal egg counts, using the Wisconsin double centrifugation technique, were used to identify eggs of nematode and cestode parasites and the cyst and oocyst of protozoans. The Baermann technique was used to test for the presence of protostrongylid lungworms whilst fecal sedimentation was used to test for the presence of flukes (trematodes). For further details regarding these methods see Zajac and Conboy, 2012, Samuel et al, 2001 and Forrester and Lankester, 1997[13, 14,15].

2.6.3 Filter papers

Filter papers were stored either frozen or in a dry cool place with desiccant packs to keep them from moulding. According to manufacturer's specification the absorbent portion of each strip holds 100uL whole blood (approximately 40uL serum) if entirely soaked. In preparation for disease testing, filter paper blood samples were processed as described in Curry et al., 2011. Disease testing was performed at veterinary diagnostic labs in Canada, the United States and Norway (Table 2). For further details on these tests, including performance, sensitivity, and specificity see Curry, 2012 and Curry et al., 2014. Note that many of the bovine serological tests used are not specifically evaluated for *Rangifer* serum or plasma. Positive results may indicate an immunological cross-reaction against viral counterparts to known bovine (or other ruminant) viruses, for which the test has been designed[16]

Table 2. Laboratories and tests used for testing pathogen exposure. Where more than one laboratory was used, both are indicated.

Agent	Test type	Laboratory
Neospora caninum	cELISA	a. Abbotsford Animal Health Lab, British Columbia Ministry of Agriculture, Canada b. Prairie Diagnositic Services (PDS) at the Western College of Western Medicine, Saskatoon, Canada
Toxoplasma gondii	a. Modified agglutination test b. ELISA	a. Dubey lab, United States Department of Agriculture, United States b. Canadian Wildlife Health Cooperative, University of Calgary, Canada
Alphaherpes-virus	a. iELISA testing for antibodies to Bovine-herpes Virus 1 b. Blocking ELISA testing for antibodies to Bovine-herpes Virus 1	a. PDS b. Research Group of Arctic Infection Biology at Tromso University, Norway
Pestivirus	a. Virus neutraliz ation assays to Bovine viral Diarrhoea virus b. ELISA	a. PDS b. Research Group of Arctic Infection Biology at Tromoso University, Norway
Paramyxo-viruses	iELISA testing for antibodies to Parainfluenza virus- 3 and Bovine respiratory syncytial virus	PDS

2.6.4 Hide samples

Samples from the neck, shoulder and the base of the tail were collected by Wildlife Health Monitors and tested for the presence of winter tick (*Dermacentor albipictus*). Each sample was subsampled four times in quadrants of approximately 100cm^2. Hides were digested in a potassium hydroxide solution until no tissue was remaining. The solution was then filtered using a 150µm sieve and the retained material was examined for ticks, and other ectoparasites, under a dissecting microscope. Ticks were classified according to developmental stage and identified to species. For further details on these methods see Kashivakura, 2013.

3. Results

To view the complete set of raw data, collected samples and list of archived samples at the University of Calgary see excel files: WHM Data, WHM Tally and WHM Sample Inventory. Some of the main results are summarized below and in appendices 2-5.

3.1 Sample size

From 2004-2013, a total of 327 samples from moose and caribou were collected by Wildlife Health Monitors in the Sahtu (Table 3a). The majority of these samples were from the Bluenose East caribou herd (121/326). Table 3b summarises when (year and month) samples were collected, and illustrates that this varies between years. To improve the ease of sample collections and the quality of the samples, the structure of the collection kits were developed throughout the program. Changes were made after discussions with hunters and included visual representation of collection methods, reduction in amount of information asked for, and switching from datasheets to tags only. Appendix 1 chronicles some of these changes.

Table 3c summarizes how many of the health indicators consistently asked for over the years were actually collected. The data/samples collected most often were recordings of location of harvest (297/327), the sex of the animal (259/327) and the metatarsal (254/327). The data/samples collected least often were hunter observations of the animal. In the first few years of the program hunters were asked to record an extensive number of observations, including the number of warble fly scars, observations of abnormalities in the eye, on the meat, the legs and more. The samples collected for each individual animal is documented in full in the excel database entitled "WHM Tally list".

Table 3a Sample size. Total number of animals sampled by species, herd and year. Neither all the data nor all samples were collected from all animals. BNE refers to Bluenose East, BNW refers to Bluenose West and WC to woodland caribou.

	Species/Herd				
Year	BNE	BNW	WC	Moose	Total
2004	20				20
2005	20	30		2	52
2006	15	1		10	26
2007	10	20		6	36
2008	6	31			37
2009	29		3	4	36
2010				17	17
2011	25			18	43
2012				7	7
2013	8	6	23	16	53
Total	133	88	26	84	**327**

Table 3b Sample size. Sample size by year, month and sex for all animals sampled from caribou herds Bluenose East (BNE) and Bluenose West (BNW).

		BNE			BNW		
Year	**Month**	Female	Male	Unknown	Female	Male	Unknown
2004	Mar	2					
	Apr	18					
2005	Jan				2	1	
	Feb	2			6		
	Mar	10			18	2	
	Apr	8					
	May					1	
2006	Apr		15				
	May					1	
2007	Mar		10				
	Sept				2	18	
2008	Jan	4					
	Jul	1		1			
	Sept					31	
2009	Apr	27		2			
2011	Apr	17	1				
	Unknown	7					
2013	Jan	1	4				
	Mar	2	1				
	Nov						2
	Dec				2	2	

Table 3c Sample size. Number of samples (health indicators) with known year of harvest collected by harvesters by species and herd from 2004-2013. HO refers to hunter observations recorded on data sheets/tags. BNE refers to Bluenose East, BNW refers to Bluenose West and WC refers to woodland caribou. Hunter observations that were recorded in the early years of the program but not included in the table below include abnormalities in the joint, liver, lungs, eyes, meat, testicles and skin on legs as well as the presence of warble scars. These observations can be viewed in the "Sahtu Tally" database. Denominators vary depending on the information recorded and sex of animals. For example, only female moose can be pregnant, and although 66 female moose were recorded, pregnancy status was only indicated for ten, and thus there would only be ten samples availablewith information on fetus sex.

	Species/Herd				
Type of sample/information	BNE	BNW	WC	Moose	Total
HO location	133/133	86/88	26/26	68/80	313/327
HO sex	123/133	86/88	24/26	43/80	259/327
HO pregnancy	73/105	12/32	0/6	10/66	95/209
HO calf	74/105	12/32	1/6	13/66	100/209
HO number of foetus	15/73	0/12	0/0	2/10	17/95
HO sex of foetus	68/73	12/12	0/0	5/10	85/95
HO back fat measurement	82/133	44/88	12/26	27/80	165/327
HO lactation status	82/105	14/32	3/26	13/80	112/327
HO hunter score of body condition	105/133	26/88	21/26	40/80	192/327
Liver sample	111/133	24/88	23/26	54/80	212/327
Kidney with fat	114/133	51/88	22/26	55/80	242/327
Metatarsal	112/133	65/88	23/26	54/80	254/327
Jaw	101/133	62/88	4/26	12/80	179/327
Incisor bar	15/133	8/88	9/26	37/80	69/327
Feces	113/133	46/88	23/26	57/80	226/327
Blood	116/133	50/88	23/26	57/80	246/327

3.2 Body condition

3.2.1 Body fat

Body condition data collected as part of the WHM program were summarised in reports in 2014 and can be viewed in full in Appendix 2. This section will consist of a brief synopsis.

Body condition indices have been measured by hunters (hunter score and back fat) and in the laboratory (kidney riney fat index and bone marrow fat index). Results are presented by sex and year for animals presumed to be adults from Bluenose East (BNE), Bluenose West (BNW), mountain woodland caribou and moose in Appendix 2. Where possible, results have been grouped in a way that allows comparisons between years. However, only for Bluenose East was there sufficient data to make comparisons in the same season between years (Appendix 2.1). The most variation appears to be in the back fat and kidney fat index with the bone marrow index being less variable.

In total, sample kits from 163 individual BNE caribou were collected between 2004-2014 (Appendix 2.1), of these 157 were assumed to come from adults. Of these 131 had data on sex, year and month of collection. These data break down further, where 101 kits had records for back fat, 125 for the kidney fat index, 90 for the bone marrow fat index and 100 had records of hunter scores of body condition. For BNE, the kidney fat index appears to indicate a declining trend for female caribou until 2013. If there is data available from similar programs across the BNE range we suggest that these data be pooled and analysed further to explore if significant trends can be detected.

For BNW caribou a total 112 sample kits were collected between 2004-2014 (Appendix 2.2), of these 109 were assumed to be adults and 107 of these were of known sex, year and month of collection. However, not all kits were complete for body condition data. Forty-four had records for back fat, 57 for kidney fat index, 51 for bone marrow fat index and 25 had records of hunter scores of body condition. Although collection of Bluenose-West body condition data was successful in the early years, due to the shift from targeting females to males and a lack of samples in the latter years, comparisons between years and analysis of trends was not possible.

Targeted sample collection for mountain woodland caribou only started in 2013, and sample kits from 39 individual mountain woodland caribou (assumed adults) have already been collected (Appendix 2.3). All these samples have complete data for collection date and sex. The number of individuals with measurements of back fat was 18, of kidney fat index 33, of bone marrow index 25, and 30 recorded hunter scores of body condition. Almost half of the samples (18/39) were collected with the assistance of a researcher during the Tulita community harvest at Caribou Flats in September 2013.

Collection of moose samples has been spread through out the years, and adequate data to infer trends are missing. Seventy-six of the kits collected were assumed to come from adults, but more than half (45/76) did not have age, or age group specified and 47% (36/76) were of unknown sex (Table 1). The total number of samples available for moose with known sex, year and month of collection that were assumed to be adults was 26 for the kidney fat index, 22 for backfat, 17 for the bone marrow fat index and 29 for hunter score of body condition. Sample collections were done by at least 27 different hunters, but hunter names for 22 of the moose sampled were not recorded (Table 2).

3.2.2 Body Size

There were fewer samples available for body size indexes compared to body condition (Table 2c). Mean body size indexes (jaw and metatarsal length) by sex and year for adult animals of the caribou herds BNE and BNW can be viewed in Appendix 3. There was not enough data available to detect trends.

Jaw measurements were only available for a handful of animals. For BNE there were a total of 35 measurements of the anterior jaw bone, 16 for females and 19 for males, but only 22 for the total length of the jaw, 4 for females and 18 for males. The anterior jawbone measurements ranged from 129-151 mm for females and 118-170 mm for males. For BNW, data was only available from males from 2008, with 25 measurements of the anterior and total jawbone. The anterior jawbone measurements ranged from 155-180 mm.

For adult BNE caribou there were a total of 59 measures of metatarsal length and circumference, 45 from females and 14 from males. For female BNE the metatarsal length ranged from 200.6-282 mm and for males 215-337 mm. BNW had fewer samples available, with 31 measurements of metatarsal length and circumference, 3 from females and 28 from males. The range of BNW metatarsal length was 240-310 mm for females and 239-365 mm for males.

3.2.3 Age

Mean ages of harvested animals, as determined by cementum age analysis, can be viewed in Appendix 4. The mean overall age of harvested animals was higher for females for both BNE, at 6.1±0.3 years (n=54), and BNW caribou, at 6.0±0.9 years (n=14), compared to a mean age for males at 4.8±0.4 years (n=24) for BNE and 5.5±0.3 years (n=47) for BNW. For moose, the average age was 3.9±0.6 (n=12) for females and 4.9±0.7 (n=9) for males.

3.3 Pathogens

3.3.1 Serology

The WHM program led to the development and application of filter-paper blood samples for wildlife serology[8, 17-19]. Appendix 5 summarises the number of samples tested and the seropositivity for assays testing for antibodies to: *Neospora caninum,* alphaherpesvirus, parainfluenza virus (PI3) and pestivirus by sex and year for caribou herds BNE and BNW, woodland caribou (WC) and moose.

Antibodies to *N. caninum* were detected in moose from the Sahtu for the first time in 2010. Since then, new and archived samples have been analysed. The highest overall sample seroprevalence (%) for *N. caninum* antibodies was found in moose at 16.1±6.9 (5/31); no BNW samples tested positive (0/47). Only samples from female BNE caribou tested positive for *N. caninum* antibodies, with a seroprevalence of 3.3±2.3 (2/64) making the overall (male and female BNE) sample seroprevalence 2.1±3.8 (2/95). For WC the overall seroprevalence was 13.0±7.0 (3/23).

Exposure to *Toxoplasma gondii* was tested using 20 BNW whole blood and filter-paper blood samples collected in 2007 and 14 samples collected in 2008, all samples tested negative. For more details see Curry, 2012.

The highest sample seropositivity (%) for alphaherpesvirus was 60.8±7.2 (28/46) for BNW, and then 45.5±10.9 (10/22) for WC whilst the lowest was 20.4±5.5 (n=54 for BNE. Two out of nine (22.2%±14.7) moose sampled were seropositive (Appendix 5.2).

Only male BNW tested positive for parainfluenza virus, with four out of 34 samples testing positive (5.5%). Of those, three were serum samples from 2007 and one filter paper sample from 2008. All other samples were negative (Appendix 5.3).

More than half the BNE and WC samples tested for exposure to pestivirus were positive, with an overall sample prevalence of 66.7±10.3 (14/21) for BNE and 57.1±20.2 (4/7) for WC. No moose tested positive for pestivirus (0/5) whilst only one out of six (16.7% .0±15.2) BNW samples were positive.

3.3.2 Fecal parasitology

Samples collected from the Sahtu also contributed to the discovery of a previously uncharacterized, genetically distinct species of protostongylid nematode, *Varestrongylus eleguneniensis,* in caribou, moose and muskoxen. For more details see Kutz et al., 2007 and Verocai et al., 2014[20].

The prevalence for some of the common parasites identified by fecal parasitology (i.e. eggs/ oocysts / larvae from helminths and protozoans) is presented in Appendix 5.6-5.9. Overall prevalence was low, with strongyle eggs being the most prevalent in caribou (Appendix 5.6) and Nematodirinae eggs the most prevalent in in moose (Appendix 5.7).

3.3.3 Other pathogens

Besnoitia tarandi is a protozoan tissue dwelling parasite that has been reported in reindeer and caribou across most of their range. Hide samples from the metatarsals were analysed by gross visualisation. The overall sample prevalence for *B. tarandi* for BNE was 56.7±6.1 % (n=67) and for BNW 53.2%±6.4 (n=62) (Appendix 5.5). As discussed in Ducrocq et al., 2013 gross visual evaluation of *B. tarandi* cysts underestimates prevalence and 2014 samples were, therefore, analysed by microscopic histology evaluation[21].

Onchocerca cervipedis, a filarioid nematode of cervids, was identified for the first time in moose in the Northwest Territories. By examining the subcutaneous connective tissues of the metacarpi, Verocai et al (2012) found lesions compatible with those caused by *O. cervipedis* in 21.4% (n=28) of moose from the Sahtu. Nematodes found in nodules were confirmed as *O. cervipedis*[12]. For more specific details of this study see Verocai et al., 2012.

In 2010-2011 moose hides were examined for ticks. Ticks were isolated from 1/24 hides from Fort Good Hope, 2/3 hides from Déline, and 2/2 hides from Tulita. None of the 25-barrenground caribou hides collected by WHMs from Déline had ticks. On average, 10% of each sampled moose hide and 15% of each sampled caribou hide was digested and tested for presence of ticks[9]. For more details regarding the winter tick study in the Sahtu see Kashivakura, 2013.

3.4 Stress

3.4.1 Dental enamel hypoplasia

The WHM program initiated a study of tooth dental enamel development and examination of linear enamel hypoplasia. Enamel hypoplasias are developmental tooth defects that are formed when the enamel is laid down (ie whilst the tooth is growing). If there is a disruption in enamel deposition, due to, for example, physiological stress caused by disease or nutritional deficiency, this can lead to improper deposition of the enamel that manifests as a horizontal furrow line on the tooth that is visible by macroscopy and canbe screened for relatively easily[10, 22]. In brief, the study found that linear enamel hypoplasias do occur in BNW and BNE caribou and could provide a useful tool for tracking physiological stress that occurred during the deposition of enamel on those teeth. This could provide an indicator of impending population declines[10]. For the full report of this study see Wu et al. 2012.

3.4.2 Stress hormones

The concentration of corticosteroid hormones in hair and faeces can be a useful indicator of an animal's physiological stress. In 2013, fecal cortisol and corticosterone levels were determined in caribou and moose from the Sahtu for the first time. The mean level of fecal cortisol (ng/g wet feces) for BNE was 18.65±2.64, for WC 13.65±0.97 and for moose 17.68±2.19. The mean level of fecal corticosterone

(ng/g wet feces) for samples from BNE was 57.66±16.49 (n=7), for WC 32.11±4.41 (n=21) and moose 77.72±13.42 (n=12).

3.5 Archived tissues

For a full list of archived tissues at the University of Calgary, including fecal, blood and hide samples see excel file WHM sample inventory.

3.6 Interview results

Workshops and structured interviews have produced a wide range of new information that compliments empirical research. Focus group interview participants indicated that they were noticing changes in caribou health. In particular, there had been an increase in the number of cases of 'green slimy wet stuff' under the skin. Wildlife health monitors also reported an increasing number of moose with poor coats and hair loss, indicating infection with the winter tick. Findings from the focus group interviews were summarized in the Climate Change Action Fund report in 2007. Additionally, in 2006 we interviewed WHM interviews using the Arctic Borderlands Ecological Knowledge Co-op interview form. Some of the notable observations were:

- No caribou-migrated to calving ground early
- Really skinny, cows and bulls both
- No calves returning to calving grounds
- Snow crusty, caribou turned back
- Hooves worn
- Lots of frogs
- Swallows gone
- Gulls – nesting in town last two years
- Geese – flying high, couldn't hunt

WHM and focus group participants also emphasized the need for more discussions between scientists and community members about wildlife healthy, with a particular and critical need to focus on the younger generation[3, 23].

4. Discussion

4.1 Introductory remarks

Monitoring has been defined as "the systematic measurement of variables and processes over time", and assumes that "there is a specific reason for that collection of data"[24]. Spellerberg (2005) summarises the main reasons to undertake monitoring. These are; (a) to provide basic knowledge about an unknown ecological system, (b) to provide accurate and scientific data to policy makers and managers, (c) to understand trends over time, such as the impact of anthropogenic

perturbations and species and habitat loss and finally, (d) to provide early detection of potentially harmful effects[24]. Monitoring of biodiversity, populations, habitats, health etc. provides this crucial information.

In the Arctic, accessing the data necessary to monitor and understand the health status of a wildlife population can be difficult for scientists and managers. Here, communities are widely scattered over a vast landscape, financial costs are often high, the weather can be unpredictable and access to wildlife can be hampered both by logistical constraints and trans-boundary issues, compounding the difficulties of monitoring elusive wildlife species. Due to many of these complications, very few baseline health indices have been available for wildlife in the Sahtu. Without baselines it is difficult to make informed management decisions.

By using a community-based approach, incorporating traditional knowledge, and engaging subsistence hunters in sample submission and as active members of the research process, the logistical difficulties, high costs and transboundary issues of bringing scientists in to do fieldwork can, to some extent, be circumvented.

There are many different models for community-based monitoring, with different degrees of community involvement. Danielsen et al (2008) provide a good overview and assess strengths and weaknesses of different types of monitoring. In brief, they discuss five categories; (i) externally driven, professionally executed monitoring with no involvement of local stake-holders, (ii) externally driven monitoring with local data collection, where analysis and interpretation are undertaken by external researchers and local stakeholder are only involved in data collection (iii) collaborative monitoring with external data interpretation, where the design and data analysis is undertaken by external scientists and local people are responsible for data collection and local management and decision making, (iv) collaborative monitoring with local data interpretation, where local stakeholders are involved every step of the way and external scientists only provide advice and training and finally, (v) autonomous local monitoring, with no involvement of external agencies [25].

The Sahtu WHM program sits in the 'iii' category as defined above. The initial impetus for the program was driven by community concerns and community members have been active partners in the data collection, and to some extent in the interpretation. There has also been extensive interaction between researchers, wildlife managers and harvesters. The local wildlife management organizations, together with government wildlife managers make the management decisions, and an external entity (the University), does the majority of the sample analyses and data interpretation, and partners with the local organizations for grant applications, and data dissemination.

The Sahtu WHM Program has met many monitoring objectives identified by Spellerberg (2005). The program has provided basic knowledge of health for caribou and moose (a), documented 'new to us' pathogens, and detected range

expansion of others (d). For some health indicators, the sample sizes are adequate for evaluating trends (c) and all data have been provided to Government wildlife agencies and Aboriginal Co-Management boards to be used in policy and wildlife management. The program has further contributed to unique research programs and peer-reviewed publications. Descriptive data of body condition, body size, ages and pathogen prevalence and diversity has been collected and summarized in the accompanying data files and appendices and can be used for further analysis of caribou health in the Sahtu. Overall, these data demonstrate that the WHM program has been somewhat successful in recording baselines of health of caribou and moose. However, data interpretation and analysis is constrained by small sample sizes. Some challenges and improvements remain to be addressed. Crucially, long-term commitment from communities, researchers and local government institutions is essential for the program to continue in a meaningful capacity.

More detailed descriptions of issues encountered, how these have been tackled, and recommendations for moving forward are discussed below.

4.2 Sample size and biases

The caribou and moose sample is obviously biased, as, when given a choice, hunters will selectively target healthy animals. However, in contrast to many studies the bias is known beforehand and can, therefore, be accounted for during interpretation of data. Inter-annual consistency of the harvested caribou can be verified by looking at the sex and age structure of the sample population every year[26]. If the age structure changes between years it could be a sign that the population structure is changing and results should be interpreted with this in mind. By establishing baselines over several years we have the opportunity to identify what the "normal" trend is and, thereby, be alerted to any 'abnormal' changes that may occur.

During the WHM program sample-sizes have been variable, both between seasons and sexes sampled. This was due to a number of different reasons.

- Collections occurred year round.

Although most caribou samples were collected from January to April and during community hunts in autumn, collections did occur year round leading to variability in sample sizes over the season.

- Harvests did not consistently target the same sex.

Initially, in 2004, harvests targeted female caribou. For research and management purposes samples from females are usually preferred, as they can provide more information on population recruitment (through pregnancy rates). Females were also the preferred targets for hunters. However, due to the population declines, in 2006 harvesters were encouraged to only target males, in order to allow the populations to recover. This switch is clearly reflected in our data (Table 2b).

- Research based on community interests increased sample sizes.

If the community were interested in the issue, more samples were generally collected, as was the case with the winter tick study. Additionally, a dedicated researcher, for example, the winter tick graduate student, can increase contact time with the community and have contagious enthusiasm for the sampling.

- Funding influenced the number of collected samples.

In 2012, there was a lack of funding to support the program, which is clearly reflected in the low sample sizes during this year. Also, during the winter tick study, increased funding allowed the program to pay hunters well, increasing the interest in the study.

- Researchers participation in community hunts increased quantity and quality of samples.

For example, in years when researchers actively participated in community hunts, such as 2007, 2008 and 2013, sample sizes were notably boosted.

- Targeted studies, with smaller sample kits, usually lead to higher sample submission.

For example, the dental enamel lesion and winter tick studies were positively received and sample collection was very successful.

The key take home message from this is that research needs to be driven by community interest. Identifying interested individuals that can act as local champions improves the collection of reliable and full sample kits. Furthermore, long-term funding and commitment is needed for this program to become established as a long-term monitoring tool. To do this we also need to build local capacity in the Sahtu.

4.3 Body condition

To get a good overall index of body condition we need to measure both body fat and body size. Measuring body fat is important for management because it significantly affects reproductive potential of caribou. More than one measurement of body fat is needed because different measures will be more or less sensitive depending on the season and the nutritional state of the animal. There is sequential mobilization of body fat from various depots. For example, back fat was found to best correlated to body fat when animals were the fattest (e.g. autumn) and metatarsal marrow fat only correlated with body fat in late winter (June) for the Porcupine caribou herd[27].

Although body mass has been suggested to be the best indicator of individual variability in body condition[28, 29], this was not measured in the WHM program because it is an impractical measure to take in the field and requires specialised equipment. However, other fat measurements can also be useful if they are taken at the right time of year. Taillon et al (2011) suggested measuring fat reserves from early fall to early spring, especially kidney fat, for evaluating individual fat reserves. For late spring to early fall she proposed that it was better to measure protein reserves (e.g peroneus muscle mass) for an accurate body condition assessment[29]. A study on red deer also validated the kidney fat index as a reflection of body condition, and stressed the importance of consistently sampling the same kidney

(left or right) with a standardised protocol[30]. Chan-McLeod et al. (1995) further commented that back fat indexes were only useful from September to November, whilst marrow fat was only useful in late winter (Jun) when animals had <9% body fat.

Body size is not as sensitive to change as body fat and can, therefore, reveal longer-term trends in body condition. This is because the skeletal size depends on the animals' environment and genetics and is relatively plastic, changing size with environmental conditions during birth and growth condition[29]. In combination, the body fat and body size indexes provide a good baseline of body condition of barren-ground caribou in the Sahtu. Further in-depth analysis of these data, such as described in Taillon et al. (2011) and Chan-McLeod et al. (1995) can help us better understand how body condition varies between sexes, seasons and potentially years. However, as outlined above, sample sizes are limited and highly variable which can prove to be a challenge for analysis.

There are three measures of body fat and two measures of body size incorporated into the WHM sampling protocol: back fat, kidney fat index, metatarsal marrow fat index and jaw length and metatarsal length. In addition, harvesters' are also asked to rank their impression of body condition. Sampling during the WHM program mainly occurs in in early fall to early spring, and, as reviewed above, the fat measurements we have collected should be appropriate for indicating body condition.

There were several issues associated with the collection of this data.

- Incorrect collection procedures may have introduced variability and uncertainty to data.

For example, hunters may measure back fat in different places. Riney Kidney fat may not have been collected according to the specified procedure, or the wrong kidney may have been collected. Errors such as these cannot be amended post-collection.

- Samples/measurements were not received.

Quite often there is no recorded back fat measurement. This could be because no ruler or pen was available or the hunters did not understand how to perform the measurement. In some cases only the front incisor bar was submitted as opposed to the whole jaw. This could be because the jaw is valued for food.

- Errors in laboratory procedures and analysis lead to missing data and increased variability in the data.

For example, although the bone marrow index is usually the most reliable measure in regards to sample submissions there were several issues in the laboratory in regards to the analysis. This was mainly due to misinterpretation of methods and damage to the samples during flooding.

The harvesters' impression of body condition has the potential to be an integrative measure of body condition[31]. Lyver and Gunn (2004) demonstrated that hunters' impressions of body condition could be somewhat reliable predictors of biological estimates of body condition using fat indices. Recording hunter impressions was, however, not without complications.

- Hunters scored body condition of animals differently.

For example, in post-hunting interviews 2005 it transpired that one hunter did indeed score an animals condition based on the amount of fat present, however, another hunter scored all animals as good body condition (even if they had very little fat) unless they had obvious signs of disease (fluid in joints, bad meat).

- Indices developed for caribou may not necessarily work for other species.

For example, hunters may assess the condition of moose differently.

Several steps were taken in an attempt to improve sample collection and consistency.

- Data sheets were switched to tyvak tags, which incorporated a ruler.

Tags take up less space and are more durable to adverse environmental conditions. Hunters could also use the ruler on the tag to measure back fat, by circling or cutting the ruler at the correct measurement.

- The amount of information requested from the hunters was reduced.
- Body condition categories were clarified.

Categories were changed to 'skinny', 'not so bad', 'fat' and 'really fat' instead of 'poor', 'fair', 'good', 'very good' to specify that we were asking for an assessment of body condition and not health.

- A one-page information sheet illustrating which samples to collect and how to collect them was added to the kits.
- The Sahtu RRCs play a more active role in kit distribution and collection.

Personnel at the RRCs were asked to confirm that all samples were collected and all information was recorded before handing out payments for samples.

Many of the issues outlined above can be solved with proper training, both of wildlife health monitors and laboratory personnel. The CARMA Protocols for sampling as well as the hunter training video are great references for correct sample collection and processing[6]. However, they do not replace personal communication and demonstrations. To accurately interpret data it is also crucial that researchers understand how hunters assess body condition and what they assess as good v.s bad body condition. Hence, the key is collaboration and close communication with hunters.

4.4 Age

Age is related to various fitness components, including body condition, fecundity and mortality, and is important for understanding both individual and population herd health. Age data allow us to ask more in-depth questions about individual and population health but are also important for detecting population trends[32]. If the

average age of animals that hunters harvest gradually changes this can be an indication that the structure of the population has changed. For example, it has been shown that changes in ungulate population density affect age structure. As population density increases, fecundity decreases whilst juvenile morality and adult female survival increases, leading to a higher average age of females [33]. Thus, if the average age of harvested animals increases this may indicate an aging population, with potentially serious consequences for recruitment.

The first incisors of caribou and moose provide a robust and reliable method to assess age of individual animals[34]. Overall, the availability of these samples has been high. This has allowed us to specify the age of individual animals. Hunters were also asked to record the maturity levels (age class) of the animals, which could be used as a backup when incisor age was not available. Unfortunately, these data were not always recorded. By having dedicated personnel receiving sample kits one can easily ask the hunters for this information and record it when they return the samples, improving data collection. With the increasing involvement of the Sahtu RRCs we are now moving toward implementing more effective organization, including standardized recording when samples are received. Overall, there appears to be little variation between the mean age of caribou harvested in the Sahtu (Appendix 4).

4.5 Pathogens

The work on caribou and moose in Sahtu provides strong support for the continued value of monitoring of pathogens in these populations. Several pathogens previously unknown to the Sahtu were identified as a direct result of this program. Many of the pathogens monitored have the potential to significantly impact on individual as well as population health and can be sensitive to a warming climate.

The results presented here provide information on sample prevalence but are difficult to extrapolate to population prevalence. However, by comparing sample prevalence between years (assuming samples are collected in a similar manner; from a similar geographic location, age group and species etc) trends may still become evident. Many factors impact on our ability to detect a disease. This includes population size, sampling technique (e.g. random, targeted, opportunistic), spatial and temporal trends in disease prevalence, and sensitivity and specificity of diagnostics tests[35, 36].

4.5.1 Serology

Overall the use of filter papers to collect blood for serology has proven to be a huge success and a great tool for community based wildlife health monitoring[17-19]. However, serological screening tests for many pathogens have not been fully validated in caribou. Sensitivity and specificity are often unknown and test results should, therefore, be interpreted with caution. There are three key elements to remember in regards to blood sampling on filter paper for screening tests to be run successfully.

- Harvesters should be trained to sample correctly.

The blood should be fresh and clean and the filter papers should be completely soaked.

- Store filter papers correctly.

After collection dry filter papers (or immediately freeze them) and store them together with desiccants to prevent the growth of mould. If samples are mouldy virus neutralisation assays cannot be performed successfully[19]

- Standardize which lab and what type of tests to use for serology so that results are comparable over-time.

Blood samples were tested for two protozoan apicomplexan parasites (*Neospora caninum* and *Toxoplasma gondii*) that are transmitted through predator-prey linkages and vertically from mother to foetus[37]. Monitoring the prevalence of these parasites is important and relevant to management decisions because they both have the potential to cause neurological disease and infertility and weak calves in their intermediate hosts (caribou and moose) and, therefore, have the potential to impact on herd health and productivity [38]. The samples testing positive for *N. caninum* were the first records of this parasite from moose and caribou from the NWT. Previous studies have reported *N. caninum* seroprevalence range of 1.4 – 15.7% (summarised in Kutz et al. (2012)) in caribou. The highest prevalence reported in Kutz et al. (2012), 15.7%, was from adult females in a declining woodland caribou herd in Yukon (M. Oakley, S. Kutz, A. Seller, R. Farnell unpubl. obs.). For caribou, the highest seroprevalence detected in the Sahtu was also from woodland caribou, where 13.6% (3/23) of adult females tested positive for *N. caninum*. This may appear high, but more samples need to be analysed for a reliable estimate. In line with our results, Curry (2012) reported that none of the BNW (n=53) samples tested positive for *N. caninum* antibodies. For moose, the 16.1% (5/31) seroprevalence detected in the Sahtu was higher than that reported elsewhere. For example, prevalence in moose from Alaska was 0.5%, (n=202 in 2001-2005)[37], in moose from Minnesota it was 13.1% (n=61)[39] and in British Columbia it was 5.7% (n=105, in 2001-2003)[40].

Toxoplasma gondii seropositive samples have been reported in *Rangifer* worldwide, with a seroprevalence range of 0.7-62.5% (summarized in Kutz et al., 2012). In 1994, 40% (6/40) of blood samples from the Bluenose caribou (sampled at Sitidgi lake 68°33'N, 132°42'W), today referred to as Bluenose-West, tested positive for antibodies to *T. gondii*[41]. However, none of the BNW WHM samples collected in 2007 and 2008 (n=34) tested positive for exposure, and neither did any of the other 19 BNW samples collected between 2007-2010[8]. A similar 'trend' was also observed for Bathurst herd, where Kutz et al. (2001) documented a 35% (n=80) seroprevalence for *T. gondii* antibodies in 1993 whilst Curry (2012) only detected a 4% (n=141) seroprevalence in samples collected 2007-2010. These changes in seroprevalence mirror the decline that occurred in these caribou herds since the early/mid 1990s and could be linked to the transmission mode of the parasite, where, prevalence may be linked to abundance of the definitive hosts (dogs, coyotes

and wolves for *N. caninum*[42, 43] and felid species for *T. gondii*[44]) which may, in turn, vary with caribou number. However, transplacental transmission may also occur and maintain the parasites in the cervid populations in absence of definitive hosts[38]. Alternatively, results could differ due to differences in sample type and test sensitivity. The most recent samples were from blood on filter paper compared to serum samples in 1993-94. The blood on filter paper has not been validated for *Toxoplasma* or for the modified agglutination test (MAT), and, thus, sensitivity of the diagnostic test used may be low. Further testing for *T. gondii* antibodies in archived samples using an ELISA test is planned.

Blood samples were also tested for exposure to herpes- (BHV-1), pesti- (BVDV) and paramyxovirus (PI3). For caribou, the highest exposure found was for pestiviruses, with BNE having the highest seroprevalence at 66.7%, (14/21), then WC at 57.1%, (4/7) and finally BNW at 16.7% (1/6). The pestivirus, Bovine viral diarrhoea virus (BVDV), is a well-studied significant pathogen of cattle where infection can lead respiratory disease, abortion and death[45]. Although the clinical signs in wild ruminants are not well documented, viremia in reindeer has been demonstrated by experimental infection[46]. Serological studies have shown evidence of exposure to pestiviruses (BVDV or a related strain) in caribou. A 69.3% (n=30) and a 60.7% (n=28) seroprevalence was reported from caribou in Quebec 1978 and 1979, respectively[47], whilst only a 3% (n=67) seroprevalence was found in caribou from Alaska in 1978-1982[48]. No positive samples were found from woodland caribou samples from Saskatchewan in 1992-1995 (n=40), Alberta in 1997-1999 (n=121)[49], or the Northwest Territories in 2003-2005 (n=103). A more recent serological survey (2007-2010) of seven caribou herds found an overall prevalence of 28% (range 0-56%, n=533), including BNW at 33% (n=33) in samples collected from 2007-2010[8]. This is a higher prevalence than we found in the Sahtu WHM BNW samples. Whilst none of the Sahtu moose samples tested positive for exposure to pestivirus, seropositive moose have been reported in Alberta in 1970 (18.2%, n=22)[50], in Alaska in 1996-1998 (2.3%, n=221) and in British Columbia in 2001-2013 (1.5%, n=105)[40], although the levels are generally lower than that reported for caribou. However, the pestivirus testing of the WHM samples had low success rate, due to cell toxicity on the plate. Therefore, sample sizes are very small, making comparisons between other studies difficult. The test failure was due to the toxicity of the samples potentially caused by mould contamination[18].

Blood samples also tested positive for alphaherpesvirus using an indirect ELISA for bovine herpes virus type 1 (BHV-1). BHV-1 is a well-described alphaherpesvirus of cattle, which causes infectious bovine rhinotrachetis (IBR), a highly contagious respiratory disease that can also lead to abortions, conjunctivitis and encephalitis in cattle[16]. Serological cross-reactions between different alphaherpesviruses are well documented and serological tests for BHV-1 can, therefore, be used to detect the presence of antibodies against alphaherpesviruses in non-bovine host species[51]. The highest seroprevalence detected in the Sahtu caribou was 60.8% (28/46) from BNW samples then 45.4% (10/22) from WC samples and finally BNE at 18.5% (10/54).

These results appear to be in a similar range to what has been found elsewhere. Seroprevalences ranging between 0-39% have been reported from caribou in Alaska in 1981-2000 (reviewed in das Neves et al. (2010)) whilst seroprevalences of 39.6% (n=30) and 14.2% (n=28) were found from caribou samples from Quebec in 1978 and 1979, respectively[47]. More recent studies have shown high seroprevalences in woodland caribou from Saskatchewan collected in 1992-1995 (55%, n=40)[52], Alberta in 1997-1999 (52%, n=121)[49] and the Northwest Territories in 2003-2004 (37.5%, n=104)[53]. Curry (2012) found an overall seroprevalence of 25% (range 0-62% n=551) in samples collected between 2007-2010 from seven herds in North America and Greenland. In line with our results, BNW, had the highest seroprevalence at 62% (n=52). For moose in the Sahtu two out of nine (22.2%) samples were seropositive for alphaherpes virus. In contrast, samples from moose in Norway (n=1774) in 2005[54], Alberta (n=22) in 1970[50] and Alaska in 1978-1981 [48] and 1996-1998 (n=220)[55] were all negative. Although we cannot accurately identify which alphaherpes virus caused the immune response we detected in caribou, it is very likely the Cervid Herpes Virus 2 (CvHV2) [16]. CvHV2 was identified as the primary agent in an outbreak of keratoconjunctivitis in Norwegian reindeer[56] and experimental infections in reindeer have been linked to neonatal death and abortion [57].

We tested for antibodies reacting to para-influenza type 3 virus (PI3). In livestock, infected animals shed the virus in nasal and ocular secretions, and although persistently infected animals have not been reported and most infections are mild, clinical disease can develop under deleterious environmental conditions or in conjuncture with other infections (such as herpes or pestivirus infections)[45]. PI3 contributes to the respiratory disease complex (together with BHV-1 and BVDV) in domesticated livestock, where they mostly cause upper respiratory infections but impacts on other organs can occur, with effects ranging from abortion and weak calves to diarrhoea and lethal disease. Antibodies to para-influenza virus have been detected in some studies, for example, Curry's (2012) survey of seven caribou herds detected an overall exposure of 7% in 2007-2010 (0-47% range n=551), including a 10% (n=52) seroprevalence in BNW. Further positive samples come from the Western Arctic herd (32%, n=241) and the Yukon in 1994, 1996 and 1997 (3.7%, n=109)[55], whilst other studies have had negative results[47, 48, 53]. We only found a low overall prevalence in BNW (8.7%), where three of the positive samples were from serum samples, and only one from filter paper samples. Its been shown that PI3 ELISA testing is more sensitive for serum samples than for filter paper samples[18].

For all serology, the results obtained depend on the type of diagnostic test used and how the samples are stored[17-19, 51]. Curry et al. (2014) found that the sensitivity and specificity of tests using blood collected on filter paper was generally high (> 85%) for detection of antibodies to *Neospora caninum* and bovine viruses using an ELISA test. All reported seroprevalences are sample prevalence, and are difficult to scale up to herd level results. However, if samples are collected during the same time periods and in consistent manner sample prevalence can be compared over time and indicate trends in disease exposure.

4.5.2 Fecal parasitology

Fecal collections from harvested animals require little effort and sample sizes are, therefore, generally good.

Fecal samples allow us to monitor the diversity and abundance of helminth parasites. Helminths are common in caribou and moose and although they do not usually cause clinical disease they can still lead to reduced body condition and fecundity. In fact, studies have shown that one of the most common gastrointestinal nematodes of caribou and reindeer, *Ostertagia gruehneri*, may have a role in regulating population dynamics[58]. Furthermore, transmission of these parasites is often density dependent, and changes in abundance may indicate population level changes in caribou numbers[59].

Faecal samples also allow monitoring of *Varestrongylus eleguneniensis,* the newly identified lungworm of caribou and moose[5] as well as other protostrongylids (*Parelaphostrongylus andersoni* and *Parelaphostrongylus odocoilei)*[38]. Protostrongylid nematodes, have an indirect life cycle with gastropods as the intermediate hosts. Infected animals generally appear healthy but severe infections can lead to difficulties with breathing and coughing and animals may appear thin and weak. The transmission of these nematodes is temperature dependent, and data has indicated that the transmission dynamics of a lungworm of muskoxen may already have been altered by climate warming, switching from a one-year to a two-year life cycle[60]. Such changes can escalate the infection pressure and it is, therefore, important to track the presence of protostrongylids in moose and caribou to detect if such changes are occurring. Another important lungworm of caribou is *Dictyocaulus eckerti*[38], but it is not detectable from frozen feces by the baermann method because it dies.

It should be noted that the overall prevalence of helminths was low. This can partly be due to the highly seasonal egg output found in many helminths, where peak burdens are most commonly observed in spring and summer[61]. Furthermore, *Ostertagia* eggs do not survive freezing and the way the samples were stored (-20°C) would have severely reduced egg counts for this species[62, 63]. In the future, analysing samples collected during the winter may, therefore, provide limited additional information, now that baselines have already been established.

Analysis of faecal samples has also led to the PCR positive identification of *Mycobacterium avium* subspecies *paratuberculosis* in the BNW caribou herd at an overall 3.8% (n=52) prevalence. This bacterium is the causative agent of Johne's disease, most common in cattle where it can lead to emaciation and wasting[64].

4.5.3 Other pathogens

Hunter submitted samples from caribou and moose has led to the identifications of a range of other pathogens. Noteworthy is the identification of *O. cervipedis* in moose from the Sahtu, demonstrating new geographic records for this parasite and a broader distribution than previously known[12]. *Onchocerca cervipedis* is filarial

nematode that generally infects subcutaneous tissue of the limbs of animals. Transmission depends on climate and the presence of suitable arthropod hosts. Although *O. cervipedis* doesn't appear to cause clinical disease in moose, significant pathology has been observed in caribou. The ecology and impacts of *O. cervipedis* at northern latitudes are currently unknown, but severe disease outbreaks caused by related arthropod-borne filarioids have been reported in reindeer in Fennoscandia and are attributed to climate warming and host range expansion[65], suggesting that *O. cervipedis* could become an important emerging pathogen in northern Canada.

In addition to investigations driven by scientific interests, observation by hunters and elders communicated during the course of the program brought other wildlife diseases of concern to the forefront of research and monitoring. In particular, there was an increase in the number of reports of moose with hair loss, signs usually associated with the infestation of *Dermacentor albipictus*, the winter tick. As a result of the moose study conducted in 2010-2011, the winter tick was isolated on two out of two hides from Tulita, two out of three hides from Deline, one out of 24 hides from Fort Good hope but was not found on the one hide examined from Norman Wells[9]. In recent communications with hunters it appears that the number of sightings of infected moose in the areas around Norman Wells and Tulita have increased in the past few years. The winter tick, a one-host ixodid tick, is a parasitic arthropod of ungulates with a seasonally synchronized life cycle. Heavy infestation with the winter tick can lead to hair loss, poor body condition, anaemia and even mortality of hosts and has been identified as a significant factor in rapid declines of moose populations[66]. The Sahtu lies beyond the previously known northern limit for the distribution of winter tick, indicating that its geographical range may be expanding, likely as a consequence of warming temperatures in the Canadian Arctic as well as increased moose populations related to local landscape change[9, 67].

Trnasmission of both *Onchocerca* and the winter tick is highly responsive to climatic conditions[67]. Another parasite identified in caribou in the Sahtu that may also be sensitive to climatic conditions is B*esnoiti. tarandi*. Infections with *B. tarandi*, a protozoan tissue dwelling parasite, can lead to alopecia, and thickening and ulceration of the skin of the head, lower limbs and the scrotum[21]. Recently, *B. tarandi* emerged as a disease causing agent in caribou herds from northern Quebec. Although the emergence of *B. tarandi* in caribou occurred at a time with broad scale temperature increases, the link between the emergence of the parasite and warming climate remains unknown. A big hurdle to elucidating this connection is that the lifecycle of this parasite has not been clearly established[11]. In light of the rapid changes that are occurring in the Arctic, continued monitoring of these parasites is a priority.

4.6 Stress

One way to monitor and predict population health is by measuring stress levels of individuals and populations. Physiological stress can be linked to life-history patterns or external drivers such as weather, parasitism and disturbance. By identifying historical, seasonal and current patterns of stress we may be able to gain

insight into causes of declines, as well as population vulnerabilities, providing information useful for the management of wildlife populations[10, 68, 69]. Stress was measured in this program using two different methods, dental enamel hypoplasia and glucocorticoid levels.

Dental enamel hypoplasias are developmental tooth defects that are linked to hereditary disorders, trauma and physiological stress, with the latter being the most common cause. By analysing tooth defects, and with knowledge of when in life enamel forms on each tooth, we can gain an understanding of the physiological stress experienced by an animal prior to birth and during the first 2 years of life. The enamel lesions are permanent, and, if the age of the animal is known (e.g., incisor aging), then the time frame (e.g., year and season) of when the stress occurred can be determined. Through jaw collections done through the WHM program, Wu et al. (2012) demonstrated, for the first time, that these defects occur in caribou. They also found that BNW had a higher number of lesions than BNE. This difference may be related to the BNW population decline in the early 2000s, a few years before the BNE decline, suggesting that animals may have been under increased physiological stress during this period. Evaluation of dental enamel hypoplasias is thus a good method for tracking population level stress, as samples are relatively easy to come by when working together with local hunters, and analysis is low-tech and requires little training[10].

Stress hormones (glucocorticoids), incorporated into hair and feces in response to physical and social stressors, have been suggested as biomarkers of overall health. However, very little is known about the level and variation of glucocorticoids in wildlife and how this relates to other health measures, such as parasitism[70]. In 2013, fecal samples from caribou and moose were analysed for the presence of glucocorticoids. Fecal stress hormones generally reflect short-term stress. As found in other studies, corticosterone metabolites were consistently detected in a higher level than native cortisol. Corticosterone levels were within a similar range as those reported in captive reindeer (originating from Siberian herds) and caribou (descended from Delta and Porcupine herds in Alaska), at the University of Alaska[71]. Before we can understand what these number mean, and if glucocorticoids can be used as biomarkers for overall health, we need to determine if there is an association between stress hormones levels and other physiological measures of health or definable external stressors (landscape disturbance, adverse weather). The collection of full health sample kits from caribou and moose will allow us to do this.

4.7 How health data can inform management decisions

The health status of an animal population depends on complex interactions, and not just the “absence of disease”. Rather, health is an outcome of biotic and abiotic factors acting on individuals and populations [72, 73]. In contrast to our knowledge of health of humans and domestic animals, few data are available to establish the “normal” or expected range for wildlife. The first step is, therefore, to establish baselines of body condition, genetics, physiological health, contaminants and

pathogen diversity and abundance[35, 36]. Once baselines have been established, comparisons with the same population at a future date can be made to determine if various disturbances (e.g. development, weather extremes) have had an effect. Changes in health indices before and after an event would suggest that the event had an influence on the health of the population[35]. These changes may reflect shifts in population health and serve as early warning signals for wildlife managers, wildlife users and public health officials[7]. Comparisons between different populations can also help determine the appropriateness of conservation management actions for individual species[35].

The WHM program has demonstrated that community-based monitoring can be successful for obtaining some baseline indices and also for targeted research studies. However, the ultimate goal is to create a system that can inform management decisions to ensure healthy and sustainable wildlife populations in the long-term.

During a presentation at a CARMA meeting in 2012, Courtier suggested a new management framework for caribou, based on the lessons learned from the George River herd. His suggestion was that management should change as herds rise, peak and recover. However, tracking caribou population cycles can be difficult. Population censuses are often used to inform management plans for caribou, with users and managers being reluctant to take action until the data from these censuses have been analyzed and major changes observed. However, as Courtier outlined, there can be temporal mismatch in when the population census occurs (every 5-10 years) and when management should be revised (annually). These mismatches are especially problematic when the herd is declining. Yet, biological indicators from individual animals may be able to detect impending population crashes well before a census would detect a shift. For example, for the George River herd traditional knowledge as well as individual body condition measures suggested that, although there were many caribou, they were in poor condition (skinny, low birth mass and fall calf mass and high percentage un-antlered individuals) several years before the population crash occurred (Courtier, 2012, unpublished). However, no action was taken until 2010 when a population census demonstrated the drastic decline of the population. If commercial and sport hunting had been limited earlier, the slope of the decline might have been shallower.

Russell et al (2013) further demonstrated that local knowledge on caribou availability and health is linked to herd population status and can be used to inform caribou management. Their study illustrated that, at a time when census data was not available, traditional knowledge gained by community-based interviews indicated that conditions for the Porcupine herd were improving (2001-2010) whilst interim agencies and boards believed the herd to be in decline. A successful survey in 2010 determined that the herd had grown since the last estimate in 2001, confirming the observations made by community members[74].

As demonstrated by these case studies, management boards struggle to make effective decisions when information about population levels are missing, highlighting the need for new approaches and methodologies for collecting information about herd status and health to complement information gained from population censuses and/or provide data to act upon in the absence of censuses.

Although the sample sizes from the WHM program were small in some years, accumulated samples gathered during several years can allow us to detect shorter and longer-term trends (2-5 years), in, for example, disease exposure[75, 76]. As reviewed in this report, research on pathogens has already been very fruitful. Now that baselines have been established we can build on Courtier's idea, and disease ecology theory, to track population trends by monitoring several biological indexes, including pathogens.

For example, *Ostertagia gruehneri* has a direct life cycle where transmission is density dependent. Studies have indicated that there is a two-year lag between host population density and parasite abundance[58, 59]. Thus, by tracking abundance of *O. gruehneri* we may be able to track host densities in the absence of population censuses.

Monitoring health and archiving samples can also help us identify new emerging threats to populations. For example, large mortality events of muskoxen in Banks and Victoria Island have been attributed to the bacteria *Erysipelothrix rhusiopathiae.* However, no regular monitoring was in place to predict this event. Also, without historic data from this area it is difficult to determine the origin and ecology of this disease. A project is currently underway to test for the presence of this bacterium in caribou from the Sahtu, only made possible by the long-term monitoring and archiving of blood samples.

It should also be noted that disease surveillance is important for wildlife management and conservation in its own right. Although diseases are natural components of ecosystems they do, in some cases, need to be managed if they are a threat to human health or conservation targets. Surveillance is needed to understand transmission patterns and document emerging epidemiological situations. Furthermore, for new diseases to be confidently identified, sound baseline knowledge of the pre-existing disease status of the population is required[35, 77].

4.8 Concluding remarks

Biological indicators of health can be complementary and predictive indicators of population health, and population trajectory and have some advantages. First, studies can be conducted more often, on an annual or bi-annual basis to coincide with management plans. Second, annual or bi-annual studies of health indicators track changes as they occur, as opposed to a census that tracks changes that have already happened.

Perhaps the most important point to note is that by using a community-based monitoring approach to conduct health monitoring local communities and hunters, who depend on caribou for their livelihood, become active members of the monitoring and research process. Hunters are the eyes on the land and are in a unique position to observe changes when they happen and hold traditional knowledge that can contribute to an integrated view of individual and population health. Community based monitoring is also cheaper and less labor intense than aerial population censuses and could be more sustainable in the long term.

In a case study review Danielsen et al (2005) attempted to evaluate the sustainability of locally based monitoring approaches and found several examples where, after substantial investment by external agencies in the "startup phase", programs could be sustained without external support for years. However, most programs reviewed were in their infancy and sustainability was, therefore, difficult to assess. The review highlighted six principles that would enhance the probability of a monitoring scheme becoming sustainable in the long-term. First, the program must provide benefits to the community, second, the benefits must exceed the cost of monitoring (e.g. time investment), third, programs must be sensitive to conflicts between government and communities and ensure that conflicts do not limit the ability/opportunity for local stakeholders to be active participants in the monitoring process, fourth, the program should build on/ work with existing institutions and management structures, fifth the work should be institutionalized at all levels (stakeholders to government) and finally data should be stored, analyzed and accessible locally, even if this means some compromise of quality[78].

Involving local stake-holders can also improve co-management strategies. Several studies have reported that the interaction between managers, researchers and users, at the local level, independent of formal administrative structures, is key for acceptance of management plans and successful collaborations[26, 74, 79]. This includes communications in both directions. Communities need a forum to share their knowledge with researchers and managers, and managers and researchers need to make sure that results are disseminated at the community level. Employing local people to collect data and running programs from a local agency improves access to stake-holders, increases interaction time between managers and stake-holders, improves the opportunities for knowledge exchange and builds trust between managers and users[26, 79]

A recent paper outlined five fundamental areas that are essential for establishing positive research relationships in northern communities; dedicating time, being present, communicating, listening, respecting, understanding, building trust, making genuine collaborative efforts and exchanging knowledge [80].

The WHM program already fulfills many of these principles. The program was created in response to community concerns and has continued to prioritize local community-driven interests. Focus group interviews have significantly contributed to the research direction and methods of the program as well as highlighted areas

that need improvement (e.g. results reporting)[8]. However, the program is still essentially externally driven, where researchers outside the study area set up, run and analyse the results from the program and local stake-holders collect data. Such approaches have been criticised for being expensive, dependent on external expertise, difficult to sustain over-time and insensitive to local management needs [81]. We believe that the program is now at a stage where moving it to a local agency and working towards a category (iv) type monitoring, i.e. the project is run locally and researchers only serve as consultants, could improve the quality of samples as well the benefits for local communities. Danielsen et al (2008) also suggested that this type of monitoring might improve the speed of decision making in matters that concern the monitoring objectives and enhance local capacity.

A second point to consider is formalizing and structuring the way traditional knowledge is incorporated, such as hunter assessments of health and body condition, by for example, conducting regular interviews with WHM monitors. This may be more easily achieve if the program is run from a local agency that has a better capacity to interact regularly with local stakeholders and record observations when the kits are returned, or at the end of the hunting season. As discussed earlier, hunters' impression of body condition are somewhat reliable predictors of body condition[31]. Furthermore, one study found that impressions of body condition during butchering and in interviews at the end of the hunting period were similar, suggesting that observations can be recorded at the end of a season reducing time, costs and imposition to hunters[26]. Although hunter observations do not provide absolute evaluations of body condition, they can provide relative comparison between years and accessible, long-term data informing on health and condition of animals, contributing to an understanding of population trends[82].

Finally, it should be noted that although community-based monitoring has the potential to generate good data, sustainably and at a low costs, many scientists are concerned about its reliability and ability to detect trends. The main concerns relate to the increased variance in data collected by local stake-holders (as opposed to trained professionals) and simplistic analysis. However, these issues can be addressed by thorough training of data collectors and continued support by an external agency (e.g. the University of Calgary) to assist in data analysis in interpretation[78].

4.9 Take-home messages

Long-term support and improved structure of the WHM program will allow future managers and researchers to efficiently gather reliable samples and data and compare it with the historical baseline-data presented in this report. Some key points identified during the past years that should be considered as the program moves forward are listed below.

General recommendations/observations:

- Research needs to be driven by community interests and management needs.

- Acceptance of community and a local organizer is key.
- Sampling needs to be standardized with-in and between community-based monitoring programs. I.e. different programs collecting body condition data on the same herds should follow the same procedures and methods so that data can be combined.
- Training of Wildlife Health Monitors and local laboratory personnel is critical for successful and correct sampling and sample processing.
- Researchers need to discuss best sampling protocols with hunters, and make sure they develop an appropriate body condition-scoring index together.
- Targeted sampling and smaller collection kits leads to more samples being collected.
- Results should be disseminated to the communities on an annual basis.

Specific suggestions

- Samples should be collected during defined time periods (e.g. September/October for community harvests and/or January-April) to focus collection efforts, decrease variability in data and allow for comparisons between years.
- To ensure data quality and increase number of samples collected we suggest collecting a smaller kit on an annual basis, complemented with biological collections every 2-3 years.
    - On an annual basis we suggest collecting: the right metatarsal, blood samples, fecal sample, incisor bar, a piece of hide from the rump, back fat measurement, hunter assessment of body condition, abnormalities as well as information on sex, age or age classification, pregnancy status, location and date of the kill and the name of the hunter.
    - Every 2-3 years we suggest collecting all of the above, as well as the whole lower jaw, the left kidney with fat, and a piece of the liver. If feasible, more in-depth monitoring of body size and collection of the peroneus muscle, as outlined in CARMA level 2 protocols is recommended.
- An assigned person must be present in the community and able to receive samples. This person must ensure proper labelling is done on reception and should fill out a datasheet to guarantee all required information is recorded. This includes:
    - Recording all metadata from all samples on a standardised datasheet (sex, age classification, pregnancy status, location, date of the kill, the name of the hunter).
    - Checking that all samples asked for were collected and are present. If samples were incorrectly collected, e.g. filter papers not soaked accurately, hunters should be informed on the correct procedures to ensure future success.
- Data should be stored in one place in a master data file with one person responsible for regular updating and maintenance. A database has now been created for the Sahtu WHM program and should be updated.

- The program needs to be run by a local wildlife agency (e.g., Government or co-management agency), with local capacity for training and sample processing.
- Data should be pooled with other similar programs across herd ranges in order to boost sample sizes.
- Data should be summarized and communicated to stakeholders annually in order to obtain feedback/interpretation, track trends and identify changes, and evaluate and adapt sampling strategies and priorities.
- A sample archiving strategy and implementation plan should be developed. Archived tissue specimens are essential as they allow future re-testing when new methods are developed or if new pathogens are identified (for example *Erysipelothrix* as discussed above).

Appendices

Appendix 1 Caribou and Moose data and information sheets

Appendix 1.1 Caribou data Sheets 2005

Caribou Health Monitoring 2005 ANIMAL ID: _____

Hunter Name:_____ **Date of Kill:**_____

Location: Name_____

Latitude _____**Longitude**_____

Sex: (✓) ☐ Cow ☐ Bull

1. Was cow producing milk? (✓) ☐ Yes ☐ No ☐ Unknown

2. Was cow with a calf? (✓) ☐ Yes ☐ No ☐ Unknown

3. Was cow pregnant? ☐ Yes ☐ No

Sex of fetus: ☐ Female ☐ Male ☐ Unknown

4. Amount of back fat: _____mm (millimetres)

Remember to turn in: (✓)

☐ Lower jaw ☐ Leg bone ☐ Left kidney
☐ Liver ☐ Fecal sample ☐ Blood (on paper strips)
☐ Any abnormalities

Hunter's overall assessment of animal's condition

For this time of the year, is the condition of the animal: **(✓)**

☐ Poor ☐ Fair ☐ Good ☐ Very Good

Any abnormalities?	Circle if found	Describe
Joints	fine swollen other	
Warbles	none 1-10 10-20 20-100 >100	
Liver	fine cysts other	
Skin on legs	fine rough other	
Eyes	fine white spots other	
Meat	fine cysts other	
Lungs	Fine cysts other	
Additional comments (e.g. abnormalities) here: **(Continue comments on back if necessary)**		

Appendix 1.2 Caribou and Moose data and information sheets 2009-2010

Caribou/Moose Health Monitoring 2009/10
ANIMAL ID: _____ Caribou or Moose (circle one)

Hunter Name:_____ **Date of Kill:**_____
Location: Name_____
Latitude _____**Longitude**_____

Sex: (✓) ☐ Cow ☐ Bull

1. Was cow producing milk? (✓) ☐ Yes ☐ No ☐ Unknown

2. Was cow with a calf? (✓) ☐ Yes ☐ No ☐ Unknown

3. Was cow pregnant? ☐ Yes ☐ No
Sex of fetus: ☐ Female ☐ Male ☐ Unknown

4. Amount of back fat: _____mm (millimetres)

Remember to turn in: (✓)

☐ Lower jaw	☐ Leg bone (left)	☐ Left kidney with fat
☐ Liver (fist size)	☐ Poo	☐ Blood (on paper strips)
☐ Lungs	☐ Testicles	☐ Any unusual stuff!

☐ Piece of hide/skin from near butt (6x6 inches – size of large hand)

Hunter's overall assessment of animal's condition
For this time of the year, is the condition of the animal: **(✓)**
☐ Skinny ☐ Not so bad ☐ Fat ☐ Really fat

Anything unusual?	Circle if found	Describe
Warbles:	none 1—10 11-20 21-100 more than 100	
Joints:	fine swollen other	
Lungs	Fine cysts other	
Liver:	fine cysts other	
Skin on legs:	fine rough other	
Eyes	fine white spots other	
Meat:	fine cysts other	
Testicles:	fine swollen other	
Additional comments here: (Continue comments on back if necessary)		

CARIBOU/MOOSE COLLECTION CHECK LIST

REMEMBER TO:	COLLECT:
Record Animal ID#	Lower Jaw
	Piece of Liver
Measure Back Fat	Left Kidney with Fat
	Left Leg Bone
Check for Milk	Poo – about 20 pellets
	Blood strips
Assess condition	Testicles
	Lungs
Examine and collect anything unusual (joints, testicles, eyes, skin, meat, liver, lungs)	Hide
	Any other abnormal tissues

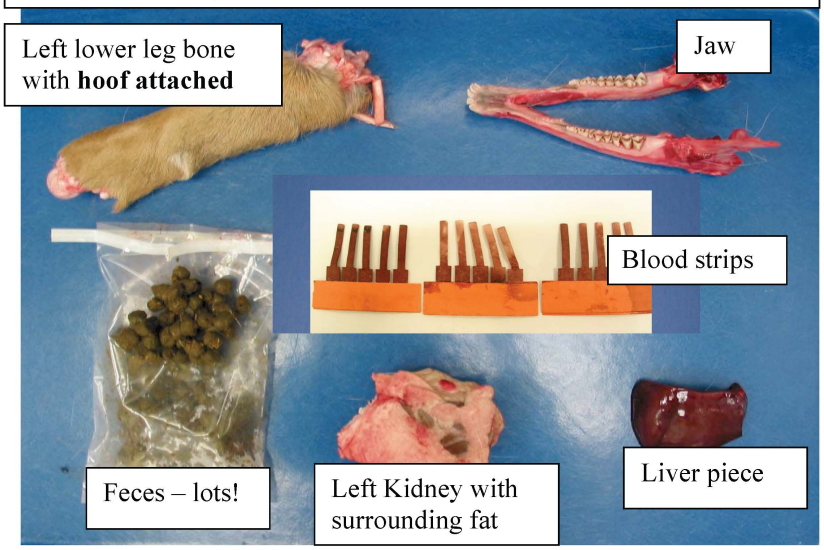
Final sample kit

Blood strips go back into envelope and then into the Ziploc bag – freeze these as soon as possible

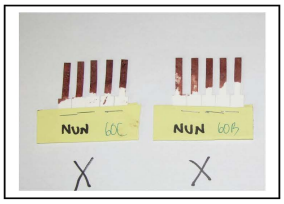
Hide – collect a piece of hide from the area to the side of the butt.

Collect anything that seems unusual as well.

Keep all samples frozen



Make sure blood strips are soaked at least all the way down to the wide part



BAD

GOOD

What do we do with the samples?

Back fat, kidney fat, and bone marrow fat provide an indication of body condition.

Blood strips –

- are used to test to see if the animal has been exposed to diseases like brucellosis, west nile virus, etc.
- we hope in the future to be able to test if the animal has been exposed to winter ticks

Liver is saved for future work on contaminants.

Leg bone –

- we look for parasites under the skin (Besnoitia – looks like salt grains, or Onchocerca – long white worms)
- we measure it as an indication of body size
- we analyze the bone marrow as an indication of how fat the animal is

Kidney –

- the amount of fat surrounding the kidney gives an indication of how fat the animal is
- the size of the kidney provides information on the body size
- the kidney is saved for future work on contaminants if needed

Jaw –

- we measure the jaw and this provides an indication of how the animal grew in its first two years of life
- the front teeth are used to determine the age of the animal

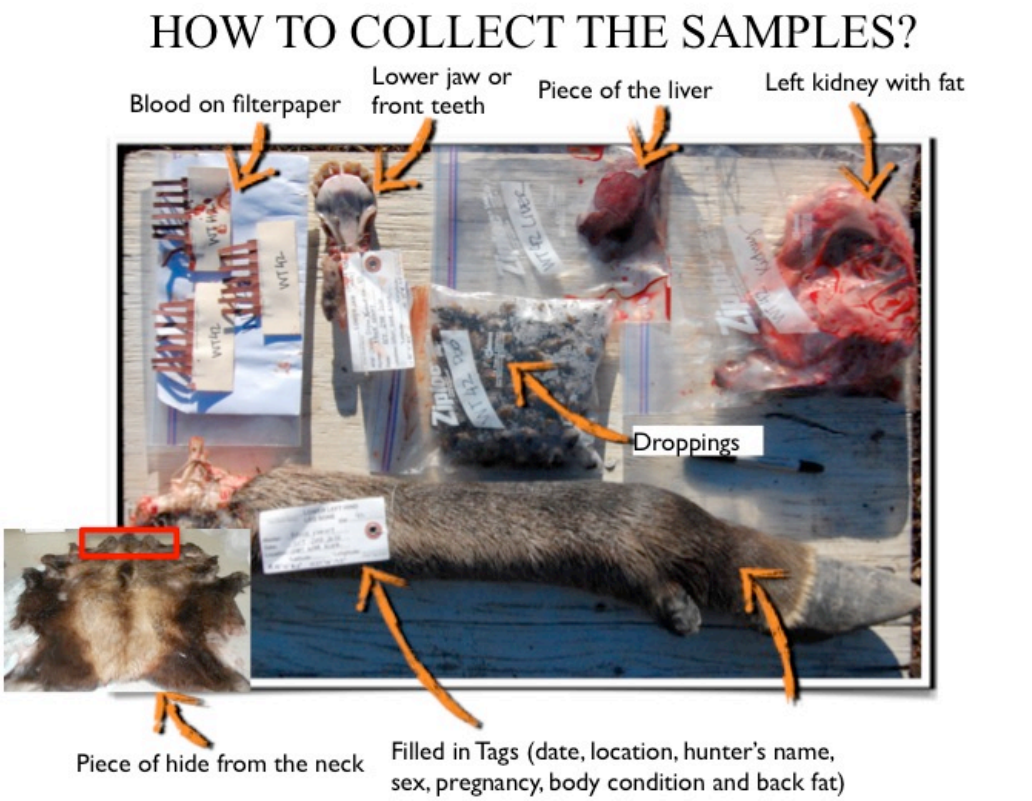
Hide

- the piece of hide is digested in a chemical solution and examined for the winter ticks

Unusual things – we keep track of this to see if there is anything changing and anything that people need to worry about

-

Appendix 1.3 Caribou and moose data and information sheets 2012



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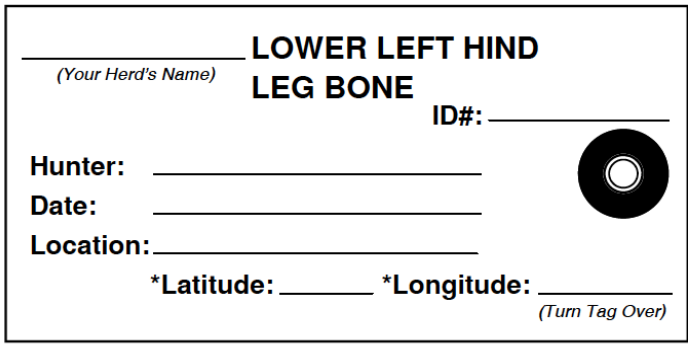

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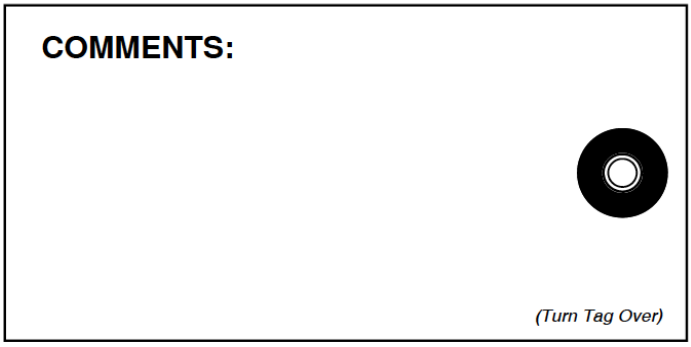

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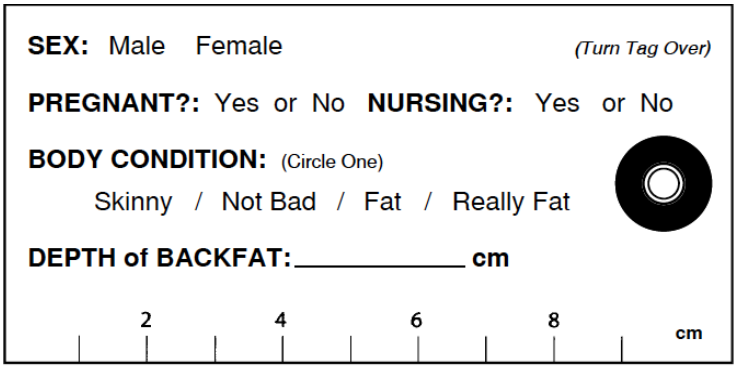

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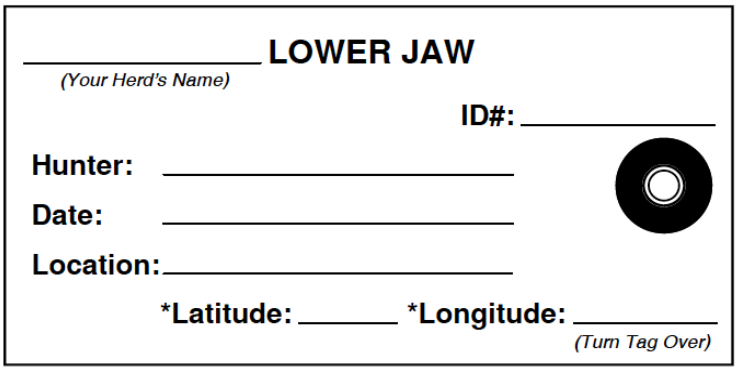


Appendix 2. Body condition

Appendix 2.1 Bluenose-East caribou body condition

Overview of Bluenose-East caribou body condition from animals harvested from 2004-2014 as part of the Wildlife Health Monitoring Program in the Sahtu Settlement Area

Anja Carlsson[1], Susan Kutz[1], Richard Popko[2], Alasdair Veitch[2], Stephanie Behrens[2], SRRC[3], SRRB[4]

[1]University of Calgary, Calgary, AB, [2]Environment and Natural Resources, Government of the Northwest Territories, NT,

[3] Sahtu Renewable Resource Councils, NT, [4] Sahtu Renewable Resources Board, NT

Background

The Sahtu Wildlife Health Monitoring (WHM) Program began in 2003 in response to community concerns about wildlife health under a regime of rapid environmental change (climate and industrial development) in the Sahtu Settlement Region, Northwest Territories. Community members indicated that they had concerns about the health and sustainability of wildlife, how wildlife health and disease may affect them, and that the next generation (youth) may not be adequately prepared to take on the emerging environmental issues in the region. Together with Environmental and Natural Resources, Government of the Northwest Territories, the Sahtu Renewable Resources Board and the Sahtu Renewable Resources Councils, the University of Saskatchewan (2003-2005) and University of Calgary (2005-present) has run the WHM program since 2004 to address these concerns [3]. The purpose of this report is to summarise the body condition data from the Bluenose-East herd collected as part of the WHM program between 2004-2014.

Methods

Local subsistence hunters were trained to collect data using standardized sampling protocols and pre-prepared sampling kits. A kit consisted of pre-labelled bags for samples, filter papers for blood collections, and tags to fill in information regarding the date and location of the kill, and the condition of the harvested animal. Samples collected included the lower jaw, the left metatarsal, fecal sample, the left kidney with fat, a piece of the liver, blood on filter papers and a piece of hide. Samples were collected and processed according to standardized protocols developed by the CircumArctic Rangifer Monitoring and Assessment Network [6, 7]. The condition of the animal was scored by the hunter according to one of four pre defined scores: 1=skinny, 2=not bad, 3=good and 4=very good. The bone marrow index (percentage marrow fat= (bone marrow dry/ bone marrow wet)*100) was calculated after breaking the metatarsus bone, extracting the marrow and weighing the wet and dry marrow as described in the CARMA protocols [6]. The kidney fat index was evaluated using a standardized technique to provide a ratio of the weight of the kidney fat to the weight of the kidney * 100; the kidney fat index was reported as a percentage

and can be >100%. Serology from filter paper, examination of abnormalities, quantifying *Besnoitia* lesions on the metatarsals, and body size measurements were also done, and will be reported elsewhere.

Results/Discussion

In total sample kits from 163 individual Bluenose-East caribou were collected between 2004-2014, of these 157 were assumed to come from adults (Table 1). However not all kits were complete and there was information and samples missing. Sample sizes, pregnancy rates and measures of body condition are summarised in the tables and graphs below. Body condition data is based on samples collected in late winter (Jan-Apr) in order to allow meaningful comparisons. Sample sizes for females are higher than that for males due to hunter preference. Although samples sizes between the years are limited, if they were to be combined with similar projects occurring across the Bluenose-East caribou herds' range sample sizes would increase, potentially allowing for the detection of trends. Almost half (42.3%) of the samples were collected by the same hunter, which may decrease the variability in the data and increase the reliability of the results (Table 2). In this program the highest sample size was for the kidney fat index, which appears to indicate a declining trend for female caribou until 2014 (Figure 2b), however further in-depth analysis would, with pooled data, would have to be conducted to confirm significant trends.

In total 131 samples were collected from Bluenose-East caribou with known sex, year and month of collection that were assumed to be adults. Of these, 101 had records for backfat, 125 for the kidney fat index, 90 for the bone marrow fat index and 100 had records of hunter scores of body condition.

Measuring and tracking wildlife health indices can provide important complimentary data to population censuses, since the former only detects changes after they have happened but by tracking health indices we have the potential to track changes as they are occurring and better identify the cause of the changes [36]. The WHM program has demonstrated that community-based wildlife health monitoring is possible and has contributed to the development of new tools that improve the sample collection procedure [10, 19].

References

1. Brook, R.K., et al., *Fostering Community-Based Wildlife Health Monitoring and Research in the Canadian North.* Ecohealth, 2009. **6**(2): p. 266-278.
2. Kutz, S., et al., *Standardized monitoring of Rangifer health during International Polar Year.* Rangifer, 2013. **33**(Sp. Iss. 21): p. 91-114.
3. CARMA. *Rangifer Health and Body Condition Monitoroing Protocols Level 1 and 2*. 2008 [cited 2014 31/3/2014]; Available from: http://www.caff.is/resources/field-protocols.
4. Ryser-Degiorgis, M.-P., *Wildlife health investigations: needs, challenges and recommendations.* BMC Veterinary Research, 2013. **9**(1): p. 223.
5. Curry, P.S., et al., *Blood Collected on Filter Paper for Wildlife Serology: Evaluating Storage and Temperature Challenges of Field Collections.* J Wildl Dis, 2014.
6. Wu, J.P., et al., *Linear enamel hypoplasia in caribou (Rangifer tarandus groenlandicus): A potential tool to assess population health.* Wildlife Society Bulletin, 2012. **36**(3): p. 554-560.

Table 1. Number of WHM samples collected from the Bluenose-East caribou herdYearlings and calves were not included in the analysis, unknown age animals were assumed to be sub-adults or older and were included in the analysis. Animals with unknown sex were not included.

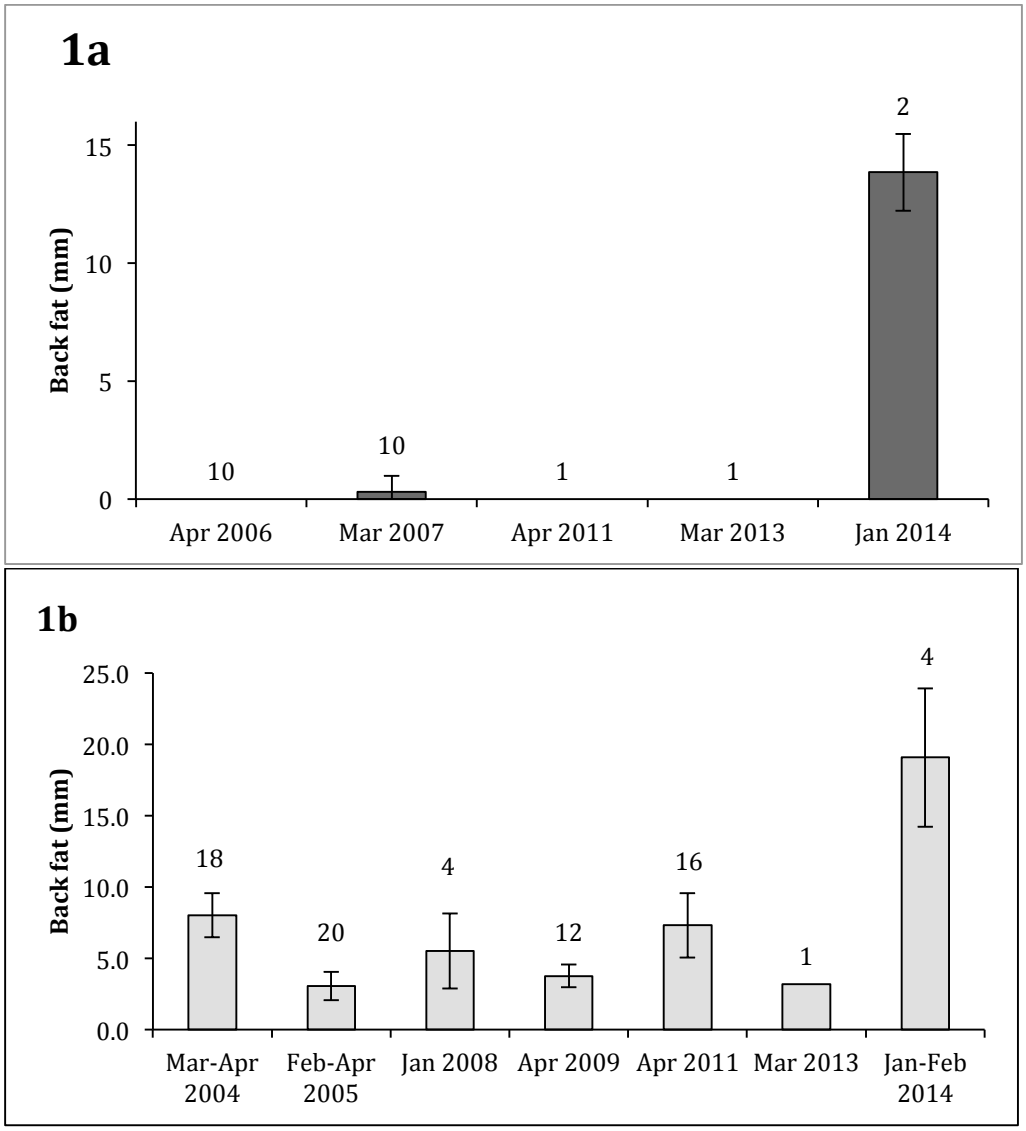
Year/Month	Females	Males	Unknown sex	Total
2004	18			18
3	2			2
4	16			16
2005	20			20
2	2			2
3	10			10
4	8			8
2006		14		14
4		14		14
2007		10		10
3		10		10
2008	5		1	6
1	4			4
7	1		1	2
2009	27		2	29
4	27		2	29
2011	24	1		25
4	17	1		18
Unknown month	7			7
2013	2	5		7
1	1	4		5
3	1	1		2
2014	7	11	10	28
1	2	4	6	12
2	5	6	4	15
5		1		1
Total	**103**	**41**	**13**	**157**

Table 2. Number of samples collected by individual harvesters from adult Bluenose-East caribou. Yearlings and calves were not included in the analysis, unknown age animals were assumed to be sub-adults or older and were included in the analysis. Animals with unknown sex were not included.

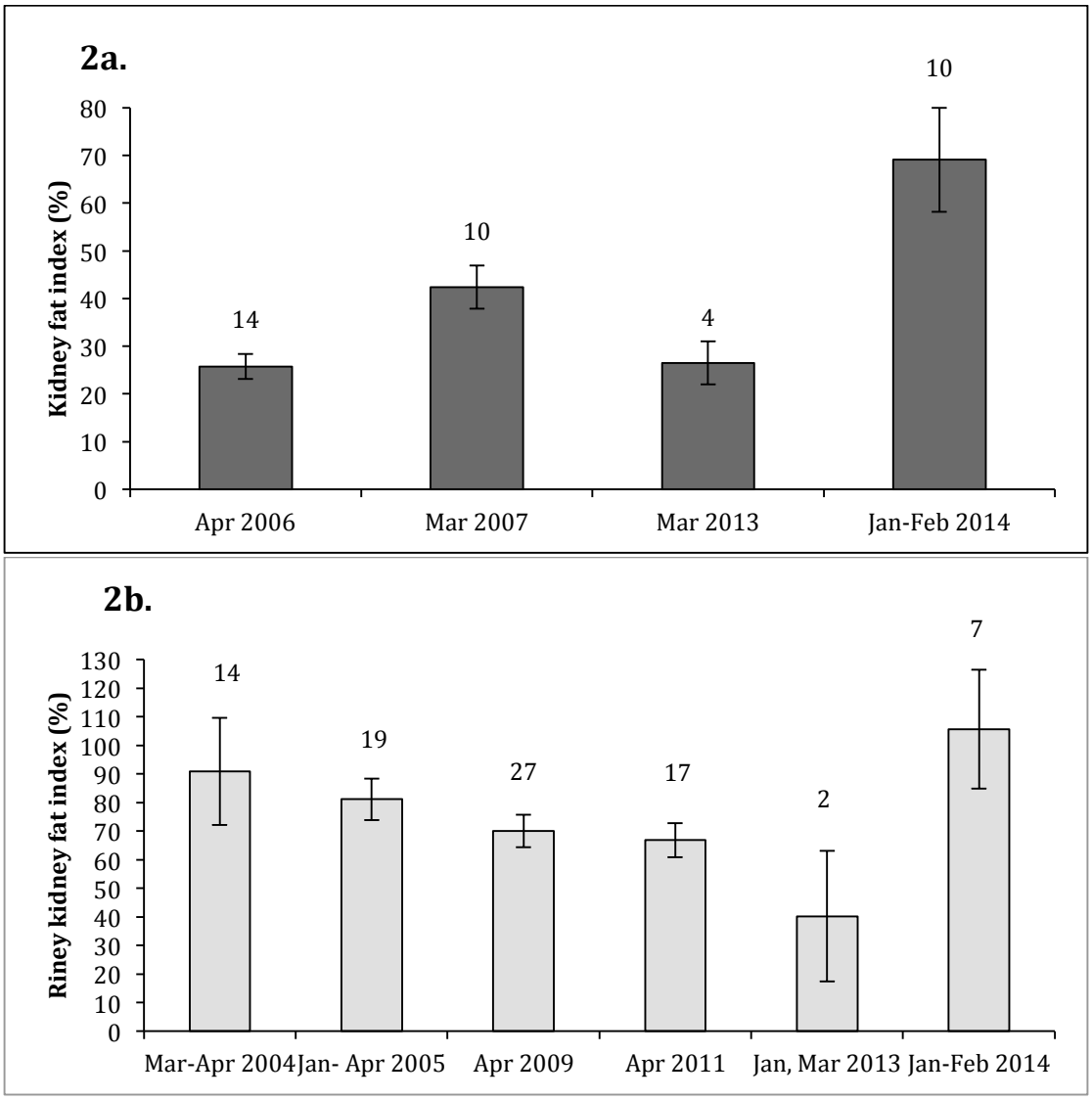
Harvester ID	No. Samples collected
7	76
9	10
11	3
14	34
17	5
19	1
20	3
28	2
30	1
35	8
36	1
38	1
41	1
47	4
49	1
52	1
53	5
57	4

Table 3. Pregnancy rates of harvested adult female Bluenose-East caribou, as recorded by harvesters. Yearlings and calves were not included in the analysis, unknown age animals were assumed to be sub-adults or older and were included in the analysis. Animals with unknown sex were not included.

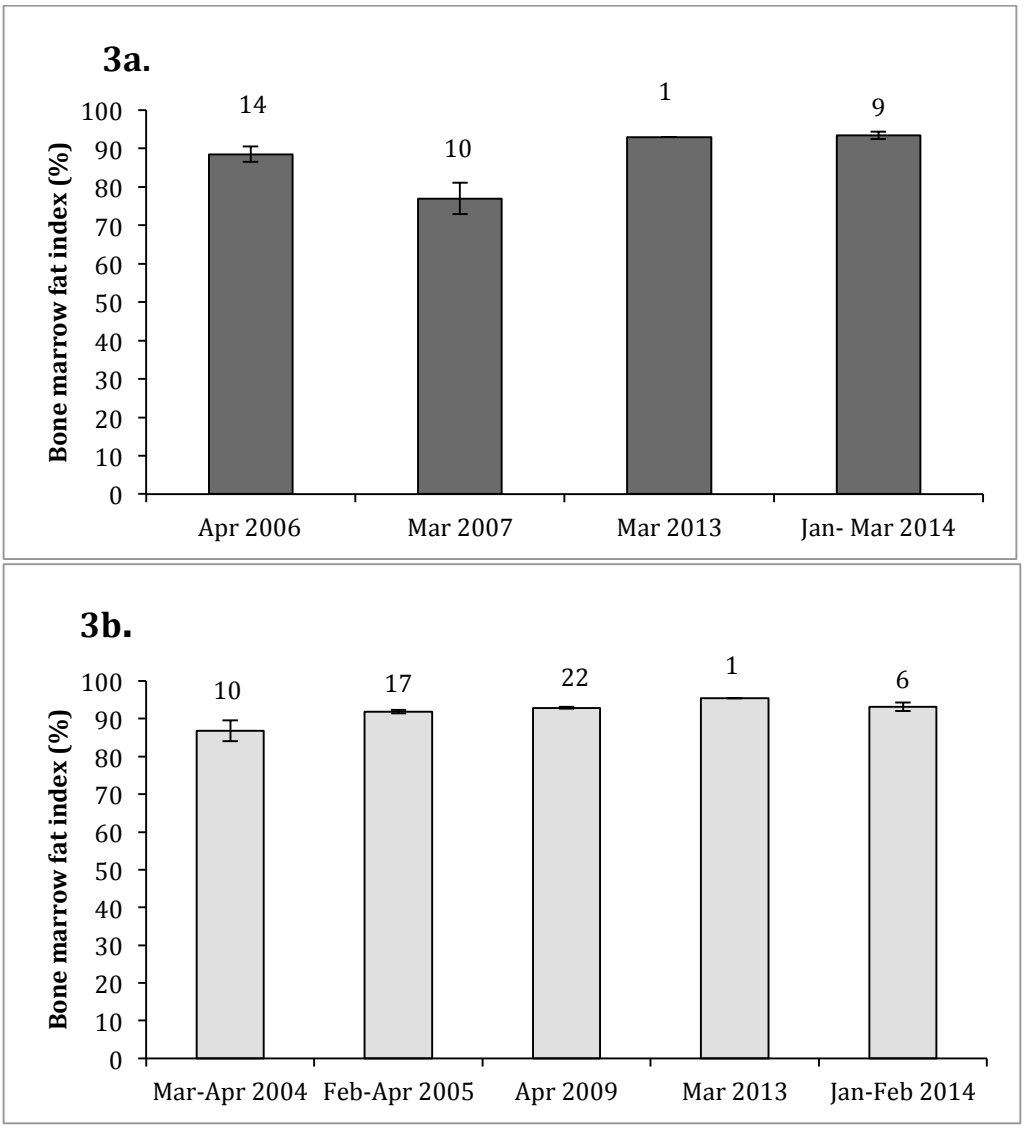
Year	Pregnancy rate (sample size)
2004	100% (16/16)
2005	90% (18/20)
2008	100% (5/5)
2009	79% (19/24)
2011	100% (17/17
2013	0% (0/1)
2014	75% (3/4)



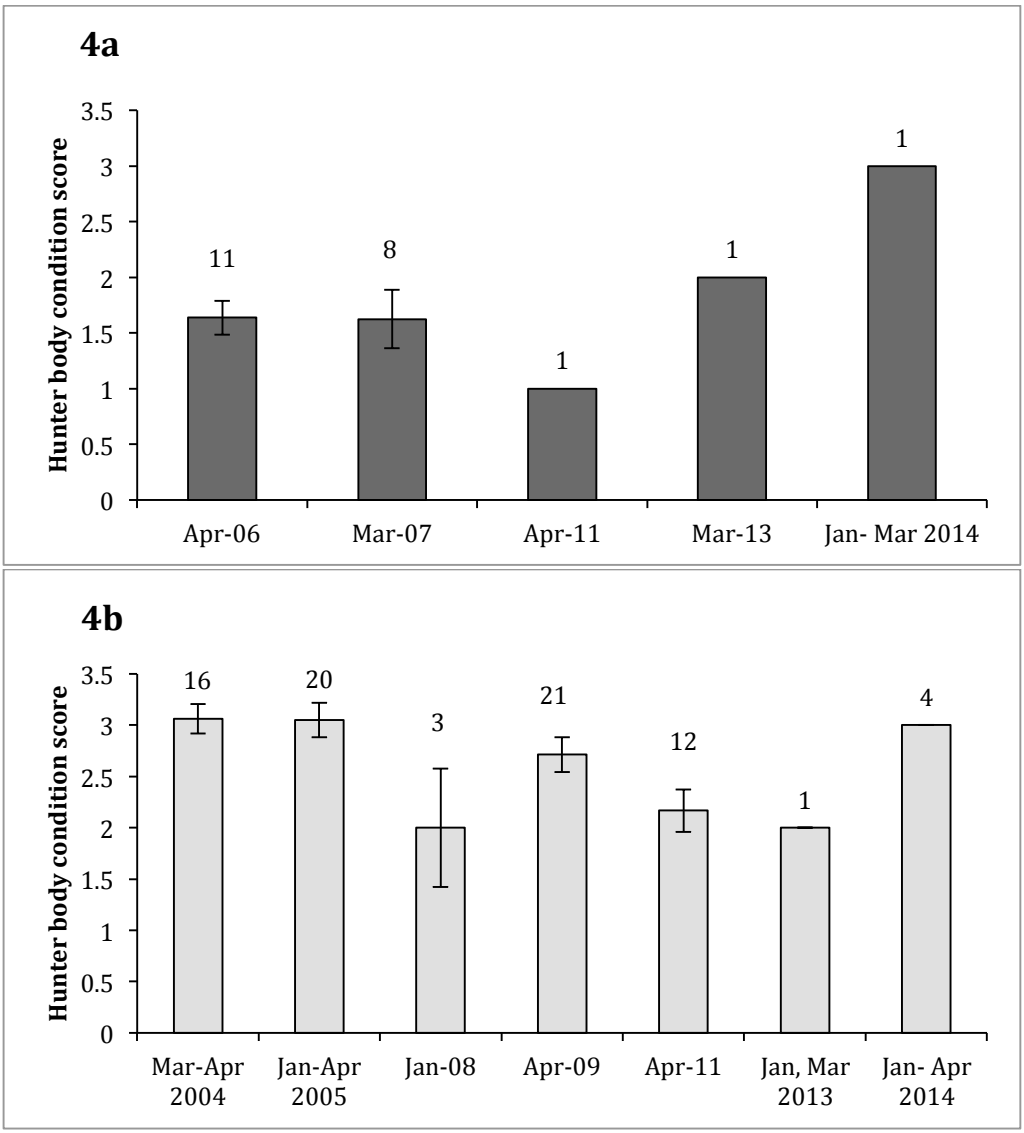
Graph 1. **Back fat** (mm) (mean where sample sizes >1)of adult male (1a) and female (1b) Bluenose-East caribou in late winter (Jan-Apr) as measured by harvesters, with standard error of the mean where appropriate. Sample sizes are indicated above the bars. Yearlings and calves were not included in the analysis, unknown age animals were assumed to be sub-adults or older and were included in the analysis. Animals with unknown sex were not included.



Graph 2. Mean Riney kidney fat index (%) for adult male (2a) and female (2b) Bluenose-East caribou in late winter (Jan-April), with standard error of the mean. Sample sizes are indicated above the bars. Yearlings and calves were not included in the analysis, unknown age animals were assumed to be sub-adults or older and were included in the analysis. Animals with unknown sex were not included.



Graph 3. Bone marrow fat index (%) (mean where sample sizes are >1) for adult male (3a) and female (3b) Bluenose-East caribou in late winter (Jan-April), with standard error of the mean where appropriate. Sample sizes are indicated above the bars. Yearlings and calves were not included in the analysis, unknown age animals were assumed to be sub-adults or older and were included in the analysis. Animals with unknown sex were not included.



Graph 3. Hunter body condition score (mean where sample sizes are >1)for adult male (3a) and female (3b) Bluenose-East caribou in late winter (Jan-April), with standard error of the mean where appropriate. Hunter score refers to four scores of caribou body condition where each animal is assigned a score by the harvester depending on body condition, the scores are as following; 1= skinny, 2= not bad, 3= good and 4= very good. Sample sizes are indicated above the bars. Yearlings and calves were not included in the analysis, unknown age animals were assumed to be sub-adults or older and were included in the analysis. Animals with unknown sex were not included.

Appendix 2.2 Bluenose-West caribou body condition

Overview of Bluenose-West caribou body condition from animals harvested from 2004-2014 as part of the Wildlife Health Monitoring Program in the Sahtu Settlement Area

Anja Carlsson[1], Susan Kutz[1], Richard Popko[2], Alasdair Veitch[2], Stephanie Behrens[2], SRRC[3], SRRB[4]

[1]University of Calgary, Calgary, AB, [2]Environment and Natural Resources, Government of the Northwest Territories, NT,

[3] Sahtu Renewable Resource Councils, NT, [4] Sahtu Renewable Resources Board, NT

Background

The Sahtu Wildlife Health Monitoring (WHM) Program began in 2003 in response to community concerns about wildlife health under a regime of rapid environmental change (climate and industrial development) in the Sahtu Settlement Region, Northwest Territories. Community members indicated that they had concerns about the health and sustainability of wildlife, how wildlife health and disease may affect them, and that the next generation (youth) may not be adequately prepared to take on the emerging environmental issues in the region. Together with Environmental and Natural Resources, Government of the Northwest Territories, the Sahtu Renewable Resources Board and the Sahtu Renewable Resources Councils, the University of Saskatchewan (2003-2005) and University of Calgary (2005-present) has run the WHM program since 2004 to address these concerns [3]. The purpose of this report is to summarise the body condition data from the Bluenose-West herd collected as part of the WHM program between 2004-2014.

Methods

Local subsistence hunters were trained to collect data using standardized sampling protocols and pre-prepared sampling kits. A kit consisted of pre-labeled bags for samples, filter papers for blood collections, and tags to fill in information regarding the date and location of the kill, and the condition of the harvested animal. Samples collected included the lower jaw, the left metatarsal, fecal sample, the left kidney with fat, a piece of the liver, blood on filter papers and a piece of hide. Samples were collected and processed according to standardized protocols developed by the CircumArctic Rangifer Monitoring and Assessment Network [6, 7]. The condition of the animal was scored by the hunter according to one of four pre defined scores: 1=skinny, 2=not bad, 3=good and 4=very good. The bone marrow index (percentage marrow fat= (bone marrow dry/ bone marrow wet)*100) was calculated after breaking the metatarsus bone, extracting the marrow and weighing the wet and dry marrow as described in the CARMA protocols [6]. The kidney fat index was evaluated using a standardized technique to provide a ratio of the weight of the kidney fat to the weight of the kidney * 100; the kidney fat index was reported as a percentage and can be >100%. Serology from filter paper, examination of abnormalities,

quantifying *Besnoitia* lesions on the metatarsals, and body size measurements were also done, and will be reported elsewhere.

Results/Discussion

In total sample kits from 112 individual Bluenose-West caribou were collected between 2004-2014 (Table 1), of these 109 were assumed to be adults and only two were of unknown sex (Table1). Sample sizes and measures of body condition are summarised in the tables and graphs below. Samples were only included in the graphs if they grouped with other observations. Sample sizes for males were higher than for females, due to hunter preference in autumn and recommendations from the government to target males. Pregnancy data was only available from 2005 for 12 adult females. All were recorded as pregnant. Twenty-eight different hunters contributed to sample collection (Table 2), more than were involved in the Bluenose-East collections. This could partly be because this program participated in two community hunts to collect samples, leading to involvement of many community members. Most of the samples were collected during the early years of the program (2005-2008), with few samples being collected since due to shifts in research focus.

In total 107 samples with known sex, year and month of collection from assumed adult Bluenose-West caribou were collected. Of these, 44 had records for backfat, 57 for kidney fat index, 51 for bone marrow fat index and 25 had records of hunter scores of body condition. Collection of Bluenose-West samples was very successful in the early years and has contributed to establishing baselines and development of new tools [10, 19]. However, due to the shift from targeting females to males and a lack of samples in the latter years comparing body condition between years and detecting trends is not possible. We recommend that communities hunting Bluenose-West caribou be consulted as to whether they would like to continue with the program, and if so an agreement as to when sampling should occur, and which sex to sample should be reached in order to improve quality of data over time. Also, if there are other programs collecting Bluenose-West health data, the results presented here should be shared consolidated with these programs, in order to form the most comprehensive picture possible.

References

1. Brook, R.K., et al., *Fostering Community-Based Wildlife Health Monitoring and Research in the Canadian North.* Ecohealth, 2009. **6**(2): p. 266-278.
2. Kutz, S., et al., *Standardized monitoring of Rangifer health during International Polar Year.* Rangifer, 2013. **33**(Sp. Iss. 21): p. 91-114.
3. CARMA. *Rangifer Health and Body Condition Monitoroing Protocols Level 1 and 2*. 2008 [cited 2014 31/3/2014]; Available from: http://www.caff.is/resources/field-protocols.
4. Curry, P.S., et al., *Blood Collected on Filter Paper for Wildlife Serology: Evaluating Storage and Temperature Challenges of Field Collections.* J Wildl Dis, 2014.
5. Wu, J.P., et al., *Linear enamel hypoplasia in caribou (Rangifer tarandus groenlandicus): A potential tool to assess population health.* Wildlife Society Bulletin, 2012. **36**(3): p. 554-560.

Table 1. Number of WHM samples collected from the Bluenose-West caribou herd. Yearlings and calves were not included in the analysis, unknown age animals were assumed to be sub-adults or older and were included in the analysis. Animals with unknown sex were not included.

Year/Month	Females	Males	Unknown sex	Total
2005	39	5		44
1	2	1		3
2	6			6
3	31	3		34
5		1		1
2006		1		1
5		1		1
2007	1	18		19
9	1	18		19
2008		29		29
9		29		29
2013	2	2	2	6
11			2	2
12	2	2		4
2014		10		10
2		4		4
3		6		6
Total	**42**	**65**	**2**	**109**

Table 2. Number of samples collected by individual harvesters from adult Bluenose-West caribou. Yearlings and calves were not included in the analysis, unknown age animals were assumed to be sub-adults or older and were included in the analysis. Animals with unknown sex were not included.

Harvester ID	No. Samples collected
0	1
2	1
3	3
5	1
6	1
8	1
12	3
13	1
15	7
20	4
27	2
29	4
32	1
35	10
36	7
37	2
39	10
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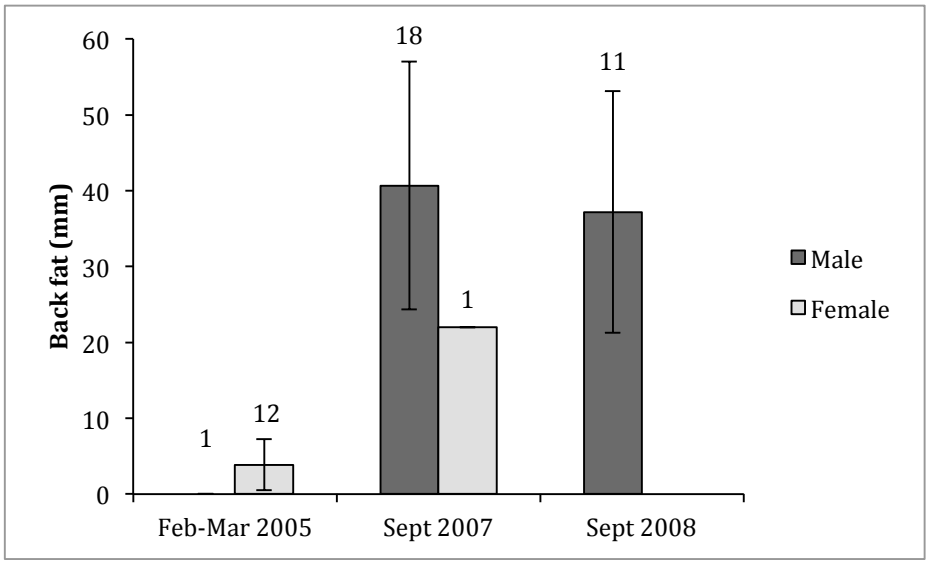
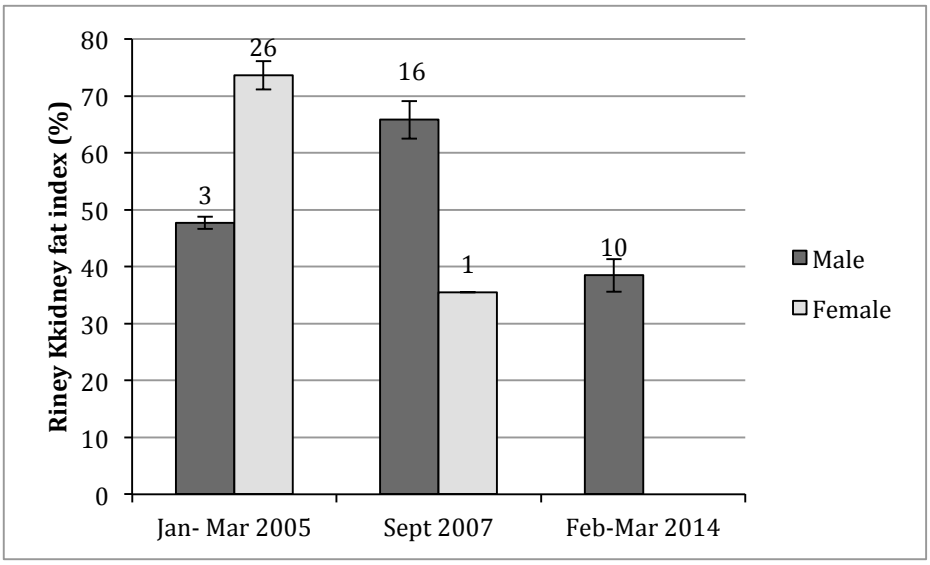
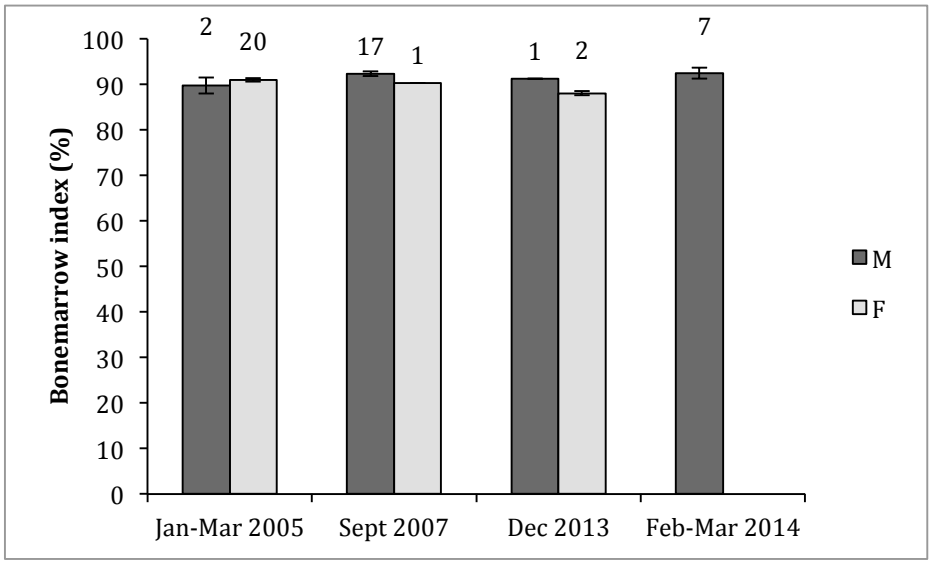
45	1
54	2
56	35
61	1
63	2
65	2
67	1
69	1
70	1
72	2



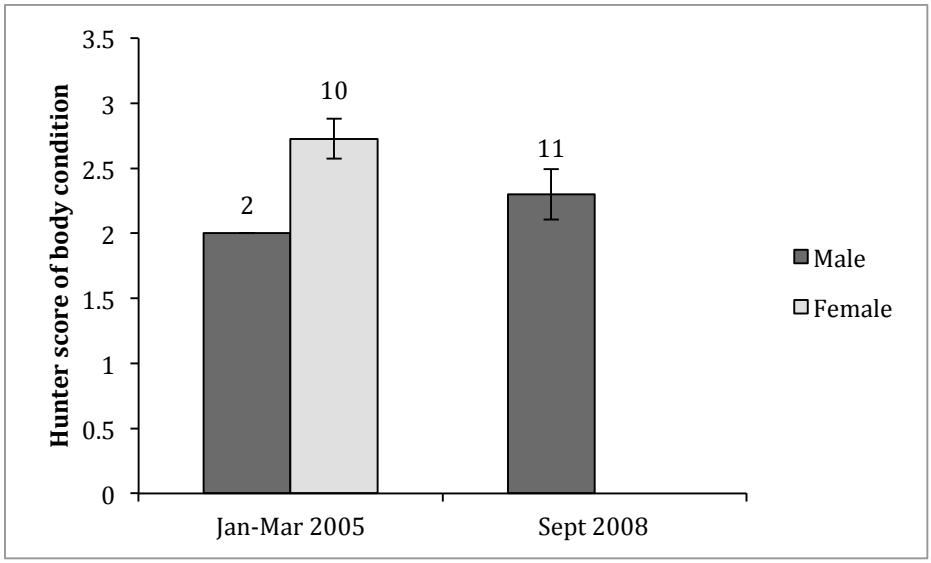
Graph 1. **Back fat** (mm) (mean where sample sizes are >1) of adult male (dark bars) and female (light bars) Bluenose-West caribou as measured by harvesters, with standard error of the mean where appropriate. Sample sizes are indicated above the bars. Yearlings and calves were not included in the analysis, unknown age animals were assumed to be sub-adults or older and were included in the analysis. Animals with unknown sex were not included.



Graph 2. Riney kidney fat index (%) (mean where sample sizes are >1) for adult male (dark bars) and female (light bars) Bluenose-West caribou, with standard error of the mean where appropriate. Sample sizes are indicated above the bars. Yearlings and calves were not included in the analysis, unknown age animals were assumed to be sub-adults or older and were included in the analysis. Animals with unknown sex were not included.



Graph 3. Bone marrow fat index (%) (mean where sample sizes are >1), for adult male (dark bars) and female (light bars) Bluenose-West caribou with standard error of the mean where appropriate. Sample sizes are indicated above the bars. Yearlings and calves were not included in the analysis, unknown age animals were assumed to be sub-adults or older and were included in the analysis. Animals with unknown sex were not included.



Graph 3. Hunter body condition score (mean where sample sizes are >1), for adult male (dark bars) and female (light bars) Bluenose-West caribou with standard error of the mean where appropriate. Hunter score refers to four scores of caribou body condition where each animal is assigned a score by the harvester depending on body condition, the scores are as following; 1= skinny, 2= not bad, 3= good and 4= very good. Sample sizes are indicated above the bars. Yearlings and calves were not included in the analysis, unknown age animals were assumed to be sub-adults or older and were included in the analysis. Animals with unknown sex were

Appendix 2.3 Mountain woodland caribou body condition

Overview of mountain woodland caribou body condition from animals harvested as part of the Wildlife Health Monitoring Program in the Sahtu Settlement Area

Anja Carlsson[1], Susan Kutz[1], Richard Popko[2], Alasdair Veitch[2], Stephanie Behrens[2], SRRC[3], SRRB[4]

[1]University of Calgary, Calgary, AB, [2]Environment and Natural Resources, Government of the Northwest Territories, NT, [3]Sahtu Renewable Resource Councils, NT, [4] Sahtu Renewable Resources Board, NT

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Methods

Local subsistence hunters were trained to collect data using standardized sampling protocols and pre-prepared sampling kits. A kit consisted of pre-labelled bags for samples, filter papers for blood collections, and tags to fill in information regarding the date and location of the kill, and the condition of the harvested animal. Samples collected included the lower jaw, the left metatarsal, fecal sample, the left kidney with fat, a piece of the liver, blood on filter papers and a piece of hide. Samples were collected and processed according to standardized protocols developed by the CircumArctic Rangifer Monitoring and Assessment Network [6, 7]. The condition of the animal was scored by the hunter according to one of four pre defined scores: 1=skinny, 2=not bad, 3=good and 4=very good. The bone marrow index (percentage marrow fat= (bone marrow dry/ bone marrow wet)*100) was calculated after breaking the metatarsus bone, extracting the marrow and weighing the wet and dry marrow as described in the CARMA protocols [6]. The kidney fat index was evaluated using a standardized technique to provide a ratio of the weight of the kidney fat to the weight of the kidney * 100; the kidney fat index was reported as a percentage and can be >100%. Serology from filter paper, examination of abnormalities,

quantifying *Besnoitia* lesions on the metatarsals, and body size measurements were also done, and will be reported elsewhere.

Results/Discussion

Targeted sample collection for mountain woodland caribou only started in 2013, and sample kits from 39 individual mountain woodland caribou (assumed adults) have already been collected (Table 1). All these samples have complete data for collection date and gender. Only nine samples from female caribou were collected, and of these only four indicated pregnancy status, with two recorded as pregnant. Fifteen different hunters collected samples.

Measures of body condition are summarised in the graphs below. The number of individuals with measurements of backfat was 18, of kidney fat index 33, of bone marrow index 25 and 30 recorded hunter scores of body condition. Almost half of the samples (18/39) were collected during the Tulita community harvest at caribou flats in September 2013. A researcher from the University of Calgary was invited to join this harvest, demonstrating that active involvement by researchers can boost samples sizes. Although kits were not complete for all the sampled caribou, the 39 samples collected in the past few years contribute significantly to forming a baseline of health indices for mountain woodland caribou. We recommend that this program continue to sample mountain woodland caribou since more data is needed to form a more robust baseline. Sampling consistently during the same season across the years is important if meaningful comparisons of body condition are to be made. Consulting hunters and communities which periods are preferred for sampling (community hunts in autum, or during hunts in late witner) and focus efforts there could be a good way forward. In addition to standardizing sampling and kits across years, establishing a well-structured system to handle incoming kits and assist hunters in documenting important information (such as date, sex, pregnancy status, score of body condition) can further improve sample quality. As outlined in other programs [26, 79], moving the "base of operations" to the Sahtu to make interactions between local stake-holders and program leads easier could also contribute to the successful collaboration and continued monitoring.

References

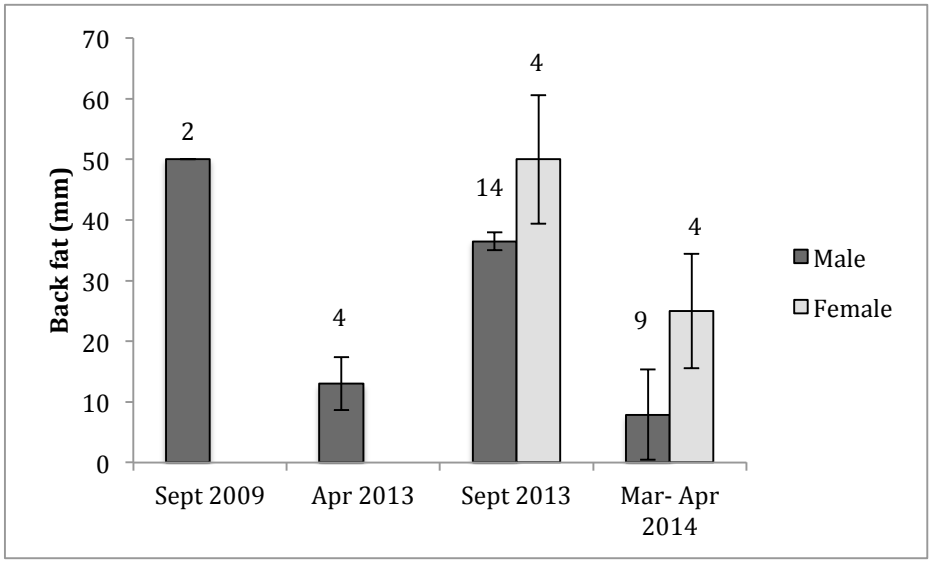
1. Brook, R.K., et al., *Fostering Community-Based Wildlife Health Monitoring and Research in the Canadian North.* Ecohealth, 2009. **6**(2): p. 266-278.
2. Kutz, S., et al., *Standardized monitoring of Rangifer health during International Polar Year.* Rangifer, 2013. **33**(Sp. Iss. 21): p. 91-114.
3. CARMA. *Rangifer Health and Body Condition Monitoroing Protocols Level 1 and 2*. 2008 [cited 2014 31/3/2014]; Available from: http://www.caff.is/resources/field-protocols.
4. Klein, D.R., et al., *Contrasts in use and perceptions of biological data for caribou management.* Wildlife Society Bulletin, 1999. **27**(2): p. 488-498.
5. Kofinas, G., et al., *Towards a protocol for community monitoring of cairbou body condition.* Rangifer, 2003(14): p. 43-22.

Table 1. Number of WHM samples collected from adult mountain woodland caribou. . Yearlings and calves were not included in the analysis, unknown age animals were assumed to be sub-adults or older and were included in the analysis. Animals with unknown sex were not included.

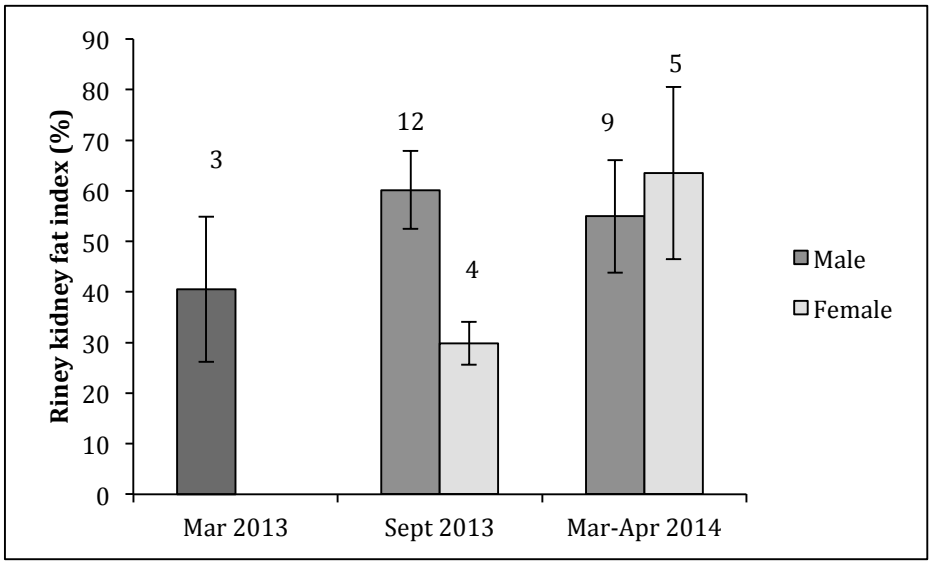
Year/Month	Females	Males	Total
2009		2	2
9		2	2
2013	4	18	22
4		4	4
9	4	14	18
2014	5	10	15
3	5	7	12
4		3	3
Total	9	30	39

Table 2. Number of samples collected by individual harvesters adult mountain woodland caribou. . Yearlings and calves were not included in the analysis, unknown age animals were assumed to be sub-adults or older and were included in the analysis. Animals with unknown sex were not included.

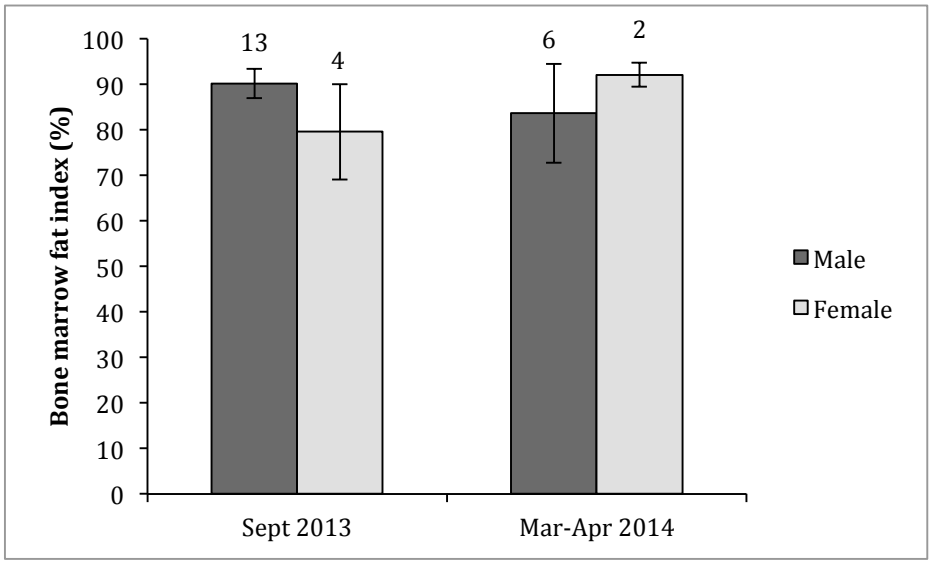
Harvester ID	No. Samples collected
10	1
21	4
24	1
25	1
26	2
29	1
35	7
44	3
50	6
55	1
57	5
59	1
60	3
68	1
74	2



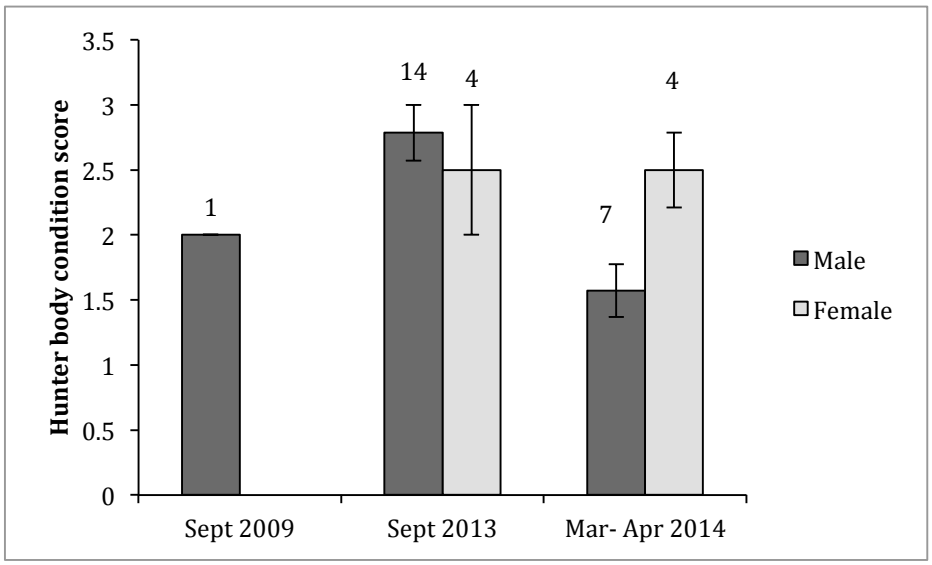
Graph 1. **Mean back fat** (mm) of adult male (dark bars) and female (light bars) mountain woodland caribou as measured by harvesters, with standard error of the mean. Sample sizes are indicated above the bars. Yearlings and calves were not included in the analysis, unknown age animals were assumed to be sub-adults or older and were included in the analysis. Animals with unknown sex were not included.



Graph 2. Mean Riney kidney fat index (%) for adult male (dark bars) and female (light bars) mountain woodland caribou with standard error of the mean. Sample sizes are indicated above the bars. Yearlings and calves were not included in the analysis, unknown age animals were assumed to be sub-adults or older and were included in the analysis. Animals with unknown sex were not included.



Graph 3. Mean bone marrow fat index (%) for adult male (dark bars) and female (light bars) mountain woodland caribou, with standard error of the mean. Sample sizes are indicated above the bars. Yearlings and calves were not included in the analysis, unknown age animals were assumed to be sub-adults or older and were included in the analysis. Animals with unknown sex were not included.



Graph 3. Hunter body condition score (mean where sample sizes are >1) for adult male (dark bars) and female (light bars) mountain woodland caribou, with standard error of the mean where appropriate. Hunter score refers to four scores of caribou body condition where each animal is assigned a score by the harvester depending on body condition, the scores are as following; 1= skinny, 2= not bad, 3= good and 4= very good. Sample sizes are indicated above the bars. Yearlings and calves were not included in the analysis, unknown age animals were assumed to be sub-adults or older and were included in the analysis. Animals with unknown sex were not included.

Appendix 2.4 Moose body condition

Overview of moose body condition from animals harvested from 2004-2014 as part of the Wildlife Health Monitoring Program in the Sahtu Settlement Area.

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Background

The Sahtu Wildlife Health Monitoring (WHM) Program began in 2003 in response to community concerns about wildlife health under a regime of rapid environmental change (climate and industrial development) in the Sahtu Settlement Region, Northwest Territories. Community members indicated that they had concerns about the health and sustainability of wildlife, how wildlife health and disease may affect them, and that the next generation (youth) may not be adequately prepared to take on the emerging environmental issues in the region. Together with Environmental and Natural Resources, Government of the Northwest Territories, the Sahtu Renewable Resources Board and the Sahtu Renewable Resources Councils, the University of Saskatchewan (2003-2005) and University of Calgary (2005-present) has run the WHM program since 2004 to address these concerns [3]. The purpose of this report is to summarise the body condition data from moose collected as part of the WHM program between 2004-2014.

Methods

Local subsistence hunters were trained to collect data using standardized sampling protocols and pre-prepared sampling kits. A kit consisted of pre-labelled bags for samples, filter papers for blood collections, and tags to fill in information regarding the date and location of the kill, and the condition of the harvested animal. Samples collected included the lower jaw, the left metatarsal, fecal sample, the left kidney with fat, a piece of the liver, blood on filter papers and a piece of hide. Samples were collected and processed according to standardized protocols developed by the CircumArctic Rangifer Monitoring and Assessment Network [6, 7]. The condition of the animal was scored by the hunter according to one of four pre defined scores: 1=skinny, 2=not bad, 3=good and 4=very good. The bone marrow index (percentage marrow fat= (bone marrow dry/ bone marrow wet)*100) was calculated after breaking the metatarsus bone, extracting the marrow and weighing the wet and dry marrow as described in the CARMA protocols [6]. The kidney fat index was evaluated using a standardized technique to provide a ratio of the weight of the kidney fat to the weight of the kidney * 100; the kidney fat index was reported as a percentage and can be >100%. Serology from filter paper, examination of abnormalities, quantifying *Besnoitia* lesions on the metatarsals, and body size measurements were also done, and will be reported elsewhere.

Results/Discussion

In total, sample kits from 85 individual moose were collected between 2004-2014. Seventy-six of these were assumed to come from adults, but more than half (45/76) did not have age, or age group specified and 47% (36/76) were of unknown sex (Table 1). Although genetic and cementum (tooth) analysis can be used to determine sex and age, these methods are costly and improved recording and organisation of samples during receipt of kits is advised. Body condition data are summarised in the tables and graphs below. Although at first glance there appears to be a lot of data, most of the bars represent single observations (Graph 1-3). In fact, overall sample sizes were very small and samples have been collected throughout the year. This makes meaningful comparisons and groupings difficult and inference about trends impossible. The total number of samples available for moose with known sex, year and month of collection that were assumed to be adults was 26 for the kidney fat index, 22 for backfat, 17 for the bone marrow fat index and 29 for hunter score of body condition. Sample collections were done by at least 27 different hunters, with the persons responsible for collecting 22 of the moose samples not being identified (Table 2). A diverse range of people collecting samples can be a good thing and demonstrates community engagement, however, the spread can also lead to increased variability in the data.

The lack of robust data for moose is partly due to the fact that moose have not been targeted as part of research studies, with the exception of the winter tick study in 2010-2011 where hide collection was the priority. Measuring and tracking wildlife health indices can provide important complimentary data to population censuses [36] but bigger sample sizes and more consistent collections are needed for this program to generate valuable data on moose body condition. However, the samples collected so far, and observation made by harvester, are valuable and have contributed to new knowledge and important baseline data of diseases present in moose, especially in regards to the detection of the winter tick in the Sahtu [9]. These data will be summarised and reported elsewhere. Continued monitoring of the winter tick and other diseases is important, especially in light of the rapid climate change that is occurring in the north [83]. Research into moose health remains a priority for the communities in the Sahtu and we, therefore, recommend that efforts are made to collect samples during a specified period of time during the year (eg late winter Jan-April) in order to decrease the variability. Consulting with the communities to understand their research priorities could also help focus monitoring efforts and decrease the number of samples collected, thereby increasing ease of collection and perhaps boosting samples sizes.

Table 1. Number of WHM samples collected from adult moose. Yearlings and calves were not included in the analysis, unknown age animals were assumed to be sub-adults or older and were included in the analysis. Animals with unknown sex were not included.

Year/Month	Females	Males	Unknown sex	Total
2005			2	2
4			1	1
NA			1	1
2006	5	3	1	9
4	2	1		3
5	1	1		2
6	2			2
12		1		1
Unknown month			1	1
2007	1	2	1	4
1	1		1	2
7		1		1
8		1		1
2009	1	2		3
9	1	2		3
2010	2	5	6	13
10	1	1	1	3
11		1		1
12			1	1
Unknown month	1	3	4	8
2011	7	3	8	18
1		1		1
2	3	1	6	10
9	1		1	2
11	2			2
12	1			1
NA		1	1	2
2012	1		6	7
1			1	1
2			1	1
3	1			1
Unknown month			4	4
2013	3	5	7	15
2	3	2	4	9
3		2	2	4
9		1		1
12			1	1
2014			1	1
2			1	1
Unknown date			4	4
Total	**20**	**20**	**36**	**76**

Table 2. Number of samples collected by individual harvesters from adult moose.. Yearlings and calves were not included in the analysis, unknown age animals were assumed to be sub-adults or older and were included in the analysis. Animals with unknown sex were not included.

Harvester ID	No. Samples collected
Unknown	22
1	1
3	1
4	1
7	2
14	1
18	1
22	1
23	1
29	2
33	2
34	1
35	2
37	2
40	1
43	1
44	1
46	1
48	1
51	2
57	1
58	1
62	2
64	4
66	6
73	14
12,29	1

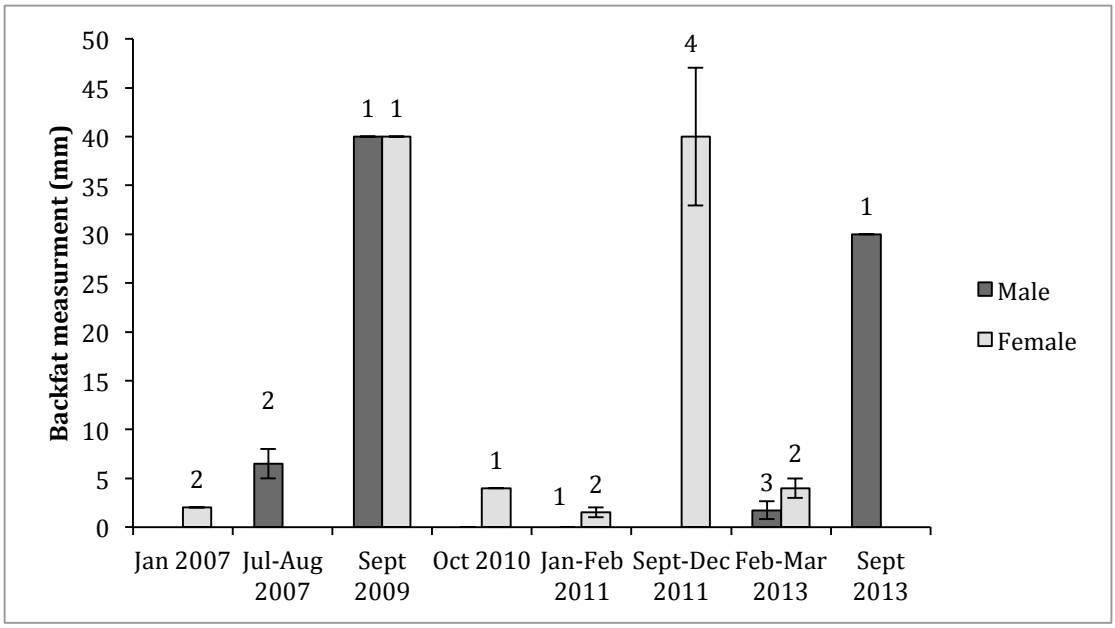

Figure 1. B**ack fat** measurements (mm) (mean where sample sizes are >1) of adult male (dark bars) and female (light bars) moose as measured by harvesters, with standard error of the mean where appropriate. Sample sizes are indicated above the bars. Yearlings and calves were not included in the analysis, unknown age animals were assumed to be sub-adults or older and were included in the analysis. Animals with unknown sex were not included.

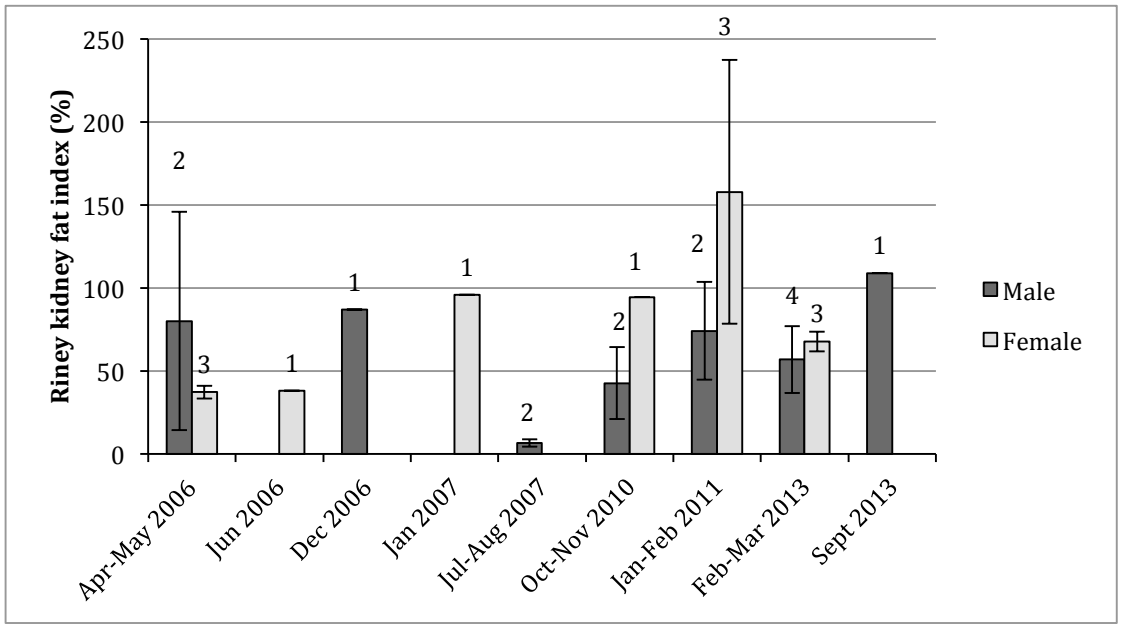

Figure 2. **Riney kidney fat index** measurements (%) (mean where sample sizes are >1) of adult male (dark bars) and female (light bars) moose as measured by harvesters, with standard error of the mean where appropriate Sample sizes are indicated above the bars. Yearlings and calves were not included in the analysis, unknown age animals were assumed to be sub-adults or older and were included in the analysis. Animals with unknown sex were not included.

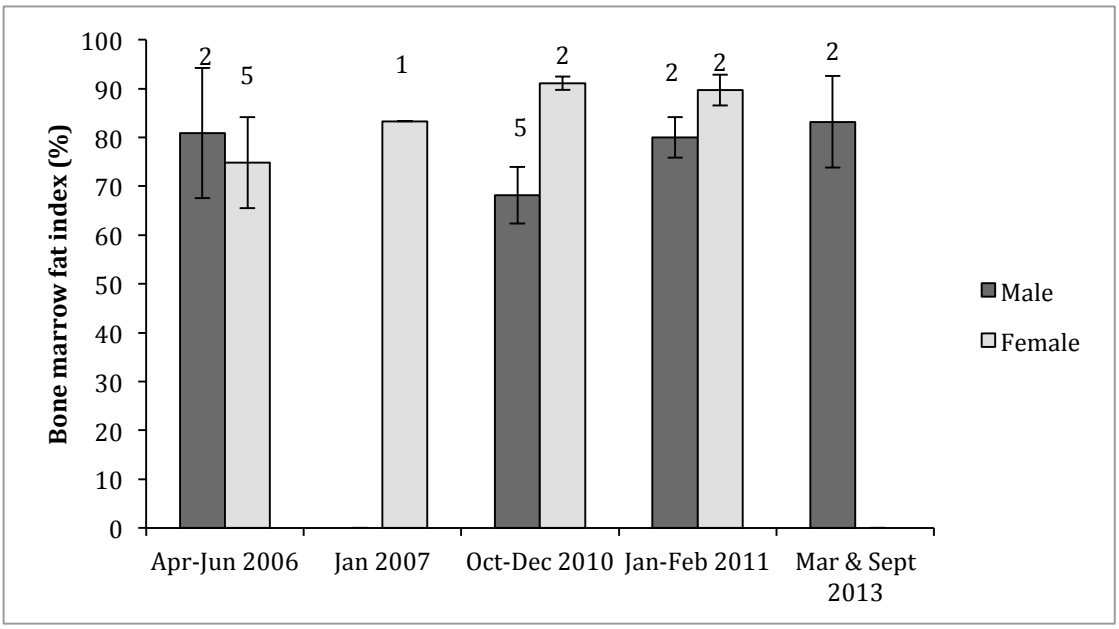

Figure 3. Bone marrow fat index (%) for adult male (dark bars) and female (light bars) moose (mean where sample sizes are >1), with standard error of the mean (where appropriate). Animals with unknown age were assumed to be adults Sample sizes are indicated above the bars. Yearlings and calves were not included in the analysis, unknown age animals were assumed to be sub-adults or older and were included in the analysis. Animals with unknown sex were not included.

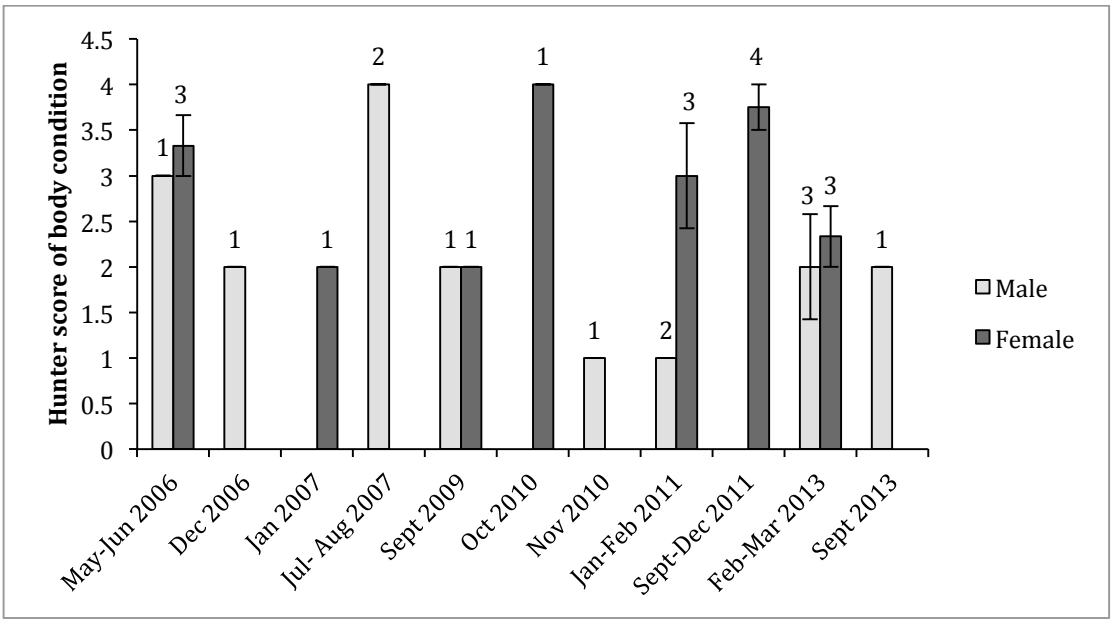

Figure 4. Hunter body condition score for adult male (dark bars) and female (light bars) moose (mean where sample sizes are >1), with standard error of the mean (where appropriate). Hunter score refers to four scores of caribou body condition where each animal is assigned a score by the harvester depending on body condition, the scores are as following; 1= skinny, 2= not bad, 3= good and 4= very good. Sample sizes are indicated above the bars. Yearlings and calves were not included in the analysis, unknown age animals were assumed to be sub-adults or older and were included in the analysis. Animals with unknown sex were not included.

References

1. Brook, R.K., et al., *Fostering Community-Based Wildlife Health Monitoring and Research in the Canadian North.* Ecohealth, 2009. **6**(2): p. 266-278.
2. Kutz, S., et al., *Standardized monitoring of Rangifer health during International Polar Year.* Rangifer, 2013. **33**(Sp. Iss. 21): p. 91-114.
3. CARMA. *Rangifer Health and Body Condition Monitoroing Protocols Level 1 and 2*. 2008 [cited 2014 31/3/2014]; Available from: http://www.caff.is/resources/field-protocols.
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5. Kashivakura, C.K., *Detecting Dermacentor albipictus (Packard, 1869), the winter tick, at the northern extent of its distribution range: hunter based monitoring and serological assay development*, in *Ecosystem and Public health*. 2013, University of Calgary: Calgary, Alberta.
6. Kutz, S.J., et al., *A walk on the tundra: Host–parasite interactions in an extreme environment.* International Journal for Parasitology: Parasites and Wildlife, 2014. **3**(2): p. 198-208.

Appendix 3 Body size measurements

Appendix 3.1 Jaw.

Mean length, with standard errors, of the anterior jawbone (mm) (JawA) and the whole jawbone (mm) (JawT) for caribou herds Bluenose East (BNE) and Bluenose West (BNW) by sex and year. Numbers in brackets refer to sample size. Yearlings and calves were not included in the analysis, unknown age animals were assumed to be sub-adults or older and were included. Animals with unknown sex were not included.

Herd	**BNE**				**BNW**	
Sex	Female		Male		Male	
Body condition index	JawA	JawT	JawA	JawT	JawA	JawT
Year						
2006			155.3 ± 2.4 (14)	287.1 ± 2.5 (14)		
2007						
2008	148.3 ± 1.3 (3)	277.7 ± 6.7 (3)			167.7 ± 1.5 (25)	300.3 ± 2.4 (25)
2011	138.7 ± 1.5 (12)		118.0 (1)			
2013	150.8 (1)	268.0 (1)	147.6 ± 3.2 (4)	267.8 ± 3.4 (4)		

Appendix 3.2 Metatarsus.

Mean length (LegL) and circumference (LegC) (mm), with standard errors, of the left metatarsal for caribou herds Bluenose East (BNE) and Bluenose West (BNW) by sex and years. Numbers in brackets refer to sample size. Yearlings and calves were not included in the analysis, unknown age animals were assumed to be sub-adults or older and were included in the analysis. Animals with unknown sex were not included.

Herd	**BNE**				**BNW**			
Sex	Female		Male		Female		Male	
Body condition index	LegL	LegC	LegL	LegC	LegL	LegC	LegL	LegC
Year								
2006			284.5 ± 0.5 (2)	100 ± 1.0 (2)			320 ± 3.9 (16)	108 ± 1.6 (16)
2007			301.4 ± 11.3 (10)	115.4 ± 3.0 (10)	310.0 (1)	95 (1)	310.0 ± 5.5 (10)	111.5 ± 4.4 (10)
2009	268.2 ± 1.7 (27)	84.2 ± 1.0 (15)						
2011	267.7 ± 4.7 (17)	84.6 ± 8.9 (17)	263.0 (1)	71 (1)				
2013	274 (1)	86 (1)	292 (1)	87 (1)	266.5 ± 26.5 (2)	87.0 ± 13.0 (2)	255.5 ±16.5 (2)	80.5 ± 7.0 (2)

Appendix 4 Age data

Mean age (years), with standard errors, for moose and caribou herds Bluenose East (BNE) and Bluenose West (BNW) by sex and year. Age was determined by cementum age analysis. Numbers in brackets refer to sample size. Animals with unknown sex were not included.

Herd/Species	**BNE**		**BNW**		**Moose**	
Sex	Female	Male	Female	Male	Female	Male
Year						
2004	5.1 ± 0.61 (16)					
2005	6.7 ± 0.5 (20)		5.9 ± 0.8 (12)	4.0 (1)		
2006		4.6 ± 0.5 (15)		3.0 (1)	6.5 ± 1.5 (2)	7.0 (1)
2007		5.0 ± 0.6 (9)	6.5 ± 5.5 (2)	6.1 ± 0.6 (18)		3.0 (1)
2008	6.8 ± 1.1 (3)			5.2 ± 0.4 (27)		
2009	6.2 ± 0.77 (13)				6 (1)	4.0 ± 0.0 (2)
2010					2.3 ± 1.2 (3)	4.0 ± 1.5 (4)
2011					3.5 ± 1.4 (6)	5.0 (1)
Overall mean	6.1 ± 0.3 (54)	4.8 ± 0.4 (24)	6.0 ±0.9 (14)	5.5 ± 0.3 (47)	3.9 ± 0.6 (12)	4.3 ± 0.7 (9)

Appendix 5 Prevalence of Pathogens

Appendix 5.1 Neospora caninum

Prevalence (%) of antibodies reacting to *N. caninum,* with Clopper Pearson Exact 95% confidence intervals (CI), by year and sex for caribou herds Bluenose East (BNE) and Bluenose West (BNW), woodland caribou (WC) and moose as detected by ELISA. The test is assessed as positive when the percentage ELISA inhibition is > 30. Numbers in brackets refer to number tested positive/sample size. Animals with unknown sex were not included.

Herd/Species		BNE			BNW			WC			Moose		
Sex		Female	Male	Overall	Female	Male	Overall	Female	Male	Overall	Female	Male	Overall
Year	**Data**												
2004	Prevalence	0		0									
	CI (%)	0-37		0-37									
	(N Pos/N Tot)	(0/8)		(0/8)									
2005	Prevalence	5.9		5.9	0	0	0						
	CI (%)	0-29		0-29	0-31	0-71	0-25						
	(N Pos/N Tot)	(1/17)		(1/17)	(0/10)	(0/3)	(0/13)						
2006	Prevalence		0	0							0	0	0
	CI (%)		0-22	0-22							0-60	0-98	0-52
	(N Pos/N Tot)		0/15	0/15							(0/4)	(0/1)	(0/5)
2007	Prevalence		0	0	0	0	0				0		0
	CI (%)		0-31	0-31	0-84	0-19	0-17				0-98		0-98
	(N Pos/N Tot)		(0/10)	(0/10)	(0/2)	(0/18)	(0/20)				(0/1)		(0/1)
2008	Prevalence	0		0		0	0						
	CI (%)	0-52		0-52		0-23	0-23						
	(N Pos/N Tot)	(0/5)		(0/5)		(0/14)	(0/14)						
2009	Prevalence	0		0									
	CI (%)	0-23		0-23									
	(N Pos/N Tot)	0/14		0/14									
2010	Prevalence										0	33.3	22.2
	CI (%)										0-71	4-78	3-60
	(N Pos/N Tot)										(0/3)	(2/6)	(2/9)
2011	Prevalence	5.9	0	5.6							14.3	100	33.3
	CI (%)	0-29	0-98	0-27							0-58	16-100	7-70
	(N Pos/N Tot)	(1/17)	(0/1)	(1/18)							(1/7)	(2/2)	(3/9)
2013	Prevalence	0	0	0				20	11.1	13.64	0	0	0
	CI (%)	0-71	0-52	0-37				5-72	10-35	3-34	0-71	0-60	0-46
	(N Pos/N Tot)	(0/3)	(0/5)	(0/8)				(1/5)	(2/18)	(3/22)	(0/3)	(0/4)	(0/6)
All Years	Prevalence	3.3	0	**2.1**	0	0	**0**	20	11.1	**13.0**	5.5	30.8	**16.1**
	CI (%)	0-11	0-11	**0-7**	0-26	0-10	**0-8**	5-75	10-35	**3-34**	0-27	9-61	**5-34**
	(N Pos/N Tot)	(2/64)	(0/31)	**(2/95)**	(0/12)	(0/35)	**(0/47**	(1/5)	(2/18)	**(3/23)**	(1/18)	(4/13)	**(5/31)**

Appendix 5.2 Alphaherpesvirus

Prevalence (%) of samples with antibodies reacting to bovine herpes virus 1 (BHV-1), Clopper Pearson Exact 95% confidence intervals (CI), by year and sex for caribou herds Bluenose East (BNE) and Bluenose West (BNW), woodland caribou (WC) and moose as detected by ELISA. The test is assessed as positive when the ELISA unit ≥4. Numbers in brackets refer to number tested positive/sample size. Animals with unknown sex were not included. All samples were from blood on filter paper except for 2007 BNW samples which were serum.

Herd/Species		**BNE**			**BNW**			**WC**			**Moose**		
Sex		Female	Male	Overall	Female	Male	Overall	Female	Male	Overall	Female	Male	Overall
Year	**Data**												
2004	Prevalence	0		0									
	CI (%)	0-98		0-98									
	(N Pos/N Tot)	(0/1)		(0/1)									
2005	Prevalence	17.6		17.7	20	0	15.4						
	CI (%)	4-43		4-43	3-56	0-71	2-45						
	(N Pos/N Tot)	(3/17)		(3/17)	(2/10)	(0/3)	(2/13)						
2007	Prevalence		20	20	100	88.9	90				50		50
	CI (%)		3-56	3-56	16-100	65-99	68-99				10-99		10-99
	(N Pos/N Tot)		(2/10)	(2/10)	(2/2)	(16/18)	1(8/20)				(1/2)		(1/2)
2008	Prevalence					61.5							
	CI (%)					32-86							
	(N Pos/N Tot)					(8/13)							
2011	Prevalence	17.6	0	16.7							0		0
	CI (%)	4+43	0-98	4-41							0-98		0-98
	(N Pos/N Tot)	(3/17)	(0/1)	(3/18							(0/1)		(0/1)
2013	Prevalence	0	40	40				40	47.1	45.5	0	50	16.7
	CI (%)	0-71	5-85	24-91				5-85	23-72	24-68	0-60	10-90	0-64
	(N Pos/N Tot)	(0/3)	(2/5)	(2/8)				(2/5)	(8/17)	(22)	(0/4)	(1/2)	(1/6)
All Years	Prevalence	15.8	25	**18.5**	33.3	70.6	**60.8**	40	47.1	**45.5**	14.3	50	**22.2**
	CI (%)	6-31	7-52	**9-31**	10-65	53-85	**60.8-90**	5-85	23-72	**24-68**	0-58	10-99	**3-60**
	(N Pos/N Tot)	(6/38)	(4/16)	**(10/54)**	(4/12)	(24/34)	**(28/36)**	(2/5)	(8/17)	**(10/22)**	(1/7)	(1/2)	**(2/9)**

Appendix 5.3 Para-influenza virus

Prevalence (%) of samples with antibodies reacting to Para-influenza virus (PI3), with Clopper Pearson Exact 95% confidence intervals (CI), by year and sex for caribou herds Bluenose East (BNE) and Bluenose West (BNW), woodland caribou (WC) and moose as detected by ELISA. The test is assessed as positive when the ELISA unit >=14. Numbers in brackets refer to number tested positive/sample size. Animals with unknown sex were not included. All samples were from blood on filterpaper except for 2007 BNW samples which were serum.

Herd/Species		**BNE**			**BNW**			**WC**			**Moose**		
Sex		Female	Male	Overall	Female	Male	Overall	Female	Male	Overall	Female	Male	Overall
Year													
2004	Prevalence	0		0									
	CI (%)	0-98		0-98									
	(N Pos/N Tot)	(0/1)		(0/1)									
2005	Prevalence	0		0	0	0	0						
	CI (%)	0-20		0-20	0-31	0-71	0-25						
	(N Pos/N Tot)	(0/17)		(0/17)	(0/10)	(0/3)	(0/13)						
2007	Prevalence		0	0	0	20	17.7				0		0
	CI (%)		0-31	0-31	0-84	4-48	4-43				0-84		0-84
	(N Pos/N Tot)		(0/10)	(0/10)	(0/2)	(3/15)	(3/17)				(0/2)		(0/2)
2008	Prevalence					7.7							
	CI (%)					0-36							
	(N Pos/N Tot)					(1/13)							
2011	Prevalence	0	0	0							0		0
	CI (%)	0-20	0-98	0-19							0-98		0-98
	(N Pos/N Tot)	(0/17)	(0/1)	(0/18)							(0/1)		(0/1)
2013	Prevalence	0	0	0				0	0	0	0	0	0
	CI (%)	0-84	0-98	0-15				0-52	0-20	0-20	0-60	0-84	0-46
	(N Pos/N Tot)	(0/2)	(0/1)	(0/23)				(0/5)	(0/17)	(0/22)	(0/4)	(0/2)	(0/6)
All years	Prevalence	0	0	**0**	0	11.8	**8.7**	0	0	**0**	0	0	**0**
	CI (%)	0-9	0-21	**0-7**	0-26	3-27	**2-21**	0-52	0-20	**0-20**	0-41	0-84	**0-30**
	(N Pos/N Tot)	(0/38)	(0/16)	**(0/54)**	(0/12)	(4/34)	**(4/46)**	(0/5)	(0/17)	**(0/22)**	(0/7)	(0/2)	**(9)**

Appendix 5.4 **Pestivirus**

Prevalence (%) of samples testing positive for antibodies reacting to bovine viral diarrhoea virus (BVDV), with Clopper Pearson Exact 95% confidence intervals (CI), by year and sex for caribou herds Bluenose East (BNE) and Bluenose West (BNW), woodland caribou (WC) and moose as detected by a viral neutralization assay. The detection limit of the assay is a titer of 20. Numbers in brackets refer to number tested positive/sample size. Animals with unknown sex were not included.

Herd/Species		**BNE**			**BNW**			**WC**			**Moose**		
Sex		Female	Male	Overall	Female	Male	Overall	Female	Male	Overall	Female	Male	Overall
Year	**Year**												
2004	Prevalence	100		100									
	CI (%)	3-100		3-100									
	(N Pos/N Tot)	(1/1)		(1/1)									
2005	Prevalence	100		100	25	0	16.7						
	CI (%)	48-100		48-100	10-81	0-84	0-64						
	(N Pos/N Tot)	(5/5)		(5/5)	(1/4)	(0/2)	(1/6)						
2007	Prevalence		20	20							0		0
	CI (%)		1-72	1-72							0-98		0-98
	(N Pos/N Tot)		(1/5)	(1/5)							(0/1)		(0/1)
2011	Prevalence	66.7		66.7							0		0
	CI (%)	22-96		22-96							0-98		0-98
	(N Pos/N Tot)	(4/6)		(4/6)							(0/1)		(0/1)
2013	Prevalence	100	67.7	75				100	50	57.1	0	0	0
	CI (%)	3-100	9-99	19-99				3-100	12-88	18-90	0-84	0-98	0-71
	(N Pos/N Tot)	(1/1)	(2/3)	(3/4)				(1/1)	(3/6)	(4/7)	(0/2)	(0/1)	(0/3)
All years	Prevalence	84.6	37.5	**66.7**	25	0	**16.7**	100	50	**57.1**	0	0	**0**
	CI (%)	55-98	9-76	**93-85**	10-81	0-84	**0-64**	3-100	12-88	**18-90**	0-60	0-98	**0-52**
	(N Pos/N Tot)	(11/13)	(3/8)	**(14/21)**	(1/4)	(0/2)	**(1/6)**	(1/1)	(3/6)	**(4/7)**	(0/4)	(0/1)	**(0/5)**

Appendix 5.5 Besnoitia tarandi

Prevalence (%) of hides samples positive *B. tarandi,* with standard errors, for caribou herds Bluenose East (BNE) and Bluenose West (BNW) by sex and year, as detected by visual inspection from hide section from the metatarsal. Numbers in brackets refer to sample size. Animals with unknown sex were not included.

Herd/Species	**BNE**			**BNW**		
Sex	Female	Male	Overall	Female	Male	Overall
Year						
2004	62.5 ±12.5 (10/16)		62.5 ±12.5 (10/16)			
2005	20 ±9.2 (4/20)		20 ±9.2 (4/20)	50 ±10 (13/26)	25 ±25 (1/4)	46.67 ±9.3 (14/30)
2006		80 ±10.7 (12/15)	80 ±10.7 (12/15)			
2007		100 (1/1)	100 (1/1)	50 ±50 (1/2)	83.3 ±9.0 (15/18)	80 9.2 (16/20)
2008					25 ±13.1 (3/12)	25 13.1 (3/12)
2009	73.3 ±11.8 (11/15)		73.3 ±11.8 (11/15)			
Overall	49.0 ±7.1 (25/51)	81.3 ±10.1 (13/16)	**56.7** ±6.1 **(38/67)**	50 ±9.6 (14/28)	55.9 8.6 (19/34)	**53.2** ±6.4 **(33/62)**

Appendix 5.6 Strongyles.

Prevalence (%) of strongyle eggs, with standard errors, in faeces by year and sex for caribou herds Bluenose East (BNE) and Bluenose West (BNW), woodland caribou (WC) and moose as detected by faecal flotation. Numbers in brackets refer to sample size. Animals with unknown sex were not included. Note that samples from BNW in 2007 and WC in 2013 were collected in autumn and not winter, and, therefore, have higher prevalence.

Herd/Species	**BNE**			**BNW**			**WC**			**Moose**		
Sex	Female	Male	Overall	Female	Male	Overall	Female	Male	Overall	Female	Male	Overall
Year												
2005	0 (0/18)		0 (0/18)	6.7 (1/15)	0 (0/4)	5.3 ±5.3 (1/19)						
2006		57.1 ±13.7 (8/14)	57.1 ±13.7 (8/14)							0 (0/4)	0 (0/2)	0 (0/6)
2007		0 (0/10)	0 (0/10)	100 ±0 (2/2)	82.4 ±9.5 (14/17)	84.2 ±8.6 (16/19)				0 (0/2)		0 (0/2)
2008	0 (0/5)		0 (0/5)									
2013	33.3 ±27.2 (1/3)	80 ±17.8 (4/5)	60 ±17.3 (5/8)				66.7 ±33.3 (2/3)	84.6 ±10.4 (2/13)	81.3 ±10.1 (16)	0 (0/4)	0 (0/2)	0 (0/6)
Overall	3.8 ±3.7 (1/26)	41.4 ±9.1 (12/29)	**20** ±6.3 **(13/55)**	17.7 ±9.5 (3/17)	66.7 ±10.5 (14/21)	**44.7** ±8.2 **(17/38)**	66.7 ±33.3 (2/3)	84.6 ±10.4 (11/13)	**81.3** ±10.1 **(13/16)**	0 (0/10)	0 (0/4)	**0 (0/14)**

Appendix 5.7 Nematodirinae

Prevalence (%) of Nematodirinae eggs in faeces, with standard errors, by year and sex for caribou herds Bluenose East (BNE) and Bluenose West (BNW), woodland caribou (WC) and moose as detected by faecal flotation. Numbers in brackets refer to sample size. Animals with unknown sex were not included.

Herd/Species	**BNE**			**BNW**			**WC**			**Moose**		
Sex	Female	Male	Overall	Female	Male	Overall	Female	Male	Overall	Female	Male	Overall
Year												
2005	0 (0/18)		0 (0/18)	6.7 ±6.7 (1/15)	0 (0/4)	5.3 ±5.3 (1/19)						
2006		0 (0/13)	0 (0/13)							100 ±0 (4/4)	50 ±50 (1/2)	83.33 ±16.7 (5/6)
2007		0 (0/10)	0 (0/10)	0 (0/2)	0 (0/17)	0 (0/19)				50 (1/2)		50 ±50 (1/2)
2008	0 (0/5)		0 (0/5)									
2013	66.7 ±27.2 (2/3)	0 (0/5)	25 ±15.3 (2/8)				0 (0/3)	7.7 ±7.7 (1/13)	6.3 ±6.25 (1/16)	75 ±25 (3/4)	50 ±50 (1/2)	66.7 ±21.1 (4/6)
Overall	7.7 ±5.2 (2/26)	0 (0/28)	**3.6** ±2.6 **(2/54)**	5.9 ±5.9 (1/17)	0 (0/21)	**2.6** ±2.6 **(1/38)**	0 (0/3)	7.7 ±7.7 (1/13)	**6.3** ±6.3 **(1/16)**	80 ±13.3 (8/10)	50 ±28.9 (2/4)	**71.4** ±12.5 **(10/14)**

Appendix 5.8 Eimeria

Prevalence (%) of *Eimeria* oocysts in faeces, with standard errors, by year and sex for caribou herds Bluenose East (BNE) and Bluenose West (BNW), woodland caribou (WC) and moose as detected by faecal flotation. Numbers in brackets refer to sample size. Animals with unknown sex were not included.

Herd/Species	**BNE**			**BNW**			**WC**			**Moose**		
Sex	Female	Male	Overall	Female	Male	Overall	Female	Male	Overall	Female	Male	Overall
Year												
2005	11.1 ±7.6 (2/18)		11.1 ±7.6 (2/18)	0 (0/15)	0 (0/4)	0 (0/19)						
2006		20 ±13.3 (2/10)	20 ±13.3 (2/10)							0 (0/4)	0 (0/2)	0 (0/6)
2007		50 ±35 (1/2)	50 ±35 (1/2)	0 (0/2)	5.9 ±5.9 (1/17)	5.3 ±5.3 (1/19)				0 (0/2)		0 (0/2)
2008	0 (0/3)		0 (0/3)									
2009	0 (0/15)		0 (0/15)									
2013	0 (0/2)	0 (0/1)	0 (0/3)				0 (0/3)	7.7 ±7.7 (1/13)	6.3 ±6.3 (1/16)	0 (0/4)	0 (0/2)	0 (0/6)
Overall	5.7 ±3.7 (2/38)	23.1 ±3.2 (3/13)	**9.8 ±4.2 (5/51)**	0 (0/17)	4.8 ±4.8 (1/21)	**2.6 ±2.6 (1/38)**	0 (0/3)	7.7 ±7.7 (1/13)	**6.3 ±6.3 (1/16)**	0 (0/10)	0 (0/4)	**0** (0/14**)**

Appendix 5.9 Protostrongylids

Prevalence (%) of Protostongylid larvae in faeces (*P. andersoni, V. V. eleguneniensis* and/or *P. odocoilei)* , with standard errors, by year and sex for caribou herds Bluenose East (BNE) and Bluenose West (BNW), woodland caribou (WC) and moose as detected by the Baermann technique. Numbers in brackets refer to sample size. Animals with unknown sex were not included.

Herd/Species	**BNE**			**BNW**			**WC**			**Moose**		
Sex	Female	Male	Overall	Female	Male	Overall	Female	Male	Overall	Female	Male	Overall
Year												
2005	22.2 ±10.1 (4/18)		22.2 ±10.1 (4/18)	0 (0/15)	25 ±25 (1/4)	5.3 ±5.3 (1/19)						
2006		40 ±16.3 (4/10)	40 ±16.3 (4/10)							0 (0/4)	0 (0/2)	0 (0/6)
2007		22.22 ±14.7 (2/9)	22.2 ±14.7 (2/9)	0 (0/2)	11.8 ±8.6 (2/17)	10.5 ±7.2 (2/19)				0 (0/2)		0 (0/2)
2008	25 ±21.6 (1/4)		25 ±231.6 (1/4)									
2009	13.3 ±9.1 (2/15)		13.3 ±9.1 (2/15)									
2010										0 (0/1)	0 (0/3)	0 (0/4)
2013	100 (3/3)	60 ±21.9 (3/5)	75 ±15.3 (6/8)				66.7 ±33.3 (2/3)	30.8 ±13.3 (4/13)	37.5 ±12.5 (6/16)	0 (0/4)	0 (0/2)	0 (0/6)
Overall	25 ±6.8 (10/40)	37.5 ±9.9 (9/24)	**29.7** ±6.8 **(19/64)**	0 (0/17)	14.3 ±7.8 (3/21)	**7.9** ±4.4 **(3/38)**	66.7 ±33.3 (2/3)	30.8 ±13.3 (4/13)	**37.5** ±12.5 **(6/16)**	0 (0/11)	0 (0/7)	**0 (0/18)**

Appendix 6 Publications

Appendix 6.1 List of publications

Brook, R.K., et al., *Fostering community-based wildlife health monitoring and research in the Canadian North.* Ecohealth, 2009. **6**(2): p. 266-278.

Curry, P., *Caribou herds and arctic communities: exploring a new tool for caribou health monitoring.* ARCTIC, 2009. **62**(4).

Curry, P.S., *Blood on filter paper for monitoring caribou health: Efficacy, community-based collection and disease ecology in circumpolar herds*, in *Ecosystem and Public Health*. 2012, University of Calgary: Calgary, Alberta.

Curry, P.S., et al., *Filter-paper blood samples for Elisa detection of Brucella antibodies in caribou.* Journal of Wildlife Diseases, 2011. **47**(1): p. 12-20.

Curry, P.S., et al., *Blood collected on filter paper for wildlife serology: Detecting antibodies to Neospora caninum, West nile virus, and five bovine viruses in Rangifer tarandus subspecies.* J Wildl Dis, 2014.

Curry, P.S., et al., *Blood Ccllected on filter Paper for wildlife serology: evaluating storage and temperature challenges of field collections.* J Wildl Dis, 2014.

Forde, T., et al., *Detection of Mycobacterium avium subspecies paratuberculosis in several herds of arctic caribou (Rangifer tarandus ssp.).* Journal of Wildlife Diseases, 2012. **48**(4): p. 918-924.

Kutz, S., et al., *Standardized monitoring of Rangifer health during International Polar Year.* Rangifer, 2013. **33**(Sp. Iss. 21): p. 91-114.

Kutz, S.J., *An evaluation of the role of climate change in the emergence of pathogens and disease in Arctic and sub-Arctic caribou populations*. 2007, Report to Government of Canada: Climate Change Action Fund.

Kutz, S.J., et al., *Parasites in Ungulates of Arctic North America and Greenland: A View of Contemporary Diversity, Ecology, and Impact in a World Under Change.* Advances in Parasitology, Vol 79, 2012. **79**: p. 99-252.

Millins, C., et al., *A needs assesment for veterinary services in the Northwest Territories*. 2008, Vetrinarians without borders.

Verocai, G.G., et al., *Defining parasite biodiversity at high latitudes of North America: new host and geographic records for Onchocerca cervipedis (Nematoda: Onchocercidae) in moose and caribou.* Parasites & Vectors, 2012. **5**.

Wu, J.P., et al., *Linear enamel hypoplasia in caribou (Rangifer tarandus groenlandicus): A potential tool to assess population health.* Wildlife Society Bulletin, 2012. **36**(3): p. 554-560.

Appendix 6.2 Fostering community-based wildlife health monitoring and research in the Canadian North.

EcoHealth
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Original Contribution

ECOHEALTH

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Fostering Community-Based Wildlife Health Monitoring and Research in the Canadian North

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Abstract: Many northern Canadians have continued a subsistence lifestyle of wildlife harvesting and, therefore, value sustainable wildlife populations. At a regional wildlife workshop in the Sahtu Settlement Area, Northwest Territories in 2002, elders and community leaders raised concerns regarding wildlife health, food safety, and the effects of climate change on wildlife. They requested that efforts be put toward training youth in science and increasing involvement of hunters and youth in wildlife research. In response, we initiated a long-term, integrated approach to foster community-based wildlife health monitoring and research. Annual trips were made to all schools in the Sahtu from 2003 to 2009 to provide hands-on learning for 250–460 students on a range of wildlife topics. In addition, interviews were conducted with 31 hunters and elders to document their local ecological knowledge of wildlife health and local hunters were trained as monitors to collect tissue samples and measurements to assess body condition and monitor health of harvested caribou (n = 69) and moose (n = 19). In 2007 the program was extended to include participation in the annual caribou hunt held by one community. Each year since 2005, a graduate student and/or a postdoctoral trainee in the veterinary or biological sciences has participated in the program. The program has evolved during the last 6 years in response to community and school input, results of empirical research, hunter feedback, local knowledge, and logistical constraints. The continuity of the program is attributed to the energetic collaboration among diverse partners and a unified approach that responds to identified needs.

Key words: wildlife health, community-based monitoring, youth education, local ecological knowledge, collaboration, disease, parasites

Appendix 6.3 Caribou herds and Arctic communities

Caribou Herds and Arctic Communities:

Exploring a New Tool for Caribou Health Monitoring

by Patricia Curry

On the ragged edge of the world I'll roam, And the home of the wolf shall be my home, And a bunch of bones on the boundless snows The end of my trail…who knows, who knows!

Robert Service

Appendix 6.4 Filter-paper blood samples for ELISA detection of Brucella Antibodies in Caribou

Journal of Wildlife Diseases, 47(1), 2011, pp. 12–20
Wildlife Disease Association 2011

FILTER-PAPER BLOOD SAMPLES FOR ELISA DETECTION OF *BRUCELLA* ANTIBODIES IN CARIBOU

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ABSTRACT: We evaluated blood collected on Nobuto filter-paper (FP) strips for use in detecting *Brucella* spp. antibodies in caribou. Whole blood (for serum) and blood-saturated FP strips were obtained from 185 killed arctic caribou (*Rangifer tarandus groenlandicus*). Sample pairs (serum and FP eluates) were simultaneously tested in duplicate using competitive enzyme-linked immunosorbent assay (c-ELISA) and indirect ELISA (i-ELISA) for *Brucella* spp. Prior work based on isolation of *Brucella* spp. revealed sensitivity (SE) and specificity (SP) of 100% and 99%, respectively, for both these serum assays in caribou. Infection status of the animals in the current study was unknown but recent sampling had revealed clinical brucellosis and .40% *Brucella* antibody prevalence in the herd. To assess the performance of FP relative to serum in these assays, serum was used as the putative gold standard. On both assays, the findings for duplicate runs (A and B) were similar. For c-ELISA run A, the FP *Brucella* prevalence (47%) was lower than serum prevalence (52%), with SE 89% (95% confidence interval [CI]: 82–95%) and SP 99% (97–100%). For i-ELISA run A, serum and FP *Brucella* prevalence rates were identical (43%), and the SE and SP of FP testing were 100% and 99% (97–100%), respectively. The findings suggest better FP test performance with i-ELISA than with c-ELISA; however, i-ELISA does not distinguish cross- reacting antibodies induced by *Brucella* vaccination or exposure to certain other Gram-negative pathogens. Results for duplicate FP eluates (prepared using separate FP strips from each animal) were strongly correlated for both protocols (r50.996 and 0.999 for c-ELISA and i-ELISA, respectively), indicating minimal variability among FPs from any individual caribou. Dried caribou FP blood samples stored for 2 mo at room temperature are comparable with serum for use in *Brucella* spp. c-ELISA and i-ELISA. Hunter-based FP sampling can facilitate detection of disease exposure in remote regions and under adverse conditions, and can expand wildlife disease surveillance across temporospatial scales.

Key words: Arctic, *Brucella*, caribou, disease surveillance, filter paper, Nobuto, *Rangifer tarandus*, serology.

Appendix 6.5 Blood collected on filter paper for wildlife serology: detecting antibodies to **Neospora caninum***, west nile virus and five bovine viruses in reindeer.*

DOI: 10.7589/2012-02-047 *Journal of Wildlife Diseases*, 50(2), 2014, pp. 297–307
Wildlife Disease Association 2014

BLOOD COLLECTED ON FILTER PAPER FOR WILDLIFE SEROLOGY: DETECTING ANTIBODIES TO *NEOSPORA CANINUM*, WEST NILE VIRUS, AND FIVE BOVINE VIRUSES IN REINDEER

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ABSTRACT: We compared Nobuto filter paper (FP) whole-blood samples to serum for detecting antibodies to seven pathogens in reindeer (*Rangifer tarandus tarandus*). Serum and FP samples were collected from captive reindeer in 2008–2009. Sample pairs (serum and FP eluates) were assayed in duplicate at diagnostic laboratories with the use of competitive enzyme-linked immunosorbent assays (cELISAs) for *Neospora caninum* and West Nile virus (WNV); indirect ELISA (iELISAs) for bovine herpesvirus type 1 (BHV-1), parainfluenza virus type 3 (PI-3), and bovine respiratory syncytial virus (BRSV); and virus neutralization (VN) for bovine viral diarrhea virus (BVDV) types I and II. Assay thresholds were evidence-based values employed by each laboratory. Comparable performance to serum was defined as FP sensitivity and specificity $80%. Filter-paper specificity estimates ranged from 92% in the cELISAs for *N. caninum* and WNV to 98% in the iELISAs for PI-3 and BRSV. Sensitivity was .85% for five tests (most $95%) but was insufficient (71–82%) for the PI-3 and BRSV iELISAs. Lowering the threshold for FP samples in these two ELISAs raised sensitivity to $87% and reduced specificity slightly ($90% in three of the four test runs). Sample size limited the precision of some performance estimates. Based on the criteria of sensitivity and specificity $80%, and using adjusted FP thresholds for PI-3 and BRSV, FP sensitivity and specificity were comparable to serum in all seven assays. A potential limitation of FP is reduced sensitivity in tests that require undiluted serum (i.e., *N. caninum* cELISA and BVDV VNs). Possible toxicity to the assay cell layer in VN requires investigation. Results suggested that cELISA is superior to iELISA for detecting antibodies in FP samples from reindeer and other *Rangifer tarandus* subspecies. Our findings expand the potential utility of FP sampling from wildlife.

Key words: Bovine herpesvirus, bovine viral diarrhea virus, filter paper, *Neospora*, parainfluenza virus, *Rangifer*, serology, West Nile virus.

Appendix 6.6 Blood collected on filter paper for wildlife serology: Evaluating storage and temperature challenges of field collections.

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Wildlife Disease Association 2014

BLOOD COLLECTED ON FILTER PAPER FOR WILDLIFE SEROLOGY: EVALUATING STORAGE AND TEMPERATURE CHALLENGES OF FIELD COLLECTIONS

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ABSTRACT: Filter-paper (FP) blood sampling can facilitate wildlife research and expand disease surveillance. Previous work indicated that Nobuto FP samples from caribou and reindeer (*Rangifer tarandus* subspecies) had comparable sensitivity and specificity to serum samples ($80% for both) in competitive enzyme-linked immunosorbent assays (cELISAs) for *Brucella* spp., *Neospora caninum*, and West Nile virus. The same sensitivity and specificity criteria were met in indirect ELISAs for *Brucella* spp., bovine herpesvirus type 1 (BHV-1), parainfluenza virus type 3 (PI-3), and bovine respiratory syncytial virus (BRSV), with adjusted FP thresholds used for PI-3 and BRSV. Comparable sensitivity and specificity values to serum were also observed for FP in virus neutralization (VN) assays for bovine viral diarrhea virus types I and II; however, reduced sensitivity is a potential limitation of FP samples in protocols that require undiluted serum (i.e., VN and *N. caninum* cELISA). We evaluated the performance of FP samples from reindeer and caribou in these nine assays after simulating potential challenges of high-latitude field collections: 1) different durations of storage and 2) different processing/storage regimes involving freezing or drying. Sample pairs (serum and FP) were collected from reindeer and caribou populations in 2007–10 and were tested in duplicate. Comparable performance to serum was defined as sensitivity and specificity $80%. In the storage experiments, FP performance was determined after 2 mo of storage dry at room temperature, and after two longer periods (variable depending on assay; up to 2 yr). After 1 yr, compared to frozen serum stored for the same period, sensitivity was $88% for all but two assays (68% BHV-1; 75% PI-3), and specificity remained .90%. A limited trial evaluated the effect of freezing FP samples as opposed to drying them for storage. There were no observed detrimental effects of freezing on FP sample performance, but rigorous investigation is warranted.

Key words: Bovine herpesvirus, *Brucella*, disease surveillance, *Neospora*, Nobuto filter paper, *Rangifer*, serology, storage time and temperature.

Appendix 6.7 Detection of Mycobacterium avium *subspecies paratuberculosis in several herds of arctic caribou (*Rangifer tarandus ssp.*)*

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Wildlife Disease Association 2012

DETECTION OF *MYCOBACTERIUM AVIUM* SUBSPECIES *PARATUBERCULOSIS* IN SEVERAL HERDS OF ARCTIC CARIBOU (*RANGIFER TARANDUS* SSP.)

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ABSTRACT: *Mycobacterium avium* subspecies *paratuberculosis* (MAP) is a common pathogen in domestic ruminants that causes granulomatous inflammation of the small intestine leading to emaciation and wasting. Clinical disease (Johne's disease) is also reported for several wild ruminant species. Between 2007 and 2009 we collected 561 fecal samples from caribou (*Rangifer tarandus* ssp.) representing 10 herds of migratory caribou, two herds of caribou from Greenland, and three populations of boreal woodland caribou. Feces were tested for MAP by bacterial culture and PCR targeting the IS*900* insertion sequence. In total, 31 samples from eight different populations representing all three ecotypes were found positive for MAP by PCR, with one sample from the Riviè re-aux-Feuilles herd also being culture positive for the type II (cattle) strain. The proportion of positive animals was particularly high in the Akia-Maniitsoq herd in Greenland, and Riviè re-aux- Feuilles and Riviè re-George herds in northeastern Canada (23.4, 11.5, and 10.0%, respectively). Our results indicate that MAP is present in several caribou herds of different ecotypes in northern Canada and Greenland and that MAP circulates within wildlife populations that do not have ongoing contact with domestic livestock. The epidemiology, pathogenicity, and effects on the health of caribou in northern ecosystems remain unknown.

Key words: Arctic, caribou, epidemiology, Johne's disease, *Mycobacterium avium* subspe- cies *paratuberculosis*, *Rangifer*.

Appendix 6.8 Standardized monitoring of Rangifer *health during International Polar Year*

13th Arctic Ungulate Conference Yellowknife, Canada
22-26 August, 2011

Standardized monitoring of *Rangifer* health during International Polar Year

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Abstract: Monitoring of individual animal health indices in wildlife populations can be a powerful tool for evaluation of population health, detecting changes, and informing management decisions. Standardized monitoring allows robust comparisons within and across populations, and over time and vast geographic regions. As an International Polar Year Initiative, the CircumArctic Rangifer Monitoring and Assessment network established field protocols for standardized monitoring of caribou and reindeer *(Rangifer tarandus)* health, which included body condition, contaminants, and pathogen exposure and abundance. To facilitate use of the protocols, training sessions were held, additional resources were developed, and language was translated where needed. From March 2007 to September 2010, at least 1206 ani- mals from 16 circumpolar herds were sampled in the field using the protocols. Four main levels of sampling were done and ranged from basic to comprehensive sampling. Possible sources of sampling error were noted by network members early in the process and protocols were modified or supplemented with additional visual resources to improve clarity when needed. This is the first time that such broad and comprehensive circumpolar sampling of migratory caribou and wild reindeer, using standardized protocols covering both body condition and disease status, has been done.

Key words: body condition; caribou; disease; health; monitoring; parasites; *Rangifer tarandus;* reindeer; standardized protocols.

Rangifer, Special Issue No. 33, 2013: 91–114

Appendix 6.9 An evaluation of the role of climate change in the emergence of pathogens and diseases in Arctic and Subarctic caribou populations

Climate Change Action Fund, Project A760. December 2007

An Evaluation of the Role of Climate Change in the Emergence of Pathogens and Diseases in Arctic and Subarctic Caribou Populations

Prepared for the Climate Change Action Fund, Government of Canada, by:
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Summary

In summary, climate change is anticipated to alter patterns and diversity of disease in northern caribou populations and negatively impact the sustainability of this important natural resource.This will act through direct effects of climate on the pathogens as well as indirect effects on the hosts. Indirect effects include changes in abundance of caribou and other hosts important for pathogen transmission (such as carnivores, other ungulate species, invertebrate intermediate hosts or vectors), changes in behaviour and habitat use that increase exposure to pathogens, and changes in immunocompetence related to the cumulative effects of climate induced 'stress'. Research programs that define the diversity, host, and geographic range of pathogens in caribou in the North are essential. There remains much 'hidden diversity' (un-described species) of pathogens in caribou – if we don't have a good understanding of the players we will not be able to anticipate their response to climate and impacts on caribou. Similarly, work is needed to identify the diversity of pathogens in 'invading' wildlife species that are extending their range further North - this remains a large knowledge gap. Once the players are defined laboratory and field research that critically investigates the transmission patterns, the impacts on caribou, and the impacts of climate on the survival, development, and activity of the pathogens is essential. From there, predictive models can be developed and tested to evaluate the impacts of climate change on the epidemiology of pathogens, and ultimately, refined to incorporate the effects on the sustainability of caribou populations. Ongoing pathogen surveillance will then serve to detect changes and identify further concerns. To support such research an infrastructure and commitment to standardized data collection, longterm
surveillance, and data and physical specimen archiving is essential. Currently, the
CircumArctic Rangifer Monitoring and Assessment Network (CARMA), together with the Canadian Cooperative Wildlife Health Centre, Environment Canada's Wildlife Specimen Bank at the National Wildlife Research Centre, and territorial wildlife agencies, are developing plans for data and specimen collection and archiving for caribou research during International Polar Year and beyond.

Appendix 6.10 Parasites in ungulates of Arctic North America and Greenland.

Parasites in ungulates of Arctic North America and Greenland: a view of contemporary diversity, ecology, and impact in a world under change.

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Advances in Parasitology (Impact Factor: 3.78). 01/2012; 79:99-252. DOI:10.1016/B978-0-12-398457-9.00002-0
Source: PubMed

ABSTRACT Parasites play an important role in the structure and function of arctic ecosystems, systems that are currently experiencing an unprecedented rate of change due to various anthropogenic perturbations, including climate change. Ungulates such as muskoxen, caribou, moose and Dall's sheep are also important components of northern ecosystems and are a source of food and income, as well as a focus for maintenance of cultural traditions, for northerners. Parasites of ungulates can influence host health, population dynamics and the quality, quantity and safety of meat and other products of animal origin consumed by people. In this article, we provide a contemporary view of the diversity of nematode, cestode, trematode, protozoan and arthropod parasites of ungulates in arctic and subarctic North America and Greenland. We explore the intricate associations among host and parasite assemblages and identify key issues and gaps in knowledge that emerge in a regime of accelerating environmental transition.

Appendix 6.11 A needs assessment for veterinary services in the Northwest Territories

This project came about through on-going contact with communities when veterinary services were voiced as needs. A side-project to the WHM program was initiated and now functions independently.

A Needs Assessment for Veterinary Services in the Northwest Territories

Produced for and funded by:

Veterinarians Without Borders Canada, September 2008

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Summary

This project focused on (i) documenting current delivery of veterinary services for domestic animals in the Northwest Territories (NT), (ii) investigating issues related to dog health and welfare and secondary effects on human and wildlife health, and (iii) exploring potential uptake of veterinary services if they were offered.

Our results indicate that, for dogs in remote regions of the NT, there are concerns associated with canine health and welfare. We identified a number of dog health and welfare issues including low levels of vaccination and deworming and poor physical conditions of dogs related at least in part to inadequate housing and possibly food provision. These concerns were mirrored by some community members attending clinics, and although we had not formally solicited opinions on needs for veterinary services during our Sahtu Community Outreach tour in the past, throughout the years many community members, including youth, have expressed an interest in better access to veterinary services. Similar sentiments have been expressed by human health care providers who are often the first source of information for dog owners. Our school and owner surveys in 2008 also confirmed a desire of at least some community members for better access to veterinary services. Representative community attitudes towards dog welfare, the role of dogs in society and veterinary services are important areas of future research. A survey of non participants to understand barriers to uptake of services is recommended.

Appropriate quantitative data to assess the effects of dogs on wildlife health and human health were unavailable to this study, due to lack of wild canid disease surveillance data and restrictions on the use of human health data. Suggestions for important areas of targeted research are outlined at the end of this report.

It is probable that at least some of the animal health and welfare issues, and possibly human and wildlife health, could be improved with better access to and uptake of animal health care services and education. One solution has been charity spay and neuter clinics provided on a sporadic basis in various communities, however, efficacy of such programs in long term improvement of animal and human health and welfare issues needs critical evaluation. For example, in some cases where spay and neuter clinics have been offered the dog population has rebounded to original levels within 6 months. Additionally, most of these initiatives have been one-off events and few have prioritized capacity-building and longterm sustainability. Appendix 8: Guiding Principles for Providing Charity Veterinary Clinics in Remote Communities provides recommended approaches for delivering such clinics.

New approaches to delivery of basic animal health care services in remote regions should be economically viable, culturally acceptable, and ultimately sustainable. Such an approach should be multi-faceted and developed in collaboration with the community and relevant stakeholders, including health care providers, wildlife officials, local municipal government and local veterinarians, to ensure acceptance, ownership, and sustainability. Importantly, barriers to uptake of services need to be investigated and solutions developed. Options for developing local capacity, perhaps modeled after the „Community-based Animal Health Care Workers‟ that have been established in remote regions of many developing nations, need to be explored. Such an approach is well established for human medicine in northern Canada where nurse practitioners deliver preventative health care services under the remote direction of medical doctors. Development of local, yet remotely supervised (by a licensed veterinarian), capacity for delivery of basic animal health services would ensure these services are accessible for 365 days/year, not just the 2-3 days that fly-in services provide.

Efforts should now be extended to fill in knowledge gaps (see following section on research recommendations) and develop and critically evaluate new approaches that will meet the animal health care and health promotion needs in a sustainable manner in these regions. This may be approached through formation of a working group that includes communities, health care providers, the municipal government, and local veterinarians, to evaluate dog issues in the NT and develop solutions and setting up demonstration projects to work through the challenges.

Appendix 6.12 Defining parasite biodiversity at high latitudes of North America

Verocai *et al. Parasites & Vectors* 2012, 5:242
http://www.parasitesandvectors.com/content/5/1/242Open Access

Defining parasite biodiversity at high latitudes of North America: new host and geographic records for *Onchocerca cervipedis* (Nematoda: Onchocercidae) in moose and caribou

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Abstract

Background: *Onchocerca cervipedis* is a filarioid nematode of cervids reported from Central America to boreal regions of North America. It is found primarily in subcutaneous tissues of the legs, and is more commonly known as 'legworm'. Blackflies are intermediate hosts and transmit larvae to ungulates when they blood-feed. In this article we report the first records of *O. cervipedis* from high latitudes of North America and its occurrence in previously unrecognized host subspecies including the Yukon-Alaska moose (*Alces americanus gigas)* and the Grant's caribou (*Rangifer tarandus granti*).

Methods: We examined the subcutaneous connective tissues of the metacarpi and/or metatarsi of 34 moose and one caribou for parasitic lesions. Samples were collected from animals killed by subsistence hunters or animals found dead in the Northwest Territories (NT), Canada and Alaska (AK), USA from 2005 to 2012. Genomic DNA lysate was prepared from nematode fragments collected from two moose. The *nd5* region of the mitochondrial DNA was amplified by PCR and sequenced.

Results: Subcutaneous nodules were found in 12 moose from the NT and AK, and one caribou from AK. Nematodes dissected from the lesions were identified as *Onchocerca cervipedis* based on morphology of female and male specimens. Histopathological findings in moose included cavitating lesions with multifocal granulomatous cellulitis containing intralesional microfilariae and adults, often necrotic and partially mineralized. Lesions in the caribou included periosteitis with chronic cellulitis, eosinophilic and lymphoplasmacytic infiltrate, and abundant granulation associated with intralesional adult nematodes and larvae. Sequences of the *nd5* region (471bp), the first generated for this species, were deposited with Genbank (JN580791 and JN580792). Representative voucher specimens were deposited in the archives of the United States National Parasite Collection.

Conclusions: The geographic range of *O. cervipedis* is broader than previously thought, and extends into subarctic regions of western North America, at least to latitude 66°N. The host range is now recognized to include two additional subspecies: the Yukon-Alaska moose and Grant's caribou. Accelerated climate change at high latitudes may affect vector dynamics, and consequently the abundance and distribution of *O. cervipedis* in moose and caribou. Disease outbreaks and mortality events associated with climatic perturbations have been reported for other filarioids, such as *Setaria tundra* in Fennoscandia, and may become an emerging issue for *O. cervipedis* in subarctic North America.

Keywords: *Alces*, Caribou, Legworm, North America, *Onchocerca cervipedis*, Moose, *Rangifer*, Subarctic, Vector-borne diseases

Appendix 6.13 Linear Enamel hypoplasia in Caribou (Rangifer tarandus groenlandicus): a potential too to assess population health

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Original Article

Linear Enamel Hypoplasia in Caribou (*Rangifer tarandus groenlandicus*): A Potential Tool to Assess Population Health

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ABSTRACT We studied the presence of linear enamel hypoplasias (LEHs; tooth defects associated with physiological stress) in caribou (*Rangifer tarandus*). A timeline of tooth enamel development was determined by radiographic examination of 48 mandibles from caribou aged 3–24 months old. We examined mandibles from the Bluenose East (*n* ¼ 56) and Bluenose West (*n* ¼ 15) caribou herds in the Northwest Territories and Nunavut, Canada, for LEHs and 21.1% (15/71) were affected. We concluded that LEHs do occur in caribou and tracking these over time may provide a tool to track population dynamics in extant wildlife. © 2012 The Wildlife Society.

KEY WORDS caribou, health, linear enamel hypoplasia, Northwest Territories, Nunavut, population dynamics, *Rangifer*, stress.

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APPENDIX II

UNIVERSITY OF CALGARY

Detecting *Dermacentor albipictus*, the winter tick, at the northern extent of its distribution range:

Hunter-based monitoring and serological assay development

By

Cyntia Kayo Kashivakura

A THESIS

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ABSTRACT

Dermacentor albipictus is an important parasite of moose and other ungulate species. In 1989, winter ticks were reported as far north as 62° N, but recent anecdotal reports of clinically affected moose in the Sahtu Settlement Area, NT suggested significant range expansion. This research aimed to determine the occurrence of *D. albipictus* on moose and caribou hides from the Sahtu submitted by local hunters, to investigate growth and development of winter ticks on captive reindeer, and to develop a serological assay to detect antibodies to ticks using cattle as a model. Winter ticks were confirmed in 5 of 30 moose at 66° N. The development of ticks on captive reindeer was similar to that reported in moose. There was no consistent pattern in antibody response after exposure to ticks. Future studies should continue monitoring to understand the potential risks of this parasite to infest caribou under a changing climate.

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To Susan, who idealized and brought this research into life.

To Mark, who makes me want to be a better person,

and to Behdzi who taught me how to love unconditionally.

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"It is not the critic who counts;
not the man who points out how the strong a man stumbles,
or where the doer of deed could have done them better.
The credit belongs to the man who is actually in the arena,
whose face is marred by dust, and sweat and blood;
who strives valiantly;
who errs, who comes short again and again,
because there is no effort without error and shortcoming;
but who does actually strive to do the deeds;
who knows great enthusiasms, the great devotions;
who spends himself in a worthy cause;
who at the best, knows in the end, the triumph of high achievement,
and who at the worst, if he fails,
at least fails while daring greatly…"

– Theodore Roosevelt –

CHAPTER 1

INTRODUCTION

The winter tick – life cycle, hosts and geographic distribution

Dermacentor albipictus (Packard, 1869) (Acari: Ixodidae), the winter tick, is a one-host tick, i.e., it completes its parasitic stage on a single host, both infesting wild and domestic ungulate species (Addison et al., 1979; Addison and McLaughlin, 1988). The winter tick life cycle takes a year to complete and follows a predictable pattern in many regions of its occurrence. Hosts become infested with winter tick larvae in the autumn and ticks remain on the host throughout the winter. Engorged females drop off from the host onto the ground in spring to lay eggs. In summer these eggs hatch and larvae emerge, which remain on the ground until late August to mid October. That is when larvae ascend the vegetation and form aggregations waiting for a potential host to pass and come in contact with these clumps (Addison and McLaughlin, 1988; Aalangdong and Samuel, 2001). Once the host is found, larvae start blood feeding and within a few weeks moult into nymphs, which undergo diapause for several months and start feeding in late January and then moult into adults in February and March. Adult ticks then feed between March and April, and engorged females drop from the host onto the ground to lay eggs in late spring.

Among affected hosts, moose (*Alces americanus* sspp.) are the most commonly and the most severely affected. However, other ungulates species such as elk (*Cervus canadensis* (Erxleben, 1777)), mule deer (*Odocoileus hemionus* Rafinesque, 1817) and woodland caribou (*Rangifer tarandus caribou* (Gmelin, 1788)) can also be affected with winter ticks (Welch et al., 1990b; Welch et al., 1991). The effects of *D. albipictus* infestations on moose can be severe: reports of an individual moose parasitized with over 100,000 ticks (Samuel and Welch, 1991) demonstrate how severely and intensely winter ticks can affect moose. The most common clinical signs observed in these animals are

damage of the guard hairs and extensive alopecia (hair loss) due to grooming behaviour in their attempt to remove ticks (McLaughlin and Addison, 1986; Mooring and Samuel, 1999). The animals that develop such signs are easily recognized in the field and popularly referred to as 'ghost moose' because of their pale colour. To date, no other ectoparasites other than winter ticks are known to cause such a clinical signs on moose (Samuel, 1989), thus several researchers have used these signs as an indication of winter tick occurrence in a region (Samuel, 1989; Kutz et al., 2009). Severely affected animals also develop other clinical signs such as poor body condition, anaemia, and eventually, mortality can also occur (McLaughlin and Addison, 1986; Musante et al., 2007; Samuel, 2007; Samuel, 2007). The impacts of winter tick infestations on moose health are so significant that it is believed that this parasite, together with other ecological factors, influences moose population dynamics. For example, the decline in moose numbers at Isle Royale National Park, Michigan, USA during the winters of 1987/88 to 1993/94 was associated with an epizootic of winter ticks (DeIgiudice et al., 1997). Similarly, from 1977 to 1982, several debilitated moose that were found annually in central Alberta were parasitized with numerous ticks and with extensive alopecia (Samuel and Barker 1979). During the winter of 1998/1999 at Elk Island National Park, Alberta, Canada, moose die-offs were also associated with heavy winter tick infestations (Pybus, 1999).

Winter ticks are widely distributed across North America, from as far south as Baja California, Mexico (31° N) (Contreras et al., 2007) to as far north as Yukon, Canada (62° N) (Samuel, 1989). Within the genus *Dermacentor*, *D. albipictus* has the northernmost distribution (Wilkinson, 1967); however, the northern limit of its distribution is still not well defined. In 1967, Wilkinson (1967) suggested that the northern limit of this parasite was at

approximately 64° N, from northern British Columbia and southern Northwest Territories in western Canada. Wilkinson's (1967) model was based on growing degree-days necessary to allow winter tick development during the free-living stages, but no winter tick specimens were ever found as far north to confirm his hypothesis. A few decades later, in the late 1980s, moose clinically affected with winter ticks were reported in the southern Yukon; however, the reports did not exceed the northern limit suggested by Wilkinson (1967) as they were all south of 62° N (Samuel, 1989). More recently, in the mid-2000s, observations of 'ghost moose' were reported as far as the Sahtu Settlement Area (further referred as the Sahtu), in the central Northwest Territories (NT) (62° - 68° N) (Kutz et al., 2009). The observations in the Sahtu considerably exceeded the northern limit of the winter tick distribution suggested by Wilkinson (1967) and the reports of clinically affected moose in Samuel (1989) study, highlighting the possibility of winter tick range expansion in the Canadian North (Kutz et al., 2009). While the increase sightings of clinically affected moose by local residents could be due to enhanced awareness, focus group interviews with local subsistence hunters who had handled hundreds of moose hides since 1970, indicated that only recently there was an increase in observation of hides with broken hair or hair loss (Kutz et al., 2009). Although observations of clinically affected moose are strong indications of winter tick occurrence, there were no sampling efforts to recover tick specimens and confirm the presence of this parasite as far as the Sahtu.

Considering the negative impacts of *D. albipictus* infestation on moose at the core of winter tick distribution range (Glines and Samuel, 1984; DeIgiudice et al., 1997; Mooring and Samuel, 1999; Musante et al., 2007), these increasing reports of affected moose in the

Sahtu raises a concern about the impacts on moose health and other ungulate species that are sympatric to moose in the region.

To date, it is unknown if boreal woodland caribou (*Rangifer tarandus caribou* (Gmelin, 1788)) from the Sahtu are infested with winter ticks as there has been no systematic surveillance of this ungulate species for the tick. Reports of boreal woodland caribou affected with winter ticks in Alberta indicate that they are susceptible to this parasite (Welch et al., 1990b) and anecdotal reports of boreal woodland caribou affected with ticks in southern regions of the Northwest Territories (Kutz et al., 2009) also suggest that winter ticks can be a concern for this ungulate species in the Northwest Territories.

The status of winter tick infestations on barrenground caribou (*Rangifer tarandus groenlandicus* (Borowski, 1780)) also remains unclear. Prior to the current study (Kashivakura et al. Chapter 2), no studies had been done to assess if barrenground caribou are infested with winter ticks. There are concerns that this parasite will invade barrenground caribou populations and cause severe consequences to their health (Kutz et al., 2009). Barrenground caribou populations are declining across their circumpolar range (Vors and Boyce, 2009). Although dramatic fluctuations in barrenground caribou numbers have been historically documented (Vors and Boyce, 2009), current climate change scenarios – together with increasing industrial development – may restrict recovery of these declining populations. A new parasite added to these existing stressors could aggravate the situation of an already declining population causing morbidity and mortality of the affected animals. Hence, determining the occurrence of this parasite in the Sahtu was the first step to better understanding the potential risks of winter ticks invading barrenground caribou populations.

Surveillance of winter ticks in the Sahtu

This research grew out of an ongoing hunter-based Wildlife Health Monitoring Program, which was initiated in 2002 in response to concerns about wildlife health raised by elders and community leaders during a regional meeting (Brook et al., 2009). Information gathered in the Sahtu through focus group interviews in 2005 (Kutz et al., 2009) provided essential context to understand the presence, or lack thereof, of winter ticks in the Sahtu. It is possible to gather valuable information by interviewing local people, however, there are disadvantages to such a method. The data rely on awareness of the interviewee and, unless the signs are remarkable, valuable information could pass unnoticed. Although clinical signs of moose infested with high numbers of winter ticks are easily recognized in the field, these signs are generally more evident in late spring when moose are infested with adult stages (Mooring and Samuel, 1999), thus ticks in the earlier developmental stages (larvae and nymphs), could be overlooked even by the most experienced hunter.

There are several alternative methods to determine the occurrence of ticks. Currently, the most popular techniques for tick surveillance are classified into two major categories: (1) methods, such as flagging and carbon dioxide-baited traps, to detect ticks in the environment during free-living stages and (2) methods that involve collecting ticks from the host through manual removal or through chemical digestion of hides in potassium hydroxide (KOH) (Ginsberg and Ewing, 1989). The flagging technique consists of dragging a large white piece of cloth (usually flannel) over the habitat in search for ticks (Ginsberg and Ewing, 1989) and the carbon dioxide-baited traps involve placing dry ice into a sealable container with a small aperture to attract ticks (Ginsberg and Ewing, 1989).

Both techniques have been used either alone or in combination to study the ecology of several species of ticks such as *Ixodes scapularis* Say 1821 (Acari: Ixodidae) and *Amblyomma americanum* (Linnaeus, 1758) (Acari: Ixodidae) (Ginsberg and Ewing, 1989). However, for winter ticks these techniques are not practical for several reasons. In addition, these methods can be logistically demanding and impractical for tick surveillance in vast and remote areas such as the Sahtu. Manually removing tick specimens from the parasitized host for species identification gives accurate information about which tick species are occurring in the region, but this method has poor sensitivity, especially for detecting larval or nymph stages. It is also impractical for wild species as it is only possible when animals are under sedation or dead. Chemical digestion of hides in KOH has been used in many studies for winter tick detection (Addison et al., 1979; Drew and Samuel, 1985; Samuel, 1989; Welch and Samuel, 1989) and has advantages over the manual removal of ticks because earlier stages of ticks are better detected with chemical digestion of hides. However, chemical digestion of hides also has its limitations as it is costly, logistically challenging, and it is possible only in dead animals. Given that hunters in the Sahtu were already hunting for subsistence, the approach taken by this research was to collect moose and caribou hides by collaborating with hunters and chemically digesting these hides in KOH, to determine the occurrence of winter ticks in the Sahtu. In Chapter 2 of this thesis, I will describe the methods used and the results obtained, the insights about winter tick distribution, and discuss the advantages and disadvantages of using hunter-based collection for winter tick surveillance.

Alternative method for tick surveillance – Serology

Despite its efficacy, the costs, labour, and the logistics involved in performing chemical digestion of hides are very high, which may make this technique unfeasible for many studies. I, therefore, tested the feasibility of developing an assay to detect tick exposure in sera, using cattle as a model species. Serum samples from woodland and barrenground caribou from the Sahtu and from southern regions of the Northwest Territories were available from the Department of Environment and Natural Resources, Government of Northwest Territories serum bank, and thus, if a serological assay was available, these samples would be ideal for evaluating historical and contemporary occurrence of *D. albipictus* across many locations in the Northwest Territories. The vast literature dedicated to study host-parasite immunity (Wikel, 1982; Brown, 1988a; Dipeolu et al., 1992; Wikel, 1996; Cruz et al., 2008; Lysyk et al., 2009) highlighted the possibility of developing a serodiagnostic tool to detect winter tick exposure in caribou sera. In this study, the process of developing a serological assay for tick surveillance involved rearing winter tick colonies to challenge captive reindeer (*Rangifer tarandus tarandus*) and domestic cattle (*Bos taurus*), collecting sera from these challenged animals, determining the tick antigen, identifying potential candidate antigens for a serological assay, and testing these sera through Western blot analysis. The detailed process of this experience including the challenges, the troubleshooting trials, the results, and the insights for future studies are described in Chapters 3 and 4 of this thesis.

Study objectives

My research was developed under the umbrella of an ongoing Wildlife Health Monitoring Program initiated in 2004 after a regional workshop meeting co-hosted by the Government of Northwest Territories and Sahtu Renewable Resources Board in 2002. In this meeting, elders and community leaders from the Sahtu raised their concerns about wildlife health, food safety and future challenges for their communities under climate change scenarios (Brook et al., 2009). In addition, the increased reports of 'ghost moose' in the region (Kutz et al., 2009) led to the initiation of this study and to intensifying efforts for winter tick surveillance on both moose and caribou in the Sahtu.

The first objective of this study was to confirm the occurrence of winter ticks in the Sahtu by using hunter-based sample collections and chemically digest these moose and caribou hides in KOH to search for ticks. In Chapter 2, I will describe in detail the findings of this study and the insights about winter tick distribution range across the Sahtu.

The second objective of this study, which is described in Chapters 3 and 4, was to test the feasibility of developing a serodiagnostic tool for tick surveillance. In Chapter 3, I describe the use of reindeer as an experimental model for winter tick studies, and in Chapter 4, I investigate the use of Western blot analysis as a substitute method to overcome the challenges encountered in chemical digestion of hides.

Finally, in Chapter 5, I discuss the significance of my research in a broader context, evaluating the importance to continue monitoring the emergence of *D. albipictus* in the Sahtu, share the knowledge gained throughout this experience, and provide recommendations for future studies.

CHAPTER TWO

HUNTER-BASED APPROACH TO DETERMINE THE OCCURRENCE OF *DERMACENTOR ALBIPICTUS* (PACKARD, 1869) (ACARI: IXODIDAE) IN THE SAHTU SETTLEMENT AREA, NORTHWEST TERRITORIES, CANADA

INTRODUCTION

Dermacentor albipictus (Packard, 1869), the winter tick, is a one-host ixodid tick (i.e., requires a single host during its parasitic stage) of wild ungulates in North America (Samuel and Welch, 1991; Welch et al., 1991). This parasite has a seasonally synchronized life cycle. Hosts become infested with winter tick larvae during autumn. Larvae develop into nymphs, and then into adults, parasitizing the host until late spring when engorged females drop onto the ground to lay eggs (Addison and McLaughlin, 1988; Aalangdong and Samuel, 2001).

Moose (*Alces americanus* sspp.) are the most commonly and the most severely affected ungulate host (Addison et al., 1979; Welch et al., 1991; Mooring and Samuel, 1999), with reports of an individual moose being parasitized with over 100,000 ticks (Samuel and Welch, 1991). Such massive infestations cause deleterious effects on the fitness and health of the host (e.g. poor body condition and anaemia due to tick feeding), and can lead to mortality of heavily infested animals (McLaughlin and Addison, 1986; Musante et al., 2007; Samuel, 2007). The most common clinical signs in heavily infested moose include damage of the guard hair and extensive alopecia often starting from neck and shoulders area (Welch et al., 1990a; Musante et al., 2007). These signs are caused by self-grooming to remove ticks and affected moose are often called ‘ghost moose’ (Mooring and Samuel, 1999).

In the late 1980s, winter ticks were known to occur only as far north as 62° N (Samuel, 1989); however, more recently, residents in the Sahtu Settlement Area (referred further as Sahtu), Northwest Territories (NT), reported sightings of ‘ghost moose’ in the

region (Kutz et al., 2009). These observations at 64 to 67^{o} N exceed considerably the northern limit suggested by Wilkinson (Wilkinson, 1967) and the reports of ghost moose by Samuel (Samuel, 1989) at 62^{o} N, highlighting the possibility of a relatively recent winter tick range expansion to northern latitudes. This apparent range expansion could have consequences both for the health of moose and woodland caribou (*Rangifer tarandus caribou* (Gmelin, 1788)), which are sympatric in the Sahtu, and may ultimately pose a risk for winter ticks to invade barrenground caribou (*Rangifer tarandus groenlandicus* (Borowski, 1780)) populations. Considering the negative consequences of *D. albipictus* infestation for moose (McLaughlin and Addison, 1986; Musante et al., 2007; Samuel, 2007), and the apparent range expansion of winter ticks to northern latitudes, confirming the presence through actual isolation of the parasite in the region was of utmost importance.

The objective of this research was to assess the occurrence of *D. albipictus* on moose and barrenground caribou from the Sahtu Settlement Area, NT by engaging subsistence hunters in sample submission.

MATERIALS AND METHODS

Hunter-based sample collections

This study was carried out from September 2010 to May 2012 in the Sahtu Settlement Area, NT (Figure 2.1). In September 2010, consultation meetings were held with local hunters in the communities of Fort Good Hope (66° 15' 24" N, 128° 37' 59" W) and Colville Lake (67° 1' 59" N, 126° 7' 00" W) to introduce the research and to encourage hunter participation in the study. As a component of an ongoing community-based Wildlife Health Monitoring Program, hunters were asked to collect whole hides from harvested

animals for tick surveillance, and other biological samples for health assessment using sample kits provided by the study. The sample kit consisted of labelled Ziploc bags® to place the collected samples, three sets of Nobuto filter papers (Toyo Roshi Kaisha, Ltd., Tokyo, Japan; distributor Advantec MFS Inc., Dublin, CA, USA) for blood collection (Curry, 2009), two Tyvek® tags to record information (hunter's name, hunt location, animal's age class and sex), and a sheet with instructions on how to collect samples, all inside a large durable nylon bag. A complete sample kit would include the whole hide, entire left kidney with fat, piece of the liver, faecal samples, metatarsus bone, incisors, and blood on filter papers (Figure 2.2). For each complete sample kit, hunters would receive financial compensation ($400 for complete sample kit and hide of moose, and $200 for complete sample kit and hide of caribou) in recognition of their efforts. Hunters were asked to concentrate sampling efforts especially from September to May, period of which winter ticks were known to be parasitizing the animals.

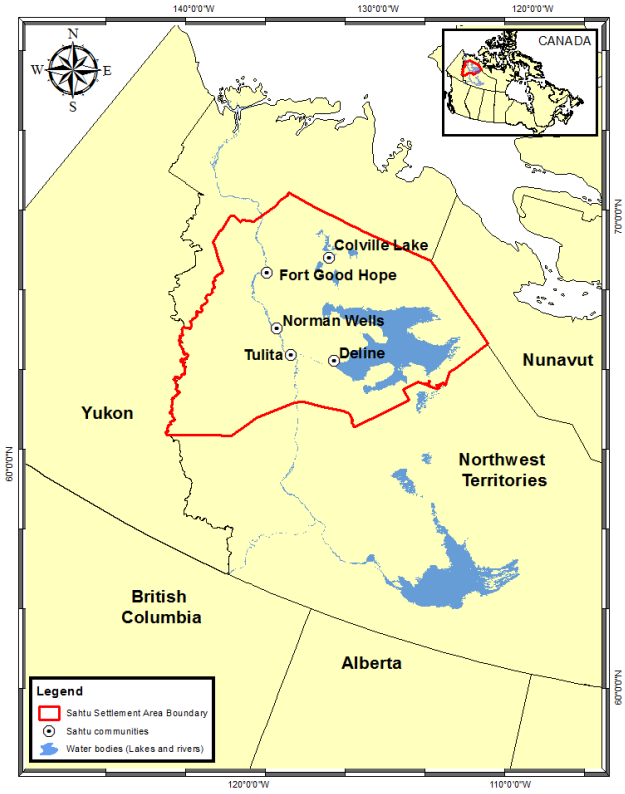


Figure 2.1. Map demonstrating the location of the Sahtu Settlement Area, in Northwest Territories, Canada. Red line represents Sahtu boundaries.

Figure 2.2. Complete biological samples requested from hunters while harvesting for subsistence included hides, blood on filter papers, incisors, faecal samples, piece of liver, left kidney with fat, and left metatarsus with a tag containing information about hunter's name, collection date, and hunting location.

In September 2010, 40 sample kits were provided to the local Renewable Resources Council (RRC) office in Fort Good Hope, and five sample kits were given directly to a hunter in Déline (65° 11' 20" N, 123° 25' 14" W). In February 2011, a second consultation meeting was held in Fort Good Hope and Déline, 25 additional sample kits were provided to the RRC in Fort Good Hope, 22 were deposited in Déline, and 10 at the Environment and Natural Resources (ENR) office in the community of Tulia (64° 54' 06" N, 125° 34' 40" W). Sample kits were also left at the ENR office in the town of Norman Wells (65° 16' 54" N, 126° 49' 45" W) in case hunters from this community were interested in participating in the study. No sample kits were deposited at the community of Colville Lake, as the hunters from this community did not engage with the research.

Once samples were collected in the field, hunters submitted the samples to their local RRC or ENR office and received the financial compensation. Samples were then directed to the ENR office in Norman Wells, shipped to the Spy Hill Campus, Faculty of Veterinary Medicine of the University of Calgary (UCVM) either by air cargo or ground transportation, and kept at -20°C in a walk-in freezer until they were analyzed.

Hide sampling method

Hides were thawed overnight, stretched to their normal shape, and measured in length and width to estimate the total area. The ratio of length: width was calculated and this ratio was used to estimate width for the hides missing measurement, and then used to calculate the area of the hide. Each hide was sampled at predilection sites for winter tick attachment (Addison et al., 1979), at Neck, Shoulders, and at the Base of the Tail (Figure 2.3). Within each area, five sections of 400 cm^2 (Section A and B (Neck area), Sections C

and D (Shoulders area), and E (Base of the Tail)) were sampled and sub-divided in four quadrants of 100 cm^2, totalling 20 quadrants per hide. The total area (in cm^2) sampled per hide was 2,000 cm^2 (400 cm^2 x 5 sections), corresponding to about 10 and 15% of the total hide area of the moose and caribou hides, respectively. After sampling was complete, each quadrant was placed individually in a Ziploc® bag and kept frozen at -20°C until digestion in potassium hydroxide (KOH).

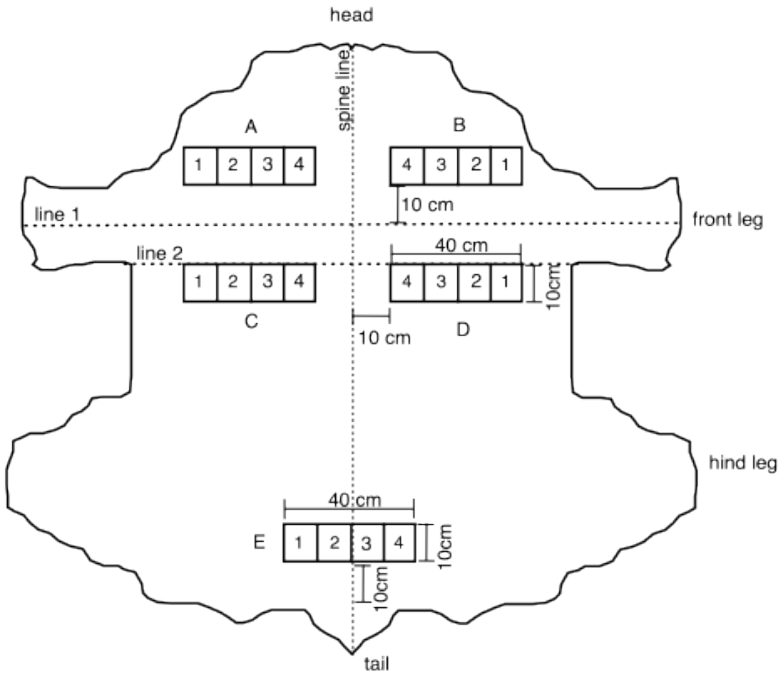


Figure 2.3. Method used for sampling moose (*Alces americanus andersoni*) and barrenground caribou (*Rangifer tarandus groenlandicus*) hides collected from the Sahtu Settlement Area, NT. All sections (A, B, C, D and E) had a measurement of 10 x 40 cm (400 cm^2) and each of these sections was sub-divided into four subsections containing 4 quadrants of 100 cm^2, resulting in a total of 20 quadrants per hide. Sections A and B corresponded to the Neck area, sections C and D to the Shoulders Area, and Section E to the Base of the Tail.

Chemical digestion of hides

The hide quadrants of 100 cm^2 were thawed overnight at 4°C and visually inspected for broken hair or hair loss, and presence of ticks. The excess muscle and connective tissue was manually removed from the hides using a scalpel blade to facilitate the digestion process and to reduce digestion time. Each quadrant was placed in 1,000 ml Erlenmeyer flask containing a preheated solution of KOH (19.85 g of KOH dissolved in 500 ml of distilled water), which was then placed into a water bath at 95°C until no tissues were grossly observed at the bottom of the flasks. The digestion time was recorded starting at the time when the flasks were placed into the water bath until no more tissues were observed. After the digestion was complete, the solution was sieved through a 150μm sieve and the material retained was gently washed with tap water into a Petri dish, and then examined for ticks under a dissecting scope at 10x magnification.

When present, the ticks were counted, classified according to the developmental stage (larvae, nymphs, or adults), identified to species (Brinton et al., 1965), and preserved in vials containing 95% ethanol. A sub-sample of the ticks recovered from each infested host was deposited at the United States Department of Agriculture National Parasite Collection (USNPC No. 106181, 106182, 106183, 106184, and 106185).

Other ectoparasites and unidentified free-living arthropods found in the hide digests were preserved in 95% ethanol but separated from the ticks for further identification (Appendix A).

Data analysis

The total number of ticks per hide, and the mean and median number of ticks observed per quadrant were calculated. The density of ticks for each quadrant was calculated by dividing the total number of ticks by its area (100 cm^2). The Chi-square (χ^2) test was used to determine if the observed and the expected frequency of ticks was the same across sampled sites in an individual animal. For this analysis, rather than analyzing each of the five sections (A, B, C, D, and E) separately, the hide was divided in three main sampling areas (Neck, Shoulders and Base of Tail) and the frequency of ticks in these locations were then compared. Since there were unequal numbers of quadrants among sampling areas, the expected frequency established for the Chi-square analysis was 40% for Neck, 40% for Shoulders and 20% for Base of the Tail Section. Chi-square analysis was also performed to compare occurrence of different tick developmental stages across sampling areas (Neck, Shoulders, and Base of Tail) within an individual animal.

RESULTS

Hunter-based sample collections

From September 2010 to May 2012, samples from a total of 45 moose and 25 barrenground caribou were collected through hunter-based collections in the Sahtu. The majority of moose samples including hides and sample kits collected were from the Fort Good Hope area (n=39). Moose hides were collected from Tulita and Norman Wells, but no other biological samples requested in the sample kits were collected from these communities. Specific to barrenground caribou, hunters from Déline were the main

contributors of caribou hides (n=25), but seven of these 25 samples were missing sample kits (Table 2.1).

Table 2.1. Number of moose hides and sample kits collected by community according to the year of collection in the Sahtu Settlement Area, NT. No sample kits were collected from Tulita and Norman Wells, and no sample kits or hides were collected from Colville Lake. All 25 barrenground caribou samples were collected from Déline in 2011.

Year	Fort Good Hope	Déline	Tulita	Norman Wells
2010	11/16*	1/1	0	0
2011	13/17	2/2	1/0	1/0
2012	0/6	0	1/0	0
Total	24/39	3/3	2/0	1/0

* Number of hides/number of sample kits collected

Complete sample kits included the hide and all biological samples of the harvested animal (as shown in Figure 2.2), and information about hunting location, hunter's name, animal's age class and sex, and overall health status, however, data and samples were missing from several submissions. Moose hides were missing for 15 of the 45 (33%) submitted kits, information about hunting location was missing in 20/45 (44%), and hunter's name was missing in 20/45 (44%) of the moose kits collected. Thus, for these hides missing information, the hunting location was recorded as the community from which they were submitted. Sample kits were missing in seven of the 25 barrenground caribou hides submitted, but all caribou hides had information about hunting location. The community of Fort Good Hope had the largest number of participants (n=10), followed by Déline (n=3), Tulita (n=2), and Norman Wells (n=1).

Chemical digestion of hides

The estimated mean area and the standard deviation of a moose hide was 2.30 m^2 (SD±0.67 m^2), and for barrenground caribou was 1.34 m^2 (SD±0.19 m^2). On average, the sampled area corresponded to 10% (SD±2%) and 15% (SD±3%) of the total area of the moose and caribou hides, respectively. A total of 600 moose and 500 barrenground caribou hide sub-sections of 100 cm^2 were digested, and on average, moose hides were fully digested in 264 (SD±41) minutes, and barrenground caribou in 170 (SD±62) minutes.

Dermacentor albipictus were found in five of 30 (16.6%) moose hides examined: two were from Déline, two from Tulita and one from Fort Good Hope (Figure 2.4 and Table 2.2). All 25 barrenground caribou hides digested in this study were negative for ticks.

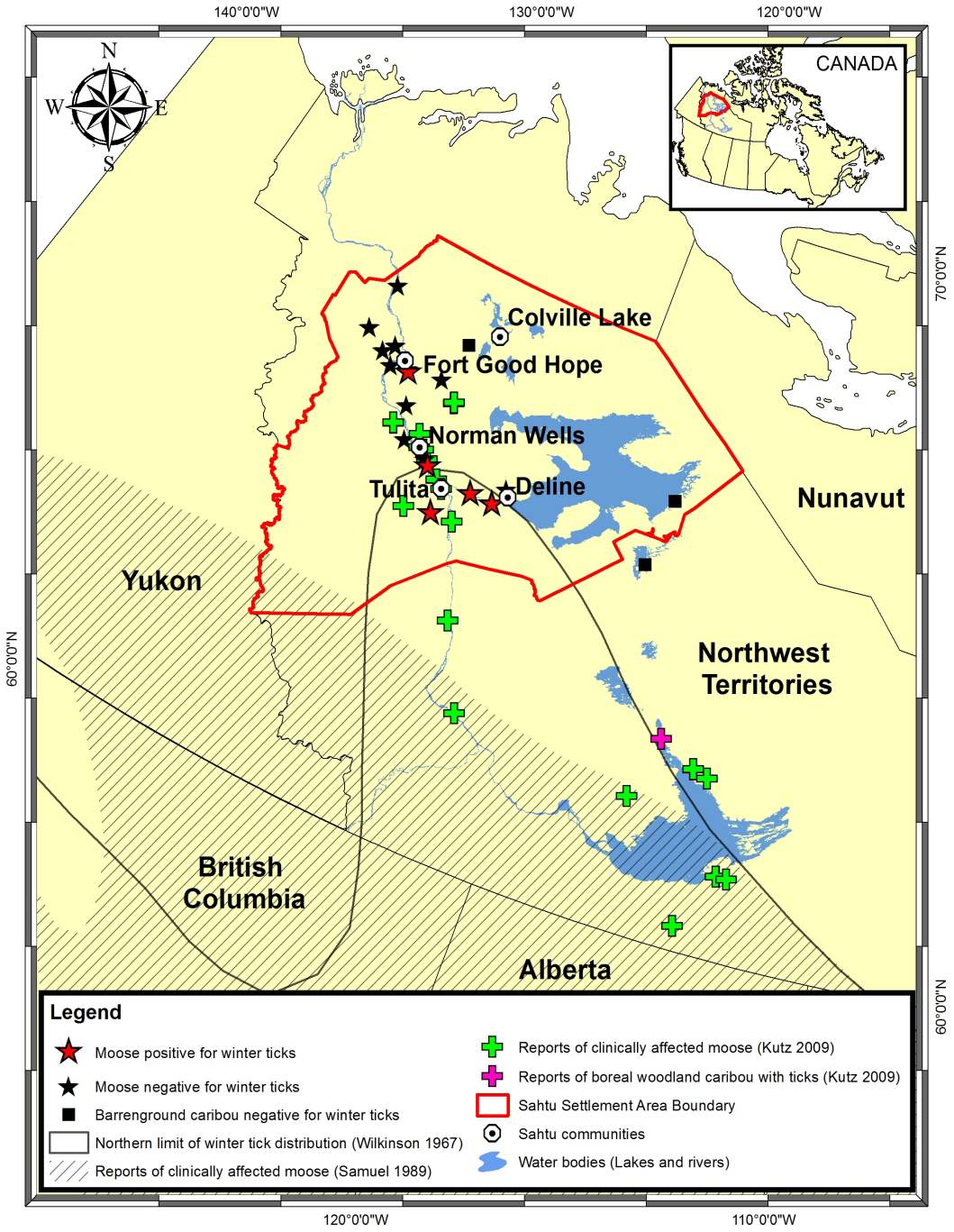


Figure 2.4. Map with northern limit of winter tick distribution suggested in Wilkinson's model in 1967 based on degree-days. Dashed line represents historical observations of clinically affected moose by Samuel (1989) and cross represent recent reports of 'ghost moose' (green cross) and reports of woodland caribou affected with winter ticks (pink cross) by Kutz (2009) in the NT. Red starts represent moose hides positive for winter ticks after analysis of hide digests and black starts represent negative moose collected from the Sahtu Settlement Area, NT. Black squares represent negative barrenground caribou hides.

Table 2.2. Geographic coordinates and collection date of moose hides positive for winter ticks according to the community in the Sahtu Settlement Area, NT.

Animal ID	Collection date	Community	Location	Latitude	Longitude
WT42	October 2nd 2010	Déline	Great Bear River	65° 01' 41"N	123° 51' 19"W
WT1029	October 12th 2011	Déline	Bennett Field (by Great Bear River)	65° 01' 48"N	124° 39' 00"W
WT1003	February 2011	Tulita	Gaudet Island	64° 32' 59"N	125° 34' 47"W
WT1012	Unknown 2011	Fort Good Hope	Fort Good Hope	66° 09' 09"N	128° 22' 47"W
WT1037	March 20th 2012	Tulita	18 mile Island (south side)	65° 06' 59"N	126° 20' 20"W

Data analysis

The overall mean density of ticks of all positive animals was 0.94 (SD±1.09) ticks/cm^2. The density per individual quadrant in these animals ranged from zero to 6.19 ticks/cm^2 (Table 2.3). The percentage of tick developmental stages varied considerably among animals (Figure 2.5), with the highest percentage of nymphs recovered from animals WT1029, WT1003, and WT1012. Animal WT42 was predominantly parasitized with larvae and animal WT1037 with adult ticks. Broken hair and alopecia was observed in animal WT1037, but was not observed in hides from animals infested with earlier tick stages.

Table 2.3. Total number of ticks found in samples of moose hide from the Sahtu Settlement Area, Northwest Territories, Canada. Each hide had 20 quadrants of 100 cm^2 sampled, corresponding to a total of 2,000 cm^2.

Animal ID	Total number of ticks / hide sampled	Mean (±SD)/ quadrant (n=20)	Median / quadrant (n=20)	Range / quadrant Min	Range / quadrant Max	Tick density (ticks/cm^2)
WT42	34	1.7 (±2.2)	1.0	0	7	0.017
WT1029	1,211	60.5 (±46)	51.5	8	151	0.605
WT1003	3,406	170.3 (±99.2)	152.5	36	388	1.703
WT1012	3,863	179.3 (±157.1)	146.0	17	619	1.793
WT1037	1,170	58.5 (±27.2)	55.0	20	115	0.585
Total						0.940

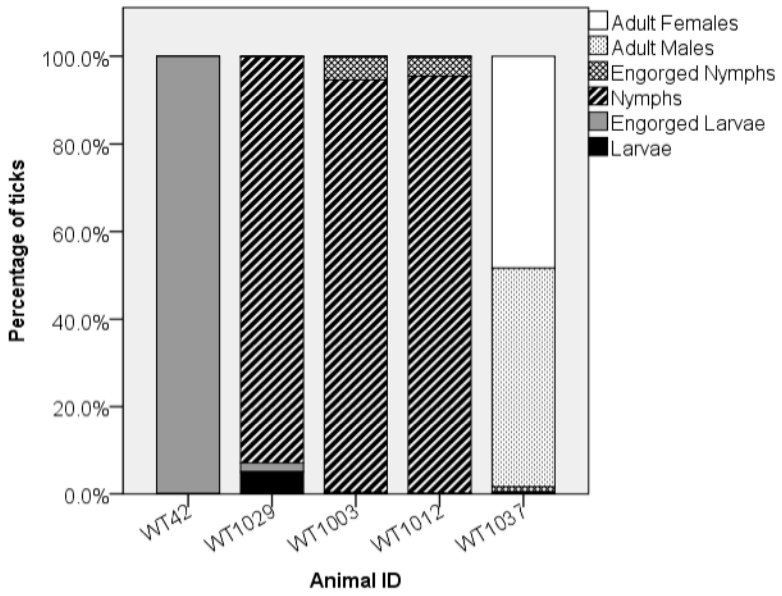


Figure 2.5. Percentage of each tick development stage observed on individual moose hides collected from September 2010 to May 2012 in the Sahtu Settlement Area, Northwest Territories, Canada.

The observed frequency of ticks in each sampling area (Neck, Shoulders, and Base of Tail) differed ($p<0.001$) from expected frequency on each individual animal; however, there was no consistent pattern across animals (Table 2.4, Figure 2.6). That is, for animal WT1012, the frequency of ticks was higher in the Neck section, but that was not true for all animals. Two animals were parasitized with higher number of ticks in the Shoulders (WT1003 and WT1037), one animal had higher number of ticks at the Base of Tail (WT42) and another animal had higher than expected numbers in both Shoulders and Base of the Tail (WT1029).

The observed frequency of different tick development stages in each sampling area also differed ($p<0.001$) from expected within an individual animal; however, once more, there was no consistent pattern across animals (Table 2.5). For animals WT1029 and WT1003, nymphs were highly concentrated in the Shoulder area and for animal WT1012 nymphs were more concentrated in Neck area.

Table 2.4. Chi-square (χ^2) analysis of the observed and expected frequency of winter ticks in each sampled section (Neck, Shoulders, and Based of Tail) according to the animal ID. Numbers in bold indicate the highest observed counts.

Animal ID	Sampling area	Observed	Expected	Chi-square (χ^2)
WT42	Neck	2	13.6	χ^2 (2) = 50.044,
	Shoulders	9	13.6	$p < 0.001$
	Base of Tail	**23**	**6.8**	
Total		34		
WT1029	Neck	143	484.4	χ^2 (2) = 473.263,
	Shoulders	**820**	**484.4**	$p < 0.001$
	Base of Tail	**248**	**242.2**	
Total		1211		
WT1003	Neck	1348	1362.4	χ^2 (2) = 401.741,
	Shoulders	**1799**	**1362.4**	$p < 0.001$
	Base of Tail	259	681.2	
Total		3406		
WT1012	**Neck**	**2639**	**1434.8**	χ^2 (2) =1685.850,
	Shoulders	658	1434.8	$p < 0.001$
	Base of Tail	290	717.4	
Total		3587		
WT1037	Neck	441	468	χ^2 (2) = 58.038,
	Shoulders	**579**	**468**	$p < 0.001$
	Base of Tail	150	234	
Total		1170		

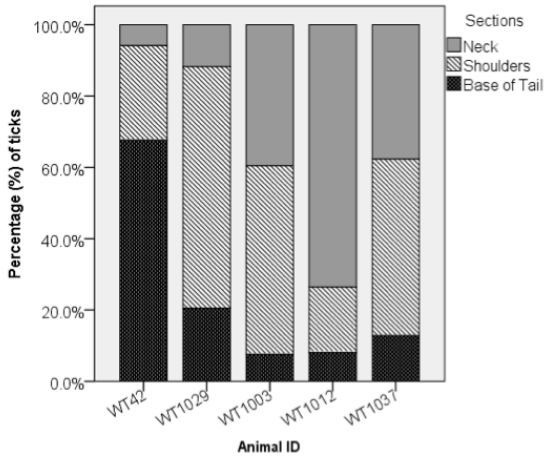


Figure 2.6. Percentage of winter ticks observed in the hide digests in each of the sections (Neck, Shoulders, and Base of the Tail) according to animal ID.

Table 2.5. Results of Chi-square (χ^2) analysis by animal ID, and observed and expected frequency of ticks in each section (Neck, Shoulders, and Based of Tail) according to tick development instars (Larval stage: larvae and engorged larvae; Nymphal stage: nymphs and engorged nymphs; and Adult stage: adult male and females). The results without enough cases will not be presented.

Animal ID	Instars	Sampling area	Observed	Expected	Chi-square (χ^2)
WT42	Larval stage	Neck	2	13.6	χ^2 (2) = 50.044,
		Shoulders	9	13.6	$p < 0.001$
		Base of Tail	**23**	**6.8**	
			34		
WT1029	Larval stage	Neck	12	34.4	χ^2 (2) = 35.512,
		Shoulders	**38**	**34.4**	$p < 0.001$
		Base of Tail	**36**	**17.2**	
			86		
WT1029	Nymphal stage	Neck	131	449.6	χ^2 (2) = 472.368,
		Shoulders	**782**	**449.6**	$p < 0.001$
		Base of Tail	211	224.8	
			1124		
WT1003	Nymphal stage	Neck	1345	1355.2	χ^2 (2) = 396.222,
		Shoulders	**1785**	**1355.2**	$p < 0.001$
		Base of Tail	258	677.6	
			3388		
WT1012	Nymphal stage	**Neck**	**2626**	**1428.4**	χ^2 (2) = 1674.800,
		Shoulders	655	1428.4	$p < 0.001$
		Base of Tail	290	714.2	
			3571		
WT1037	Adult stage	Neck	437	460.4	χ^2 (2) = 59.558,
		Shoulders	**570**	**460.4**	$p < 0.001$
		Base of Tail	144	230.2	
			1151		

DISCUSSION

This study provides the first definitive evidence, based on recovery and identification of ticks, that *D. albipictus* occurs in the Sahtu Settlement Area. These findings confirm previous anecdotal observations of ghost moose reported by Kutz (2009).

Unlike other areas of winter tick occurrence where nearly all moose examined were infested with *D. albipictus* (Samuel and Welch, 1991), in this study only five of the 30 moose hides examined (16.6%) were positive for winter ticks. Although the density of ticks observed in this study was lower (0.94 ticks/cm^2) than in other provinces of western Canada (1.43 ticks/cm^2) (Samuel and Welch, 1991), it was not statistically significant (One sample t test, *p*-value= 0.231). Nonetheless, it is important to consider that the number of ticks/cm^2 in this study may not represent the actual density of ticks on the animal because the hides in this study were sampled at predilection sites of winter tick attachment, and because only 10% of the whole hide was sampled to search for ticks. Despite the limited sample size, the low sample prevalence (number of positive cases divided by the total samples tested) observed in this study may suggest recent invasion of this parasite in northern latitudes. In other regions of winter tick occurrence (e.g. provinces of Alberta, British Columbia, and Manitoba) the sample prevalence also appears higher than that observed in this study (Samuel and Welch, 1991).

The low sample prevalence observed in this study could be associated with the low density and diversity of hosts, and/or a restrictive climate limiting development of free-living stages of winter ticks in northern latitudes. Parasite abundance is linked to host availability (Arneberg et al., 1998), and in locations where the host density is low and less diverse, the survival and transmission of parasites (basic reproductive rate - *Ro*) may be limited, which could possibly be the case of winter ticks in the Sahtu. Moose occur at much lower densities (maximum recorded 0.16 moose/ km^2) in the Northwest Territories than, for instance, in central Alberta, where density of moose is 7.5 times higher (1.20 moose/ km^2) (Stenhouse et al., 1995). Yet, despite the lower density it is likely that moose are the main

host sustaining winter tick populations in the region as few alternative ungulate hosts species are available for winter ticks in the Sahtu– white-tailed deer (*Odocoileus virginianus* Zimmermann, 1780) and mule deer (*Odocoileus hemionus* Rafinesque, 1817) are extremely rare, and elk (*Cervus canadensis* (Erxleben, 1777)) are absent from the region (Veitch, 2001; Kutz et al., 2012). Woodland caribou are also susceptible to the winter ticks, but the role of this ungulate species in maintaining the parasite in the Sahtu is not known.

In addition to host density, climate can also affect survival, development, and transmission of free-living stages of winter ticks at northern latitudes. However, under current climate scenarios, i.e., milder winters with earlier snow melt and later freezing in the fall, survival and transmission of free-living stages of *D. albipictus* in the Sahtu may increase. More specifically, engorged female ticks may encounter better conditions in late spring, produce more eggs, and larvae may persist longer in the environment, and thereby increase the risk of transmission to new hosts. Interestingly, even with the very limited number of positive cases, the results of this study suggest that winter tick instars are emerging at the similar time of the year as in other regions of Canada where winter ticks commonly occur (Addison and McLaughlin, 1988; Drew and Samuel, 1989).

Insights about hunter-based sampling in the Sahtu

Large-scale sampling, especially collection of valuable biological samples is very challenging in northern wildlife research due to limited access to resources, high costs for sampling, and logistics. Hunter-based approaches for caribou body condition and health assessment have been described for more than a decade in northern Canada (Kofinas et al.,

2003; Lyver, 2005; Brook et al., 2009), but have been challenged by a lack of active community engagement in the research process (Brook and McLachlan, 2008; Curry, 2009). Although challenges were encountered in hunter-based collections for winter tick surveillance in the Sahtu, without the hunter's involvement, it would have been extremely difficult to have access to moose and barrenground caribou hides. The fact that this research was created under the umbrella of an ongoing Wildlife Health Monitoring Program facilitated acceptance and increased participation of hunters in the winter tick study.

The inconsistency in data collection, however, hindered obtaining accurate information of the moose hide from Fort Good Hope that was positive for ticks. Based on this experience, there is certainly room for improvement in hunter-based collections in the Sahtu. Perhaps, a few adjustments of the sample kit to make it a more intuitive collection package would facilitate proper collection of biological samples and improve documentation of valuable data (e.g., hunting location, animal age, sex). In addition, intensive training of the new participants in the research and ongoing reinforcement of their knowledge about proper sample collection would result in more consistent and standardized biological samples. Nonetheless, despite these challenges, hunter-based sample collection is still the best approach to continue monitoring *D. albipictus* in the Sahtu because it allows engagement of local hunters in the research process and exchange of traditional and scientific knowledge. In addition, collection of moose and barrenground caribou hides would have been impossible without the participation of hunter and collaborators, and therefore, this approach is strongly recommended for future studies. However, it is important to emphasize that any program such as this that involves hunter-based collection

should include intensive training and ongoing reinforcement of their sample collection knowledge. On the other hand, it is also important to take into consideration that hunter-based sample collection is a relatively new approach implemented in the Sahtu. Few hunters have the background in science and in scientific collection methods, thus inconsistency in collection would be expected during the initial learning process.

Evaluations of hide sampling and chemical digestion of hides in KOH

Hide sampling

The use of chemical digestion of hides in KOH was fundamental to detect earlier stages of winter ticks, which are normally overlooked by naked eye. This technique has been effectively used for decades for winter tick surveillance (Addison et al., 1979; Samuel, 1989; Welch and Samuel, 1989; Samuel and Welch, 1991).

The main goal of this research was to determine presence of winter tick in the Sahtu. Although digestion of the entire hide would be the most sensitive method for detecting ticks, this was not logistically or financially feasible. Instead, hides were sampled at tick predilections sites, as described in Samuel (2004). With the exception of one animal (WT42), all analyzed quadrants of the positive animals (WT1029, WT1003, WT1012, WT1037) had ticks, suggesting consistency among sites, at least with respect to detection (not density). Although it is not possible to be certain that the sampling strategy was 100% sensitive (i.e., no false negatives), it is likely that it provided a reasonable index of occurrence of winter ticks in the Sahtu.

The process of sending entire hides to the University of Calgary and then sampling them at the predilection sites increased considerably the costs in transportation and also

challenged the logistics. It would have been more practical and cost-effective if sampled sections were sent for analysis instead of the entire hide, thus for future studies, the recommendation is to request hunters to focus hide sampling only at the Neck and the Base of the tail. It is likely that based on the results of this study one piece of 10x40 cm from each area would provide a reasonable index for winter tick presence and dramatically reduce the costs in transportation and facilitate the logistics in future studies. In addition, given that many people from the Sahtu still use moose and caribou hides for tanning and for traditional clothing, asking hunters to collect small pieces of hides would cause less damage on hide for tanning, which would probably make it hunters more willing to collaborate with the research and facilitate collection and transportation of the samples.

This sampling method does not provide an accurate estimate of the actual density of ticks on the hide because the sampling was biased towards the predilection sites, and also because this sampling protocol only encompassed 10% of the whole hide. To obtain accurate estimation of the tick density, random sampling of at least 15% of half of a moose hide is recommended to reduce bias and increase accuracy between actual tick density and estimated tick density (Welch and Samuel, 1989).

Chemical digestion of hides

Despite its efficacy, the procedure of chemical digestion of hides was labour-intensive and time-consuming. For example, the total time required to chemically digest all 1,100 sub-samples of moose (n=600) and barrenground caribou (n=500) hides with approximately 7- 12 sub-samples digested per day, was 210,617 (SD± 68) minutes (approximately 3,500 hours). If there were an alternative method that has the same

detection capacity as chemical digestion of hides, but less time-consuming, the analysis of the samples would have been more efficient.

CONCLUSION

The results of this study show the first evidence of *D. albipictus* occurrence in the Sahtu Settlement Area based on recovery of ticks, and these reports represent the northernmost reports of *D. albipictus* distribution in Canada.

Hunter-based sample collection was fundamental to have access to moose and barrenground caribou hides from the Sahtu. Even though inconsistency in sample and data collection hindered obtaining accurate information of these moose and caribou hides, it is still the best approach for surveying ticks in remote and vast areas such as the Sahtu.

CHAPTER THREE

EXPERIMENTAL INFESTATION OF CAPTIVE REINDEER (*RANGIFER TARANDUS TARANDUS* LINNAEUS, 1758) WITH WINTER TICKS (*DERMACENTOR ALBIPICTUS* (PACKARD, 1869))

INTRODUCTION

Dermacentor albipictus (Packard, 1869), the winter tick, is a one-host ixodid tick first reported on moose from Nova Scotia (Packard, 1869) but known to parasitize domestic cattle, horses and other wild ungulates species (Drummond et al., 1969). Moose (*Alces americanus*) are the most susceptible and the most severely affected host by *D. albipictus* (Welch et al., 1991). Clinical signs and pathology commonly associated with winter tick infestations on moose include extensive alopecia, anaemia due to tick feeding, poor body condition, and mortality of severely affected animals (McLaughlin and Addison, 1986; Musante et al., 2007; Samuel, 2007).

Winter ticks are widely distributed across Canada, have the northernmost distribution among the *Dermacentor* species (Wilkinson, 1967), and their range appears to be expanding. Recent anecdotal reports of clinically affected moose as far north as the Sahtu Settlement Area in the Northwest Territories, suggested that *D. albipictus* was present in this area (Kutz et al., 2009). My subsequent work (Chapter 2), confirmed the occurrence of winter ticks on moose from this region in the Northwest Territories through recovery and identification of ticks in hide digests. These findings considerably exceed the previously suggested northern limit of winter tick distribution (Wilkinson, 1967), and raise a concern about the potential for this parasite to invade and establish on other sympatric ungulate species, such as woodland (*Rangifer tarandus caribou* (Gmelin, 1788)) and barrenground caribou (*Rangifer tarandus groenlandicus* (Borowski, 1780)).

The impacts of winter tick infestations on hair coat, blood parameters, and body condition of moose are well documented (Glines and Samuel, 1984; McLaughlin and

Addison, 1986; Glines and Samuel, 1989; Welch et al., 1990a; Mooring and Samuel, 1999). Similarly, winter tick growth and development on moose and other ungulate species such as elk (*Cervus canadensis* (Erxleben, 1777)), mule deer (*Odocoileus hemionus* (Rafinesque, 1817)), and white-tailed deer (*Odocoileus virginianus* Zimmermann, 1780) has been widely studied through experimental infestations (Drummond et al., 1969; Addison and McLaughlin, 1988; Drew and Samuel, 1989; Welch et al., 1991; Welch et al., 1991). In contrast, although it is known that *Rangifer* species are susceptible to *D. albipictus* in captive and wild situations (Welch et al., 1990b), little is known about the development and effects of ticks on these ungulate species.

The purpose of this research was to develop an experimental reindeer-winter tick model that could be used to (1) produce winter tick-challenged sera from captive reindeer for further immunodetection studies, and to (2) investigate the growth and development of the winter ticks on captive reindeer.

MATERIALS AND METHODS

Winter ticks – rearing free-living stages under laboratory conditions

Engorged female winter ticks were collected from a road-killed moose near Elk Island National Park, AB (53°36′11″ N, 112°53′19″ W) and transported on 18 of May 2010 to the Veterinary Parasitology lab at the University of Calgary. Using double-sided tape, ticks were attached to a Petri dish for oviposition and these dishes placed into a plastic container with moist paper towel at room temperature (~23°C) (Figure 3.1) (Prata et al., 1999). When oviposition was complete, i.e., female ticks were dead, the egg mass was transferred into 15 ml plastic test tubes (BD Biosciences, Mississauga, Ontario) with a fine

fabric mesh on top and kept at room temperature until hatched into larvae. These were then individually counted using a vacuum apparatus and placed into new plastic tubes containing 750 larvae/tube. Each tube was covered with a fine fabric mesh (Figure 3.2) and maintained at room temperature (~23°C) at 95% humidity until used to infest the reindeer calves.

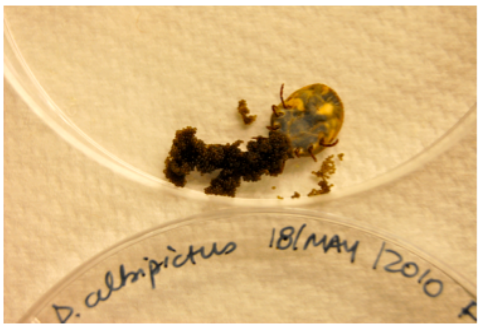
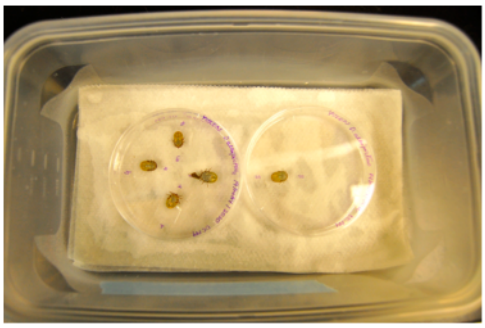
(A)  (B) 

Figure 3.1. Laboratory conditions under which the engorged female ticks were maintained to ovipost. (A): Engorged female winter tick attached to a Petri dish to ovipost and (B): Petri dishes with ticks inside a plastic container with moist paper towel.

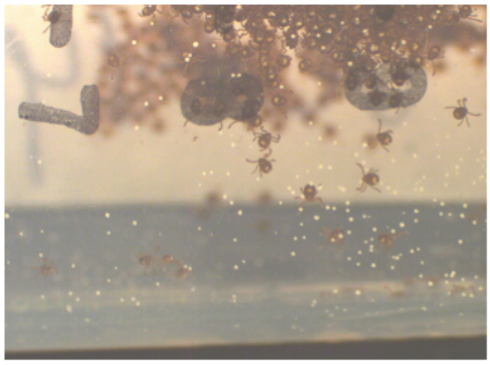
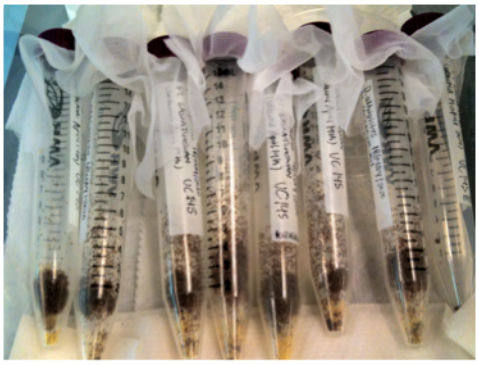
(A)  (B) 

Figure 3.2. Laboratory conditions under which winter tick eggs were kept for larvae to emerge. (A): Winter tick larvae in detail and (B): Conditions under which winter tick larvae were maintained until being used to infest the captive reindeer calves.

Reindeer infestation

On 20 of September 2010, before the experimental trial, four captive born and reared reindeer calves (two males and two females) between 5 to 6 months old were treated with subcutaneous (sc) administration of doramectin (200µg/kg) (Pfizer Animal Health,

Pfizer Canada Inc, Kirkland, QC) to clear any pre-existing parasite infection or infestation. On 18 of November 2010, calves were separated from the herd and moved from the Wildlife Research and Teaching Facility to the Veterinary Sciences Research Station (VSRS) at the University of Calgary for this experiment. At the VSRS facility, reindeer calves were housed in individual outdoor pens allowing visual contact to each other to minimize stress, and monitored daily for health conditions. A week before the infestation, "tick cages" were placed on the back of the animals to ensure that the animals were adapted to the cages before exposing them to the ticks. The "tick cages" consisted of a stockinet sleeve (QMD Medical, Montreal, Quebec, Canada) glued with non-toxic contact cement to a shaved area on the back of the animal (LePage® Pres-Tite® Green Contact Cement) (Lysyk and Majak, 2003).

Each animal had four regions (each 10 cm diameter) on the dorsal mid-line (two near the shoulders and two at the rump) in which the hair was shaved to a length of approximately 3 cm, and to which the cages were glued (Figure 3.3). On 26 of November 2010, each calf was infested with 3,000 winter tick larvae equally distributed in four tick cages (750 ticks/cage) and with the top securely fastened with black Velcro® strips to prevent ticks from escaping. The main purpose of these cages was to facilitate tick collection by confining ticks to a small area and to prevent contamination of the facility where the animals were housed. Successive steps of the infestation procedure are fully described in Appendix B.

Tick attachment success and development stage were monitored weekly from infestation (Day 0) until Day 153 post-infestation. Ticks were examined by parting the hair

along one 10 cm cranio-caudal and one 10 cm latero-lateral transect; attached ticks were counted, classified according to developmental instars, and recorded in the data sheet.

Figure 3.3. Stockinet sleeves were attached with non-toxic glue along the midline of the back of the animal (two near shoulders and two at the rump area). Ticks were placed inside the sleeves and the top was securely fastened with black Velcro® strips.

Re-infestation

At 153 days after the initial infestation (29 of April 2011), each reindeer calf was re-infested with adult ticks collected from two culled moose (an adult male and a female calf) from Peace River, AB (56°14′89″ N, 117°17′17″W). Each reindeer was infested with 210 adult winter ticks (200 flat females and 10 adult males) that were equally distributed in two tick cages (105 total per cage). The four adult ticks observed (2 males and 2 females) from the initial infestation were not removed from the infested animals for the re-infestation procedure. Tick attachment was monitored every other day and, once engorged,

female ticks were manually removed, kept in plastic tubes and transported to the laboratory for saliva extraction (Chapter 4).

The experiment was terminated on 27 of May 2011 (29 days following the re-infestation and 182 days since the initial infestation). All ticks observed in the cages on the animals at that point were manually removed. Calves were then visually inspected for ticks on other parts of their bodies, treated with subcutaneous administration of doramectin (200μg/kg) (Pfizer Animal Health, Pfizer Canada Inc., Kirkland, QC), and kept in quarantine for 42 days, when they were moved back to the Wildlife Facility to join the main research herd. All procedures described were in accordance with the University of Calgary Animal Use Protocol Guidelines (Protocol number: BI10R-14).

Blood sample collection

Blood samples were collected from the reindeer under manual restraint and by jugular venipuncture, starting at the day of infestation (Day 0), and continuing monthly until a year after the first exposure to winter ticks. Blood samples were collected in three Vacutainer red top glass tubes (10 ml) and in one Vacutainer purple top plastic tube (4 ml) containing Ethylenediaminetetraacetic acid (EDTA). In the laboratory, sera were separated from the whole blood by centrifuging the red top tubes at 3,000 rpm for 10 minutes, distributed in cryotubes of 1.5 ml, and stored at -20°C for future use in immunodetection trials. The blood sample containing EDTA was sent to IDEXX Laboratories for Complete Blood Count (CBC) analysis to investigate if there were any alterations on the blood cell counts due to experimental infestation.

RESULTS

Winter ticks – rearing free-living stages in laboratory

The oviposition period of the engorged females ticks collected from Elk Island National Part was from 21 of May to approximately 24 of June 2010 (35 days). Pre-oviposition period could not be calculated because there was no information about the date when ticks were collected from the road-killed moose. Larvae were first observed on 28 of June and hatching was complete by 8 of July (11 days).

Reindeer infestation

A week after the initial infestation large numbers of larvae were dead inside the top of the stockinet sleeve. The first engorged larva was observed at 14 days post-exposure, the first nymph at 21 days, and the first adult tick (male) at 125 days after the initial infestation (Table 3.1 and Figure 3.4). At 146 days post-infestation, only four adult ticks (two males and two females) were recorded on reindeer. More ticks were observed and counted on Reindeer 1 and 2 than on Reindeer 3; no ticks were ever recovered from Reindeer 4 (Figure 3.5).

Table 3.1. Date and days after initial infestation of the first and last observations of tick development instars on four experimentally infested reindeer.

Tick instars	First observation	Last observation
Engorged larvae	11 of December 2010 (14)*	18 of December 2010 (21)
Un-engorged nymph	18 of December 2010 (21)	11 of March 2011 (104)
Engorged nymph	18 of March 2011 (111)	25 of March 2011 (118)
Adult male	1 of April 2011 (125)	29 of April 2011 (153) [++]
Adult female	4 of April 2011 (146)	29 of April 2011 (153) [++]

* Date (days after infestation)
[++] Date adult tick were last observed before re-infestation of reindeer

Re-infestation

The experiment was terminated after 29 days following re-infestation and 182 days since the initial infestation. The first engorged female was observed 8 days (Day 161) after the re-infestation. A total of 35/802 ((200 x 4 animals) + 2 female ticks from initial infestation) of engorged female ticks was collected at the end of the re-infestation experiment (Figure 3.4). This number corresponds to only 4% of the initial number of females applied on all four reindeer for the re-infestation experiment. No information was collected about the attachment success of male ticks.

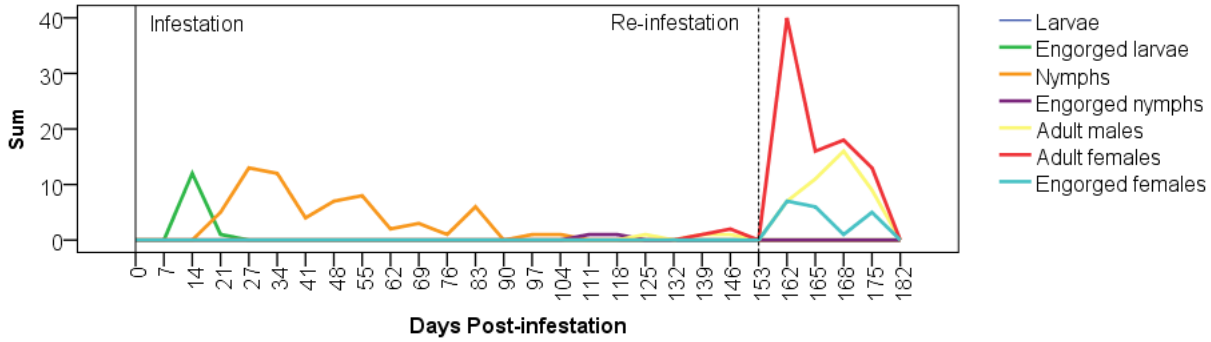


Figure 3.4. Total number of tick observations recorded according to tick development instars in all infested reindeer throughout the experimental infestation.

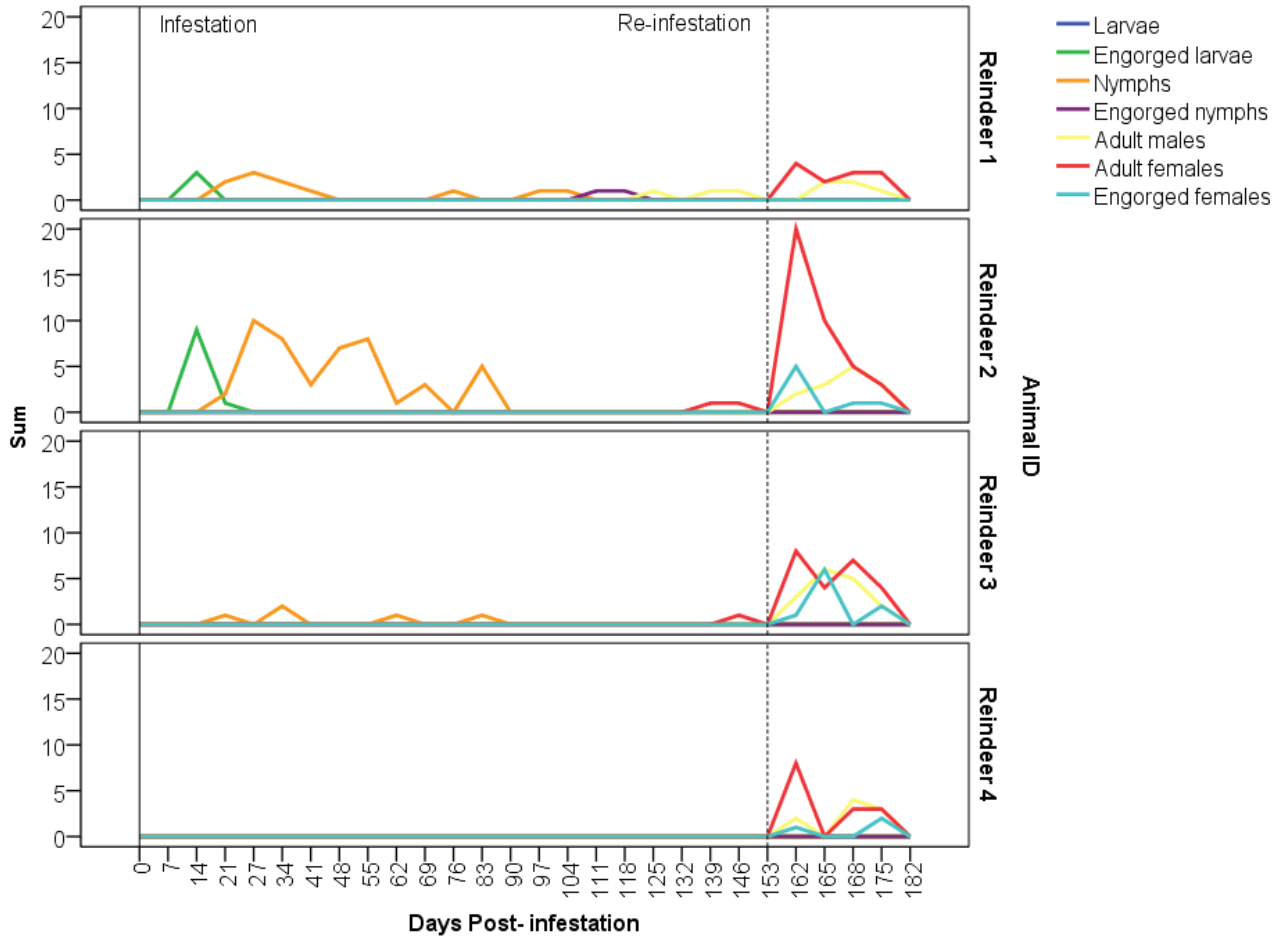


Figure 3.5. Total number of ticks recorded, according to tick development instars and reindeer, throughout the experimental infestation.

Blood sample collection

No significant alterations were observed in blood parameters of reindeer infested with winter ticks (Appendix C) when compared to 'normal' reindeer blood values available in literature (Timisjärvi et al., 1981; Nieminen and Timisjärvi, 2010).

DISCUSSION

Tick cages have been used for many decades on cattle, sheep, and rabbits to rear ticks (Gregson, 1966), but no studies have been previously done using this technique on

captive reindeer. This was the first study to use such methodology on captive reindeer for tick rearing and despite the long period (182 days) that the animals had to remain with the tick cages in place, the animals did not seemed uncomfortable with the tick cages and they did not try to remove the cages by grooming. One of the concerns of keeping the tick cages for such a long time was the occurrence of dermatological reaction to the contact cement; however, no such reactions were observed at the tick placement sites throughout the infestation, likely because the tick cages were attached to the partially shaved hair and not directly to the skin. Because the cages were glued to hair, and because of the long infestation period, the tick cages did require constant maintenance to ensure they were properly attached on the back of the animals. Despite these challenges, the use of this technique is still strongly recommended for future studies with reindeer. Very few adult ticks (2 females and 2 male) were recovered after the initial infestation and, likely, the visualization of this small number was possible because the tick cages limited the movement of these ticks.

Insights about experimental winter tick infestation on captive reindeer

The causes of the high larval mortality after the initial infestation are not clear; however, this likely had a major impact on the total number of ticks recovered. Several factors such as the subcutaneous administration of doramectin (Pfizer Animal Health, Pfizer Canada Inc, Kirkland, QC) before infestation, viability of the tick larvae, the number of ticks used to infest the reindeer, and even the host susceptibility could have possibly influenced for these results.

Doramectin is an endectocide drug that belongs to the group of the avermectins with a wide spectrum activity (Lifschitz et al., 1999; Kanbur et al. 2008). Avermectins are products derived from the fermentation of the fungus *Streptomyces avermitilis* with great antihelminthic and insecticidal proprieties. Ivermectin and abamectin also belong to this group and are commercially available in several formulations for veterinary use.

The persistence of the drug in the sera and its efficacy varies with the route of administration. For example, serum concentrations of a single subcutaneous administration of a long-acting formulation of ivermectin (630μg/kg) in cattle remained above the threshold necessary to control feeding ticks (≥8 parts per billion) for 42.6 days (Davey et al., 2010). In another study, ivermectin was detected in cattle sera (> 5ng/ml) after 27.5 days of intramuscular and subcutaneous administration (Lifschitz et al., 1999). More specifically, for the reindeer, serum concentration of ivermectin was still detectable, but close to zero, after 21 days of topical (500μg/kg), oral (200μg/kg) and subcutaneous (200μg/kg) administration (Oksanen et al., 1992). Even though it is likely that after 67 days the serum concentration of doramectin would be close to zero as observed by Oksanen et al. (1992), it is difficult to affirm with confidence if in this study the high larvae mortality was associated with the administration of doramectin before infestation, as sera concentration was not measured.

A positive correlation between the response time of winter tick larvae to host stimuli and the progression of the transmission season has been previously reported (Samuel et al., 2000). That is, the later in the transmission season, the longer the response time (in seconds) of winter tick larvae to host stimuli (CO_2 and thermal stimuli). This correlation appears to be associated with the depletion of energy reserves, and increase in

reaction time to stimuli, and decrease success in infesting a host as larvae become older (Samuel et al., 2000). This could be a possible explanation for the high larval mortality and low attachment rate in this study. The larvae used in this experiment were 4 months old and the infestation was done late in the season (26 of November 2010).

Furthermore, Samuel et al. (2000) showed a negative association between cold temperatures and the response time of larvae to host stimuli, i.e., the lower the temperature the longer was the response time of larvae from inactive to active state (Samuel et al., 2000). Hence, another reason for the high mortality observed in this study could be related to the exposure of tick larvae to cold temperatures after being placed on the reindeer. That is, before infestation, larvae were maintained in the laboratory at room temperature (~23°C) and then, approximately 30 minutes after the infestation procedure, which was also done at room temperature, reindeer were moved to outside pens. The mean ambient temperature at the date of the infestation (26 of November 2010) was 0.8°C, with the maximum and the minimum temperature 5.6°C and -4.0°C, respectively (National Climate Data and Information Archive, Environment Canada), thus larvae may have experienced subzero temperatures and died before reaching the host skin. The initial assumption was that the remaining hair inside the tick cages would protect the ticks from the extreme temperatures during winter, but it is hard to measure if the hair had any influence in protecting them or if it was more an obstacle for the ticks to reach the skin.

The total number of winter tick larvae used in this experiment was lower than the numbers used in other experimental studies with cattle, moose, or other ungulate species (Drummond et al., 1969; Addison and McLaughlin, 1988; Drew and Samuel, 1989; Glines and Samuel, 1989; Welch et al., 1990a; Welch et al., 1991). It is thus not surprising to have

recovered a small number of ticks at the end of this experimental study. In a study by Welch (1991), after infesting a moose with 52,900 winter tick larvae, only 8% (4,217) were recovered as engorged females, and only a few males and partially engorged females were recovered from a white-tailed deer infested with more than 17,000 winter ticks. Perhaps, if a greater number of winter tick larvae were administered on each reindeer, the total adults ticks recovered at the end of this experiment would have been higher. However, it is important to highlight that Welch (1991) exposed the whole body surface of the animal to ticks and thus could use a larger number of ticks than if they were confined to only a few cages. Because this study was the first to experimentally infest captive reindeer with winter ticks, a more conservative approach was taken by using a lower number of ticks and by restricting these ticks in cages. This, together with high mortality of larvae, may have contributed to the outcome of this experiment.

In regards to the re-infestation, a very small number of engorged female ticks were recovered at the end of the experiment. It is difficult to determine if the interruption in feeding of the adult ticks and the time these ticks spent in the laboratory under controlled conditions before being placed on the reindeer had any influence on their attachment success on the reindeer. However, studies have demonstrated that female *Rhipicephalus appendiculatus* Neumann, 1901 (Acari: Ixodidae) ticks that had feeding interrupted for four weeks were still able to successfully re-attach to the second host, to reach engorgement weight and lay large egg masses similar to female ticks that did not have interrupted feeding (Wang et al., 1999), thus other factors are likely to have contributed to the recovery of such a small number of engorged female ticks.

Feeding, maturation, and fecundity of tick species are influenced by host species (Gregson, 1966; Addison and McLaughlin, 1988; Welch et al., 1991) and this may have played a role in the recovery of engorged female winter ticks in this study. For instance, females of *Hyalomma rufipes* Koch, 1844 (Acari: Ixodidae) are able to feed and engorge on dogs and rabbits, but these ticks only produce viable larvae if they are fed on ruminants (Gregson, 1966). More specifically for *D. albipictus*, engorged females recovered from less suitable hosts, such as mule deer and elk, were smaller in size at the end of experimental infestation than engorged females collected from moose (Welch et al., 1991). It is known that *Rangifer tarandus* ssp. are highly susceptible to winter ticks in captive situations; however, the suitability of reindeer as host for winter ticks in comparison to other ungulate species such as moose, elk, or white-tailed-deer has not been yet determined.

Winter tick development on reindeer

The growth and development of winter ticks in reindeer was consistent with the pattern observed in moose: short parasitic larval stage, prolonged nymphal stage (due to diapause), and observation of adult ticks from January to March (Addison and McLaughlin, 1988). According to Addison and McLaughlin (1988), winter tick larvae moulted into nymph stages between 10 - 22 days after infestation, nymphs had a prolonged stage (22 – 160 days after infestation), and the first adults were observed at 104 days post-infestation. Despite the low number of ticks recovered in this study, tick development could be followed throughout the experimental infestation. On the experimentally infested reindeer, winter tick larvae were engorged by day 14 post-infestation, moulted into nymphs by day 21, and the first adult observed was at day 125. Due to small number of ticks recovered in this study, the variability in the development of each tick instar could not be determined.

CONCLUSION

This was the first study attempting to experimentally infest captive reindeer with *D. albipictus* and as with any first attempt, several lessons were learned throughout the process. Numerous aspects of the infestation procedure can be improved and some of these insights are shared in the following paragraph.

First, withdrawing administration of doramectin before experimental infestation could prevent harm to ticks. Alternatively, if the doramectin administration is necessary to control other pre-existing arthropods or nematodes species, measuring the concentration of the drug in sera and making sure that it is below 8 ppb before infestation could minimize harm to ticks. Second, timing the experimental infestation earlier in the season with younger larvae may help reduce larval mortality at infestation, improve attachment rate, and consequently, increase number of recovered ticks. Also, housing the host in room temperature after infestation procedure until the tick larvae are acclimatized may possibly contribute to attachment success and establishment of tick larvae.

Third, increasing the dosage of ticks administered on each reindeer may contribute to recovery of a higher number of adult ticks at the end of the experiment. Finally, administering ticks on the whole body of the animal instead of restricting them into tick cages would permit increasing the dosage of ticks and allow better distribution of the ticks on the animal.

CHAPTER FOUR

WESTERN BLOT ANALYSIS TO INVESTIGATE THE ANTIBODY RESPONSE OF CATTLE (*BOS TAURUS*) EXPERIMENTALLY INFESTED WITH WOOD TICKS (*DERMACENTOR ANDERSONI*) AND WINTER TICKS (*DERMACENTOR ALBIPICTUS*)

INTRODUCTION

Ticks are obligate bloodsucking parasites of mammals, birds, reptiles and amphibians (Anderson and Magnarelli, 2008) found worldwide, with great importance to veterinary and public health (Sonenshine et al. 2002). Ticks cause great health and economic impacts in wild species and domestic livestock (Brown, 1985; Frisch, 1999; Jongejan and Uilenberg, 2004) as vehicles of several bacterial, viral and protozoan diseases, and by causing severe dermatological reactions, increased grooming and altering foraging behaviour on animals due to tick feeding. Hence, methods for tick surveillance need to be sensitive and efficient to provide accurate information about tick occurrence in a cost-effective manner to allow better planning and management strategies for tick control.

The currently available methods for tick surveillance can be classified into two major categories: (1) techniques that involve detecting free-living tick stages in the environment through flagging and carbon-dioxide-baited traps and (2) the methods that involve collecting ticks directly from the host either though manual removal or through chemical digestion of hides in potassium hydroxide (KOH) (Ginsberg and Ewing, 1989). These are classic methods and have been used efficiently by many researchers (Wilson et al., 1972; Ginsberg and Ewing, 1989; Jensen, 2000; Cançado et al., 2008; Kensinger and Allan, 2011); however, performing these techniques in vast and remote areas, such as northern Canada, can be logistically challenging, time consuming, and impractical in many situations. Alternative techniques could potentially reduce the costs related to logistics, particularly transportation, and thereby considerably increase the efficiency of the surveillance.

Serological diagnosis is a potential alternative method for tick surveillance. Hosts develop acquired immunity to ticks, and although there are many studies on the immunological responses to ticks (Kemp et al., 1986; Wikel, 1988; Pruett, 1999; Manzano-Roman et al., 2006), there are few that apply this knowledge for tick surveillance (Canals et al., 1990). Canals et al. (1990) developed an enzyme-linked immunosorbent assay (ELISA) to detect specific antibodies in pigs infested with *Ornithodoros erraticus* (Lucas, 1849) (Acari: Argasidae). The technique was sensitive enough to detect infestations with as few as 10 ticks; however, it was low in specificity (Canals et al., 1990).

Many factors related to the complexity of host immunity to ticks, such as the host species and host individual variability, duration of the immune response, variability in tick antigens among tick species and between feeding stages, may be hindering the development of a serodiagnostic tool and limiting the production of a protective and cost-effective vaccine to control ticks (Kemp et al., 1986; Ferreira et al., 1996; Cruz et al., 2008). Nonetheless, many researchers have detected host antibody response against tick extracts using common serological techniques such as immunodetection (known as Western blot) in laboratory experiments (Pruett et al., 2006; Cruz et al., 2008). This highlights the possibility of using this technique as an alternative method for tick surveillance.

The objective of this study was to use Western blot to investigate antibody response of cattle after exposure to *Dermacentor andersoni* Stiles, 1908 (wood ticks) and *Dermacentor albipictus* (Packard, 1869) (winter ticks), and to determine which tick salivary proteins were consistently found to be immunogenic. I first compare and describe similarities and differences in salivary proteins of wood tick and winter tick. I then investigate cattle antibody response to tick salivary proteins in Western blot using a

colorimetric detection method, and then describe the troubleshooting trials and the optimizations attempted to find a protocol for chemiluminescence detection method.

MATERIALS AND METHODS

Production of tick-challenged sera

Source of ticks

Adult specimens of *D. andersoni* (wood tick) were obtained from a tick colony maintained at the Lethbridge Research Centre, Agriculture Agri-Food Canada (Lysyk and Majak, 2003). Tick larvae and nymphs were reared on domestic rabbits and held at 25^{o}C and 95% relative humidity until they moulted to adults, which were then later used to infest bovine calves (Group 1). Adult specimens of *D. albipictus* (winter tick) were collected on 10 of March of 2011 from two culled moose (an adult male and a female calf) from Peace River, Alberta (AB). Ticks were maintained at 10^{o}C and 95% relative humidity for approximately two months and then used to infest bovine calves (Group 2) at the Lethbridge Research Centre.

Cattle infestation

Eight male Holstein calves (3-4 months old), naive to ticks, were housed individually in indoor pens at the Lethbridge Research Centre and separated into two groups of four animals to be experimentally infested with female wood ticks (Group 1) and female winter ticks (Group 2). Each calf had one tick cage attached on its back for the infestation procedure (detailed description of the tick cage placement on the back of the animal and infestation procedure is provided in Lysyk and Majak (2003) (Appendix B). On

11 of May 2011 (Day 0), the animals from Group 1 were exposed to 50 wood ticks each, and the Group 2 animals were exposed to 56, 56, 57, and 57 winter ticks (Table 4.1). The tick attachment success was monitored every other day and ticks were manually removed from cattle once they were engorged. On Day 14, the experiment was terminated and the ticks that were remaining on the animals manually removed.

Blood samples were collected by jugular venipuncture on the day of infestation (Day 0) before exposure to ticks (Pre- = pre-exposure), and monthly, until three months post-exposure (P1= one month post-exposure, P2= two months, P3= three months post-exposure to ticks). The serum samples were separated from the whole blood and stored at -20^{o}C until use. All protocols were in accordance with both the Canadian Council of Animal Care Guidelines and the Lethbridge Research Centre Animal Care Committee (Protocol number: LRC 1119).

Table 4.1. Number of wood and winter ticks to which each cow was exposed in 2011.

Tick species	Number of ticks	Animal ID
Dermacentor andersoni	50	Group 1: 117, 129, 136, 140
Dermacentor albipictus	56, 56, 57, 57	Group 2: 115, 121, 124, 138

Archived bovine sera

Archived sera of 12 cattle exposed to wood tick were also available for use in this study. In 2009, 12 bovine calves, naive to ticks, were experimentally infested with adult wood ticks at the Lethbridge Research Centre for other research purposes. These animals were divided into four groups and infested with 25 (n=6), 50 (n=2), 100 (n=2), or 150 (n=2) adult wood ticks (Lysyk, personal communication) (Table 4.2) and in all cases ticks were left on the animals until 14 days post infestation. Blood samples from these animals were

collected before exposure to ticks (Pre-), and then monthly, until three months after exposure (P1, P2, and P3).

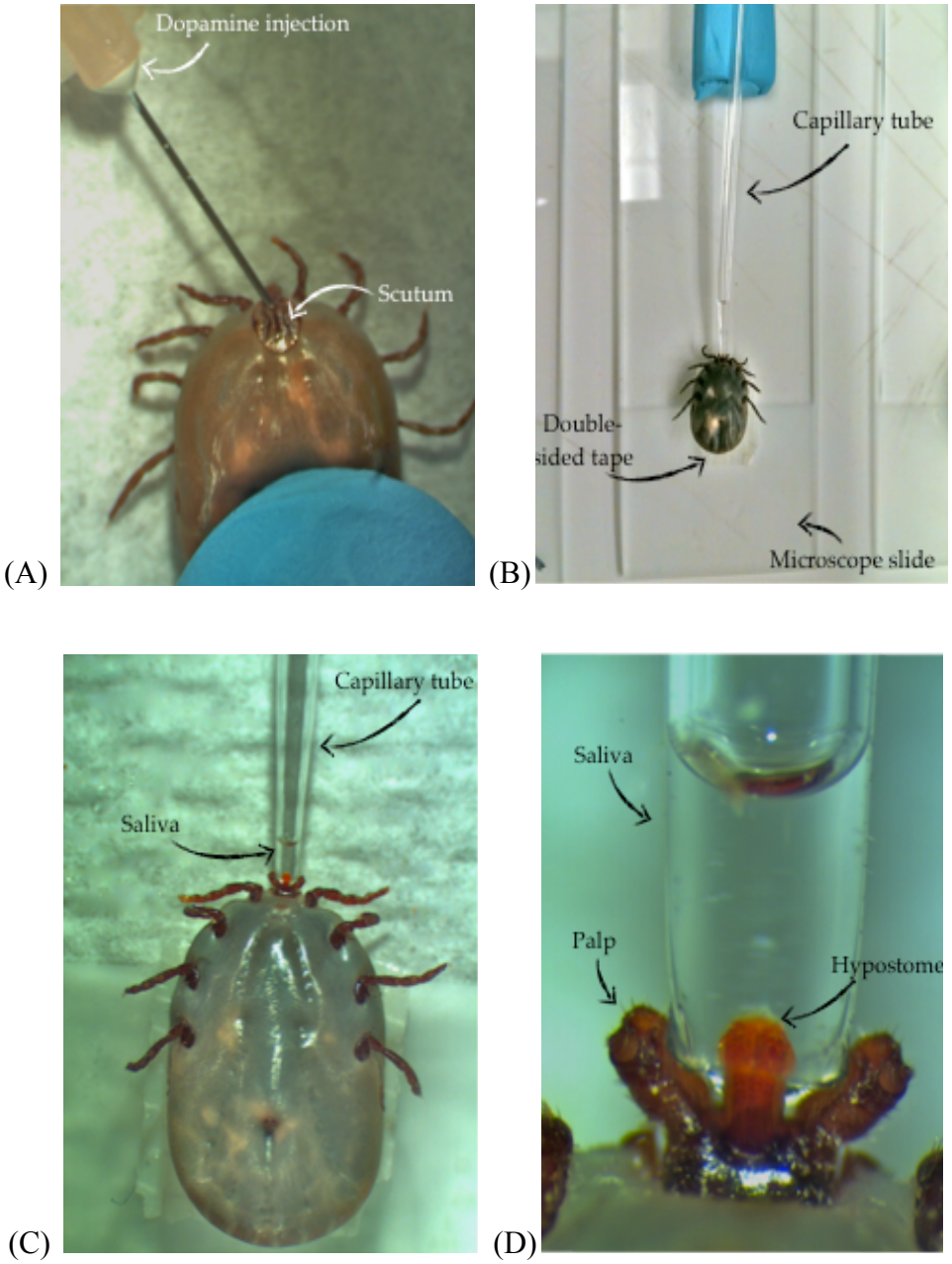
Table 4.2. Number of wood ticks to which each cow was exposed in 2009.

Tick species	Number and Sex of ticks	Animal ID
Dermacentor andersoni	75 females and 75 males	915, 920
	50 females and 50 males	919, 931
	25 females and 25 males	926, 933
	25 females	923, 925, 929, 932, 941, 946

Comparison of proteins present in wood tick and winter tick saliva using Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE)

Collection of tick saliva

Saliva was extracted from partially engorged females immediately after ticks were removed from the experimentally infested animals. Each tick was weighed before saliva extraction and salivation was induced by injecting 10 μl per 100 mg of tick body weight of Dopamine solution (5mM dopamine hydrochloride (Sigma Life Science, SIGMA-ALDRICH, St. Louis, Missouri, USA) in 0.9% sodium chloride solution) underneath the scutum of the tick (Kaufman, 1978). After injection, ticks were placed on their backs and attached onto a microscope slide using a double-sided tape. Under a dissecting microscope at 10x magnification, a capillary tube (10μl) was placed over the chelicerae and hypostome for saliva collection (Kaufman, 1978) (Figure 4.1).



(A) (B) (C) (D)

Figure 4. 1 Procedure for tick saliva extraction. (A) Injection of 10µl of dopamine solution (5mM dopamine hydrochloride (Sigma Life Science, SIGMA-ALDRICH, St. Louis, Missouri, USA) in 0.9% sodium chloride solution) underneath the scutum of the engorged female tick; (B) Placement of the tick onto the microscope slide after dopamine injection; (C) Placement of the capillary tube on their mouthparts, and (D) detailed picture showing the location the capillary tube is placed for saliva collection.

Wood tick saliva was extracted from ticks collected from the experimentally infested cattle in 2011. Winter tick saliva was extracted from ticks collected from (a) two naturally infested moose (adult male and female calf) from Peace River, AB in 2011, and (b) ticks collected from the experimentally infested cattle and reindeer (Chapter 3) in 2011. The saliva collected from these ticks was categorized by tick species, *D. andersoni* or *D. albipictus*, and the host species to which they were attached, i.e., moose, reindeer or cattle. In each category, saliva from multiple ticks was pooled to standardize the protein concentration among samples, separated into aliquots of 100µl, and stored at -80^{o}C until use. The protein concentration in tick saliva was measured using a commercially available protein assay kit (Bio-Rad DC Protein Assay kit, Bio-Rad, Hercules, California, USA) according to the manufacturer's protocol.

Tick saliva protein preparation

In a 1.5 ml cryotube, one part of tick saliva (1 - 10µl) was diluted in a commercial sample loading buffer (Life Technologies - Invitrogen, Burlington, Ontario, Canada) as follows: 4 parts of Nu Page® LDS sample buffer, 10 parts of Nu Page® reducing agent, and 1.5 part of molecular grade water. The top of the vial containing the samples was securely fastened with a Beaker Buddy boiling rack (USA Scientific, Ocala, Florida, USA) and placed into an 800 ml Beaker containing boiling water for 10 minutes. The vial was then removed from the beaker and the prepared sample was placed into a bucket containing ice until use.

Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE)

The prepared sample containing tick saliva protein was loaded (10µl/ well) in a gradient (4-12%) 10 wells SDS-PAGE gel (Nu Page® 4-12% Bis-Tris precast Mini-gels, Life Technologies - Invitrogen, Burlington, Ontario, Canada) and proteins in tick saliva were resolved for 35 minutes at 200V. To visualize the separated protein bands of the tick saliva, the gels were removed from the cassette and stained in Simply Blue™ Safe Stain (Life Technologies - Invitrogen, Burlington, Ontario, Canada) for 15 minutes. Excess stain was removed by washing the gel with distilled water in gentle agitation until protein bands were clearly visualized. The gel was then digitalized using a scanner and saved as a picture format file (Experiment 1). The molecular weight of the protein bands observed in tick saliva was determined by visually comparing them to the molecular weight marker (Broad Range, Novex Sharp Pre-Stained Protein Standard, Bio-Rad).

Optimization of Western blot to investigate antibody response of 2009 and 2011 sera after tick exposure

The main purpose of Western blot analysis is to detect specific polyclonal antibodies reactive to proteins in a complex system. A Western blot procedure is composed of these subsequent steps:

(1) Sample preparation – proteins in tick saliva

(2) Separation of proteins in tick saliva using SDS-PAGE

(3) Protein Transfer (from gel to a Nitrocellulose or PVDF membrane)

(4) Blocking non-specific sites of antibody-binding with blocking solution

(5) Wash away excess blocking solution

(6) Incubation with primary antibody (challenged sera – bovine sera exposed to ticks)

(7) Wash away excess primary antibody

(8) Incubation with secondary antibody (commercially available sera raised against challenged sera – goat antibody raised against bovine antibody and conjugated to Horseradish Peroxidase (HRP))

(9) Wash away excess secondary antibody

(10) Incubation with substrate solution (that will react to enzyme (HRP) providing colour (colorimetric) or light (chemiluminescence) to the reaction

(11) Wash away excess substrate solution (only for colorimetric)

(12) Analysis of the results

Detailed description of the procedure used in this study's experiments is described below.

Western blot of 2009 sera using colorimetric detection method

Following SDS-PAGE (described above), the gel was equilibrated in Transfer Buffer (25mM Tris, 192 mM Glycine, 20% v/v Methanol) and the protein from the gel was transferred to a nitrocellulose membrane (0.45 mm pore aperture) using a wet-transfer system (Mini Trans-Blot® Electrophoretic Transfer Cell, Bio-Rad, Hercules, California, USA) for one hour at 100V. After the protein transfer was complete, the membrane was placed into the blocking solution containing 3% Gelatin and gently agitated for one hour at room temperature and then washed three times for five minutes each in Tris-buffered saline (TBS) solution. The membrane containing tick saliva was then cut into several strips of approximately 0.5 cm wide, placed in a multi-channel tray and each, incubated with pre- or post-exposure sera (primary antibody) of the challenged animals (Animal ID 915, 919, 920, 923, 925, 926, 929, 931, 933, 932, 941, and 946). Sera were diluted to 1:1,000 (1µl serum

and 1,000µl of Gelatin 1%) and incubated overnight at room temperature, and in gentle agitation (Experiment 2). Pre-exposure sera were considered as negative controls and compared to post-exposure sera to investigate for differences in protein bands. Different protein bands observed after exposure to ticks were assumed to be product of an antibody response to tick infestations.

After incubation with cattle sera, the membrane strips were washed three times in TBS for five minutes each and incubated in secondary antibody solution – Goat anti-bovine IgG (Heavy and Light chains) conjugated to HRP (SouthernBiotech, Birmingham, Alabama) – at 1:10,000 dilution for one hour at room temperature (~23°C). Another vigorous wash (three times for five minutes each) with Tween 20 TBS (TTBS) followed the secondary antibody incubation. The membrane strips were then incubated for up to 30 minutes with colorimetric substrate solution (Opti 4-CN™Substrate Kit, Bio-Rad, Hercules, California, USA) until signals (reaction of antibody with protein bands) could be visualized, washed with double distilled water for 15 minutes, and dried on filter paper. After the membrane was completely dry, it was digitalized, and stored in a plastic wrap.

Western blot of 2011 sera using colorimetric and chemiluminescence detection method

Colorimetric detection method

The same procedure described for *2009 sera* was performed for the *2011 sera*, except that the serum samples of the animals tested were from Group 1 (Animal ID: 117, 129, 136, and 140) and Group 2 (Animal ID: 115, 121, 124, and 138) (Experiment 3 and 4). In addition, wood tick saliva was resolved in SDS-PAGE to test the animals from Group 1 (cattle exposed to wood ticks), while winter tick saliva was resolved in SDS-PAGE to test

Group 2 (cattle exposed to winter ticks). The concentrations of the primary antibody, blocking solution, secondary antibody incubation and washing times were similar to those described for *2009 sera*.

Chemiluminescence detection method

For the chemiluminescence detection method, the protocol was similar to the colorimetric detection method; however, because it was more sensitive, several optimizations in blocking solution and changes in concentrations of primary antibody and secondary antibody were necessary.

An initial experiment (Experiment 5) was performed with the colorimetric protocol using the same blocking solution and the same concentration of primary and secondary antibody, but instead using the chemiluminescence substrate solution (ECL Plus Western Blotting Detection Reagent, GE Healthcare, Mississauga, Ontario). The membrane strips were incubated in substrate solution for 5 minutes, covered with a plastic wrap, inserted in a film cassette and developed in a negative film in the dark room. Based on the results of that experiment, other trials were subsequently conducted to overcome the challenges encountered. Such experiments aimed at investigating the optimal antigen concentration (Experiment 6) by testing different concentrations of wood and winter tick saliva in SDS-PAGE. In Experiment 7, the efficiency of two blocking solution Gelatin 3% and Super Block® Blocking Buffer (Thermo Fisher Scientific, Burlington, Ontario) were tested to reduce dark background and, based on results from previous experiments, complete trials with pre-and post-exposure sera from Group 1 and Group 2 were also conducted using chemiluminescence (Experiment 8 and 9). Even after these experiments, more

optimizations were still needed, which were testing different concentrations of secondary (Experiment 10) and primary (Experiment 11) antibody. Experiment 10 was conducted to investigate which dilution would be optimal to reduce dark background and to assess cross-reactivity of antigen with secondary antibody, and Experiment 11 was performed to investigate the optimal dilution of primary antibody necessary to capture a clear signal (visualization of antibody reaction to protein bands).

RESULTS

Production of tick-challenged sera

Cattle infestation

At 14 days post-infestation, a total of 186 engorged female wood ticks, and 45 engorged female winter ticks were recovered from the infested cattle.

Comparison of proteins present in wood tick and winter tick saliva using Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE)

Collection of tick saliva

A total of 186 partially engorged female wood ticks were collected from the cattle infestation and provided approximately 2.25 ml of saliva. A total of 342 engorged female winter ticks collected from two culled moose (n=297) from Peace River, AB, and from experimentally infested cattle (n=45) provided approximately 1.7 ml of tick saliva. The protein concentration tested using the protein assay kit (Bio-Rad DC Protein Assay kit, Bio-Rad, Hercules, California, USA) was approximately 2 mg/ml for winter tick saliva and 4 mg/ml for wood tick saliva.

Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE)

Wood and winter ticks fed on cattle, moose and reindeer all had proteins with molecular weight of 70 kDa, 90 kDa, 110 kDa, and 210 kDa in their saliva extracts. Proteins with a molecular weight of 25 kDa were present in saliva from winter ticks, but not in saliva from wood ticks. Interestingly, proteins with the molecular weight of 15 kDa were observed in the saliva extracted from both wood and winter tick fed on cattle but not in the saliva extracts of ticks that fed on moose or reindeer (Figure 4.2 and Table 4.3).

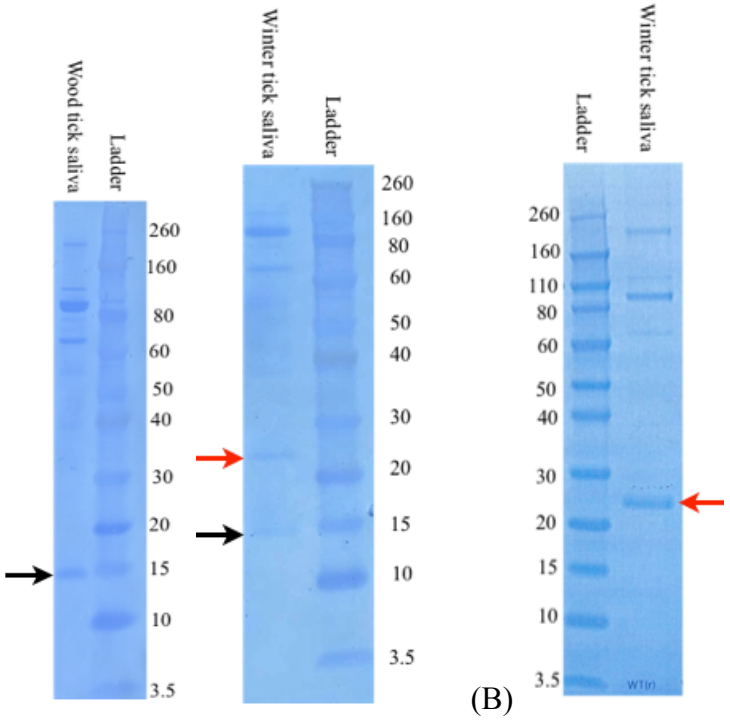


Figure 4. 2. Experiment 1: Comparison of protein bands in wood tick and winter tick saliva stained with Simply Blue™ Safe Stain. (A): wood tick and winter ticks fed on cattle; (B): winter ticks fed on moose and reindeer. Both species of ticks had salivary proteins at 70 kDa, 90 kDa, 110 kDa, and 210 kDa, but only ticks fed on cattle had proteins at 15 kDa (black arrow). Salivary protein at 25 kDa was only present in winter tick saliva (red arrow). Specifications: Ladder: Broad Range (Novex Sharp Pre-Stained Protein Standard, Bio-Rad), Antigen: Wood tick (8 µg saliva/well), winter tick (5.7 µg saliva/well); Pre-cast 4-12% SDS –Page Gel stained with Simply Blue™ Safe Stain.

Table 4.3. Experiment 1: Comparison of protein bands observed in wood tick and winter tick saliva in both SDS-Page Gel and Nitrocellulose membrane stained with Simply Blue™ Safe Stain.

Tick species (host)	Wood tick saliva (Fed on cattle)	Winter tick saliva (Fed on cattle)	Winter tick saliva (Fed on moose and reindeer)
Molecular weight of proteins observed in SDS-Page	210	210	210
	110	110	110
	90	90	90
	70	70	70
	-	25	25
	15	15	-

Optimization of Western blot to investigate antibody response of 2009 and 2011 sera after tick exposure

Western blot of 2009 sera using Colorimetric detection method

Sera from all animals (n=12) from *2009 sera* reacted to tick salivary proteins at 30 kDa, 35 kDa and 60 kDa in pre- and post-exposure sera. However, post-exposure sera of six animals (Animal ID 915, 919, 920, 925, 929 and 931) reacted also with tick salivary proteins at 40 kDa. The post-exposure sera of one animal (Animal ID 929) in particular, reacted to numerous tick proteins at three months (P3) after exposure to ticks (Figure 4.3, Experiment 2).

Western blot of 2011 sera using Colorimetric and Chemiluminescence detection method

Colorimetric detection method

There was no difference among pre- and post-exposure sera in any of the cattle exposed to wood ticks and tested against wood tick saliva (Group 1), but similar to sera

from 2009, antibody reactions to tick salivary proteins at 30 kDa, 35 kDa and 60 kDa were observed in both pre- and post-exposure sera (Figure 4.4, Experiment 3). For Group 2, no difference was observed between pre- and post-exposure sera of the cattle exposed to winter ticks and tested against winter tick saliva; sera of experimental animals reacted to tick proteins at 25 kDa and 60 kDa in both pre- and post-exposure sera. The only distinct observation was the sera of one animal (Animal ID 115) that showed reaction to the protein at 35 kDa before and after exposure to ticks, which gradually decreased through the P1, P2, and P3 time periods (Figure 4.5, Experiment 4).

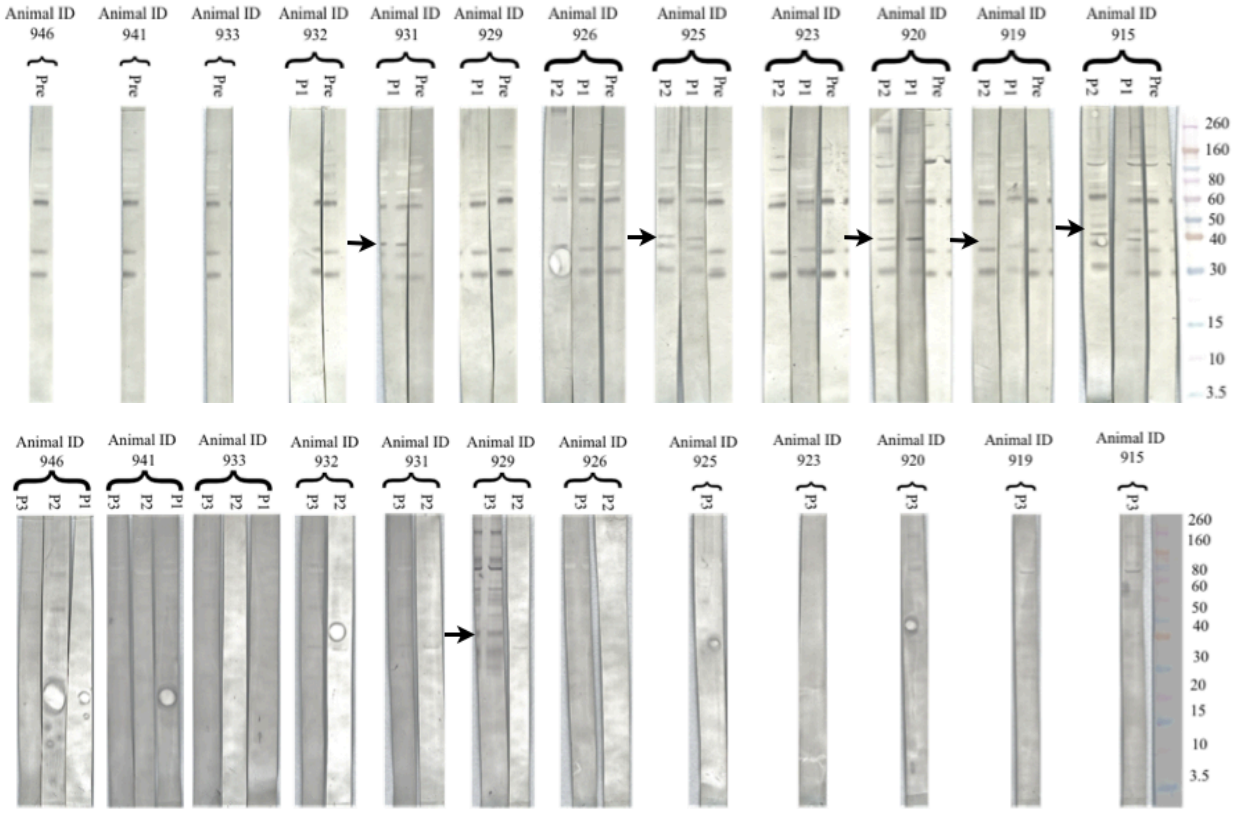


Figure 4.3. Experiment 2: Western blot analysis of *2009 sera* (archived bovine sera) to investigate antibody response to wood tick salivary proteins. Pre- represent pre-exposure sera and P1, P2 and P3 represent post-exposure sera (P1= one month, P2= two months, and P3= three months after exposure to ticks). Pre- and post-exposure sera of all animals reacted to salivary proteins at 30 kDa, 35 kDa and 60 kDa, but reaction to protein 40 kDa was only observed in post-exposure sera in six of the animals tested (black arrow).
Specifications: Antigen: Wood tick (11μg saliva/well); Ladder: Broad Range (Novex Sharp Pre-Stained Protein Standard, Bio-Rad); Pre-cast 4-12% SDS –Page Gel; Nitrocellulose membrane; Blocking solution: Gelatin 3%; Primary antibody: cattle exposed to wood tick (1:1,000); Secondary antibody: Goat anti-bovine HRP (1:10,000); Colorimetric detection method: Opti 4-CN (Bio-Rad).

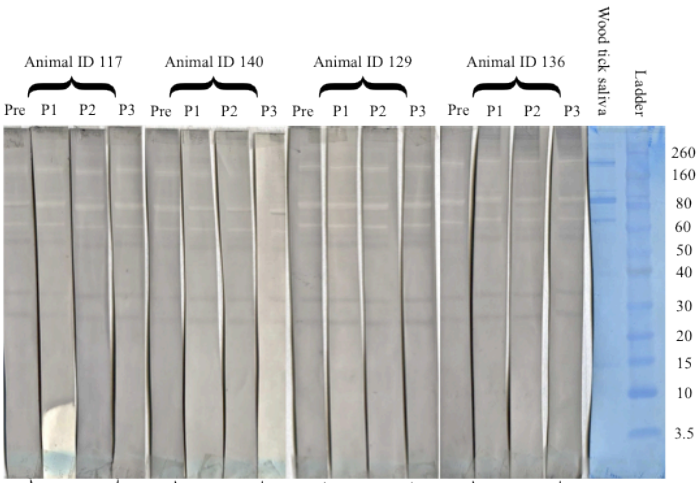


Figure 4.4. Experiment 3: Western blot analysis of the *2011 sera* from Group 1 to investigate antibody response to wood tick salivary proteins. Pre- represent pre-exposure sera and P1, P2 and P3 represent post-exposure sera (P1= one month, P2= two months, and P3= three months after exposure to ticks). Presence of ghost bands at 70 kDa, 90 kDa, 110 kDa. There was no distinct antibody reacting to tick proteins in post-exposure sera other than those observed in pre- and post-exposure sera at 30 kDa, 35 kDa and 60 kDa.
Specifications: Antigen: Wood tick saliva (11 µg saliva/well); Ladder: Broad Range (Novex Sharp Pre-Stained Protein Standard, Bio-Rad); Pre-cast 4-12% SDS –Page Gel, Nitrocellulose membrane; Blocking solution: Gelatin 3%; Primary antibody: cattle exposed to wood ticks (1:1,000); Secondary antibody: Goat anti-bovine HRP (1:10,000); Colorimetric detection method: Opti 4-CN (Bio-Rad).

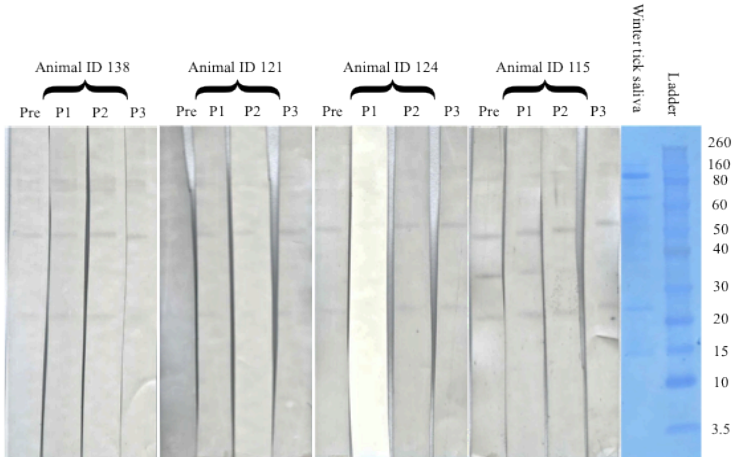


Figure 4.5. Experiment 4: Western blot analysis of the *2011 sera* from Group 2 to investigate antibody response to tick salivary proteins. Pre- represent pre-exposure sera and P1, P2 and P3 represent after exposure (P1= one month, P2= two months, and P3= three months after exposure). Pre- and post-exposure sera of all animals reacted to tick salivary proteins at 25 kDa and 60 kDa. Sera of animal 115 reacted also to the protein at 35 kDa.
Specifications: Antigen: Winter tick saliva (8 µg saliva/well); Ladder: Broad Range (Novex Sharp Pre-Stained Protein Standard, Bio-Rad); Pre-cast 4-12% SDS –Page Gel; Nitrocellulose membrane; Blocking solution: Gelatin 3%; Primary antibody: cattle exposed to winter ticks (1:1,000); Secondary antibody: Goat anti-bovine HRP (1:10,000); Colorimetric detection method: Opti 4-CN (Bio-Rad).

Chemiluminescence detection method

In Experiment 5, the Western blot developed in a film showed ghost bands and dark and blotchy background, and therefore, antibody reaction to tick salivary proteins were not visible (Figure 4.6). In Experiment 6, different concentrations of tick saliva (13, 15, and 18μg/ well for winter ticks and 15, 18, and 26μg/ well for wood ticks) were tested, but dark and blotchy background obscured visualization of the antibody reaction to tick proteins, especially in the wood tick experiment (Figure 4.7, Table 4.4). To overcome this dark and blotchy background issue, Experiment 7 was done to test the efficiency of two blocking solutions. There was considerable reduction in background and better visualization of the antibody reaction using Super Block® Blocking Buffer (Thermo Scientific, Burlington, Ontario) compared to Gelatin 3%; however, a few blotchy areas were still present after film development (Figure 4.8, Table 4.5). Based on these results, complete trials with *2011 sera* of Group 1 were conducted using the Super Block® Blocking Buffer (Thermo Scientific, Burlington, Ontario) (Experiment 8, Figure 4.9). Pre- and post-exposure sera of all animals reacted to tick salivary protein at 100 kDa but reactions to the protein at 250 kDa were observed only in animals 140 and 117 in both pre- and post-exposure sera. Although antibody reactions could be better visualized in Experiment 8, the dark background was still an issue (Figure 4.9), indicating that other aspects in the system needed optimization. Considering this, Experiment 9 was conducted with secondary antibody dilution at 1:80,000 – as described in Lysyk et al. (2009) using the sera of the Group 2. However, unlike Lysyk's (2009) protocol, the membrane was blocked with Super Block® Blocking Buffer (Thermo Fisher Scientific, Burlington, Ontario) and incubated overnight in primary antibody at 1:2,000 dilution. Blotchy areas were still present in the blot, but the dark

background was dramatically reduced in comparison to previous experiments facilitating visualization of antibody reaction to tick salivary proteins (Figure 4.10). There was no difference in antibody reaction to tick salivary proteins between pre- and post-exposure sera of the animals tested; however, similar to the results from colorimetric method, sera of animal 115 reacted to the protein at approximately 35 kDa fading gradually as post-exposure months progressed.

With regard to the optimization of secondary and primary antibodies, the results of Experiment 10 showed that dilutions at or above 1:40,000 were better for reducing dark background and the absence of protein bands indicated no cross-reactivity between the antigen and the secondary antibody (Figure 4.11). With the secondary antibody concentration set to 1:40,000, the results of the Experiment 11 showed that for the primary antibody, the dilutions up to 1:2,500 still allowed visualization of the protein bands (Figure 4.12). Optimizations of other aspects attempted in the Western blot system are described in the Figure 4.13.

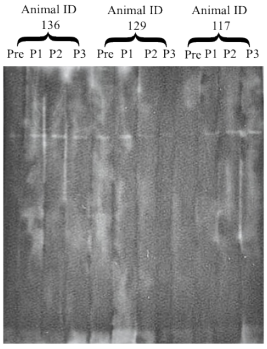
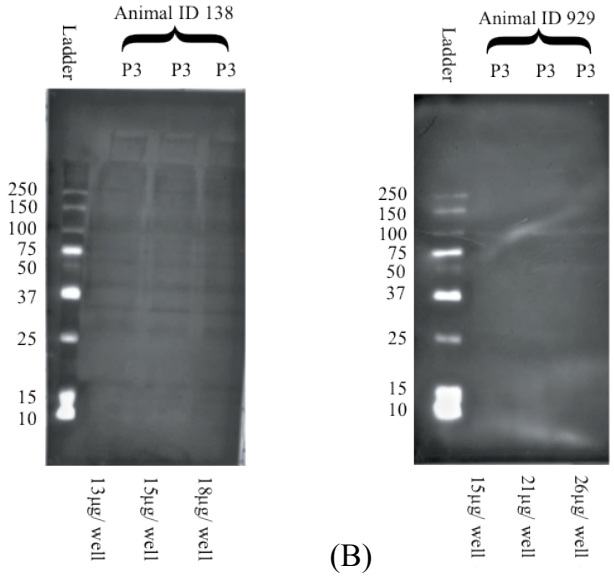


Figure 4.6. Experiment 5: Experiment using chemiluminescence detection method following the standardized protocol for colorimetric detection method in cattle. Dark and blotchy background obscured visualization of antibody reaction to tick salivary proteins.
Specifications: Antigen: Wood tick (3µg saliva/well); Ladder (not visible): Broad Range (Novex Sharp Pre-Stained Protein Standard, Bio-Rad); Pre-cast 4-12% SDS-Page Gel; Nitrocellulose membrane; Blocking solution: Gelatin 3%; Primary antibody: cattle exposed to wood ticks (1:1,000); Secondary antibody: Goat anti-bovine HRP (1:10,000); Chemiluminescence detection method: ECL Plus (GE).

Table 4.4. Experiment 6: Western blot analysis to investigate the optimal antigen concentration for winter tick and wood tick saliva necessary to capture a signal.
Specifications: Ladder: Pre-stained protein standard (Precision Plus Protein™ Kaleidoscope™ Standard, Bio-Rad); 10% SDS–Page Gel; Nitrocellulose membrane; Blocking solution: Gelatin 3%; Secondary antibody: Goat anti-bovine HRP.

SDS-Page	Antigen (tick saliva)	Concentration (µg of saliva/well)	Primary antibody	Secondary antibody	Substrate
10%	Winter tick	13 15 18	Cow exposed to winter ticks (Animal ID 138) (1:1,500)	1:10,000	ECL Plus
10%	Wood tick	15 21 26	Cow exposed to wood ticks (Animal ID 929) (1:1,500)	1:10,000	ECL Plus

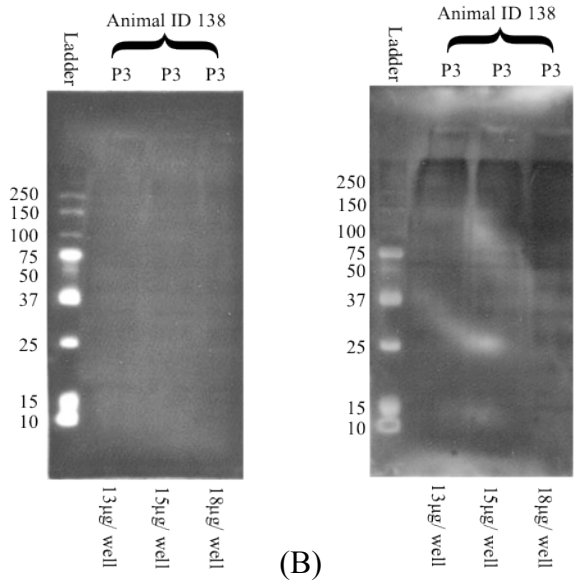


(A) (B)

Figure 4.7. Experiment 6: Investigation of the antigen concentration for (A) winter tick and (B) wood tick saliva necessary to capture a signal. Dark and blotchy background hindered visualization of antibody reaction to tick salivary proteins, especially on figure (B).
Specifications: Antigen: (A): winter tick saliva (13µg saliva/well, 15µg saliva/well, and 18µg saliva/well); (B): wood tick saliva (15µg saliva/well, 21µg saliva/well, and 26µg saliva/well); Ladder: Pre-stained protein standard (Precision Plus Protein™ Kaleidoscope™ Standard, Bio-Rad); 10% SDS–Page Gel; Nitrocellulose membrane; Blocking solution: Gelatin 3%; Primary antibody: cattle exposed to (A): winter ticks (Animal ID 138) and to (B): wood ticks (Animal ID 929) (1:1,500); Secondary antibody: Goat anti-bovine HRP (1:10,000); Chemiluminescence detection method: ECL Plus (GE).

Table 4.5. Experiment 7: Western blot analysis comparing the efficiency of two blocking solutions to reduce dark background and non-specific antigen-antibody bindings.
Specifications: Ladder: Pre-stained protein standard (Precision Plus Protein™ Kaleidoscope™ Standard, Bio-Rad); Pre-cast 4-12% SDS–Page Gel; PVDF membrane; Secondary antibody: Goat anti-bovine HRP.

SDS-Page	Antigen (tick saliva)	µg of saliva/well	Blocking solution	Primary antibody	Secondary antibody	Enzyme Substrate
10%	Winter tick	13 15 18	Gelatin 3%	Cow exposed to winter ticks (Animal ID 138) (1:1,500)	1:10,000	ECL Plus
10%	Winter tick	13 15 18	Super Block®	Cow exposed to winter ticks (Animal ID 138) (1:1,500)	1:10,000	ECL Plus



(A) (B)

Figure 4.8. Experiment 7: Western blot analysis comparing the efficiency of two blocking solutions to reduce dark background and non-specific antigen-antibody bindings. (A) Dark background and faint signals observed when blocked with Gelatin 3%; (B) Background was still dark and blotchy, but antibody reaction to tick salivary proteins were better visualized when blocked with Super Block® Blocking Buffer (Thermo Scientific).
Specifications: Antigen: With winter tick saliva (13µg saliva/well, 15µg saliva/well, and 18µg saliva/well); Ladder: Pre-stained protein standard (Precision Plus Protein™ Kaleidoscope™ Standard, Bio-Rad); Pre-cast 4-12% SDS–Page Gel; PVDF membrane; Blocking solution: (A): Gelatin 3% and (B): Super Block® Blocking Buffer (Thermo Scientific); Primary antibody: cattle exposed to winter ticks (Animal ID 138) (1:1,500); Secondary antibody: Goat anti-bovine HRP (1:10,000); Chemiluminescence detection method: ECL Plus (GE).

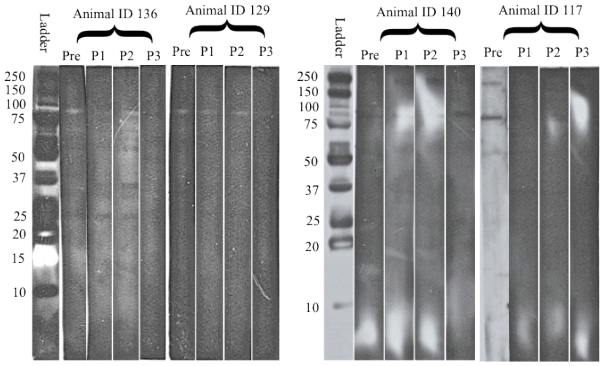


Figure 4.9. Experiment 8: Western blot analysis of the sera from cattle experimentally infested with wood ticks (Group 1) to investigate antibody response to wood tick salivary proteins before (Pre-) and after exposure (P1= one month, P2= two months, and P3= three months after exposure) to wood ticks using chemiluminescence detection method. Dark background does not allow good visualization of the antibody reaction to tick salivary proteins hindering interpretation of the results.

Specifications: Antigen: Wood tick (6 µg saliva/well); Ladder: Pre-stained protein standard (Precision Plus Protein™ Kaleidoscope™ Standard, Bio-Rad); Pre-cast 4-12% SDS–Page Gel; PVDF membrane; Blocking solution: Super Block® Blocking Buffer (Thermo Scientific); Primary antibody: cattle exposed to wood ticks (Animal IDs 136, 129, 140, 117) (1:1,500); Secondary antibody: Goat anti-bovine HRP (1:10,000); Chemiluminescence detection method: ECL Plus (GE).

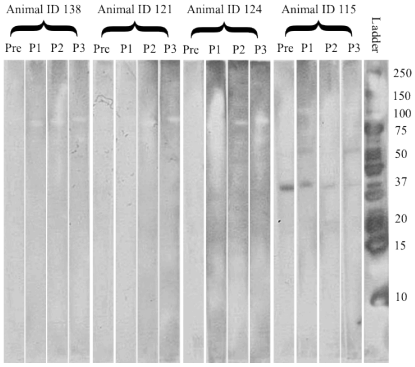


Figure 4.10. Experiment 9: Western blot analysis of the sera from cattle experimentally infested with winter ticks (Group 2) to investigate antibody reaction to tick salivary proteins before (Pre-) and after exposure (P1= one month, P2= two months, and P3= three months after exposure) to winter ticks using chemiluminescence detection method. Pre- and post-exposure sera of animal 115 reacted to tick salivary protein at 35 kDa, which was consistent with the results from colorimetric detection method.

Specifications: Antigen: Winter tick (6µg saliva/well); Ladder: Pre-stained protein standard (Precision Plus Protein™ Kaleidoscope™ Standard, Bio-Rad); Pre-cast 4-12% SDS–Page Gel; PVDF membrane; Blocking solution: Super Block® Blocking Buffer (Thermo Scientific); Primary antibody: cattle exposed to winter ticks (Animal IDs 138, 121, 124, 115) (1:2,000); Secondary antibody: Goat anti-bovine HRP (1:80,000); Chemiluminescence detection method: ECL Plus (GE).

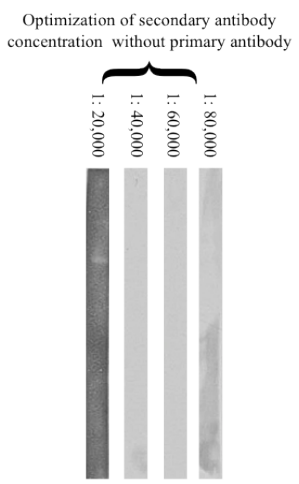


Figure 4.11. Experiment 10: Optimization of secondary antibody concentration without primary antibody to investigate the optimal dilution necessary to reduce dark background, and to assess cross-reactivity between antigen and secondary antibody. Note that dark background is reduced dramatically when used secondary antibody at 1: 40,000 dilution.
Specifications: Antigen: Wood tick (6 μg saliva/well); Ladder: Pre-stained protein standard (Precision Plus Protein™ Kaleidoscope™ Standard, Bio-Rad) (not visible); Pre-cast 4-12% SDS–Page Gel; Blocking solution: Super Block® Blocking Buffer (Thermo Scientific); PVDF membrane; Primary antibody: non applicable; Secondary antibody: Goat anti-bovine HRP (1:20,000, 1:40,000, 1:60,000 and 1:80,000); Chemiluminescence detection method: ECL Plus (GE).

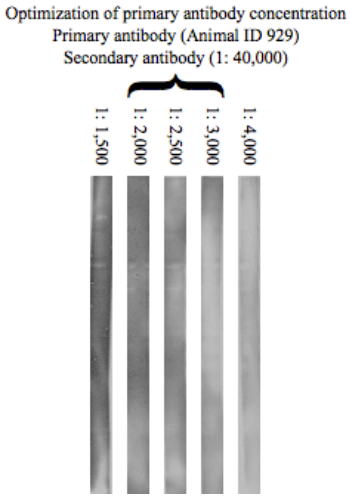


Figure 4.12. Experiment 11: Optimization of primary antibody concentration to investigate the optimal dilution of primary antibody necessary to capture a clear signal. Note that the dark background is reduced as the dilution increases, but at 1:2,500 dilution it is still possible to see protein bands.
Specifications: Antigen: Wood tick (6 μg saliva/well); Ladder: Pre-stained protein standard (Precision Plus Protein™ Kaleidoscope™ Standard, Bio-Rad) (not visible); Pre-cast 4-12% SDS –Page Gel; PVDF membrane; Blocking solution: Super Block® Blocking Buffer (Thermo Scientific); Primary antibody: Cattle exposed to wood tick (Animal ID 929 - 1:1,500, 1:2,000, 1:2,500, 1:3,000, and 1:4,000); Secondary antibody: Goat anti-bovine HRP (1:40,000); Chemiluminescence detection method: ECL Plus (GE).

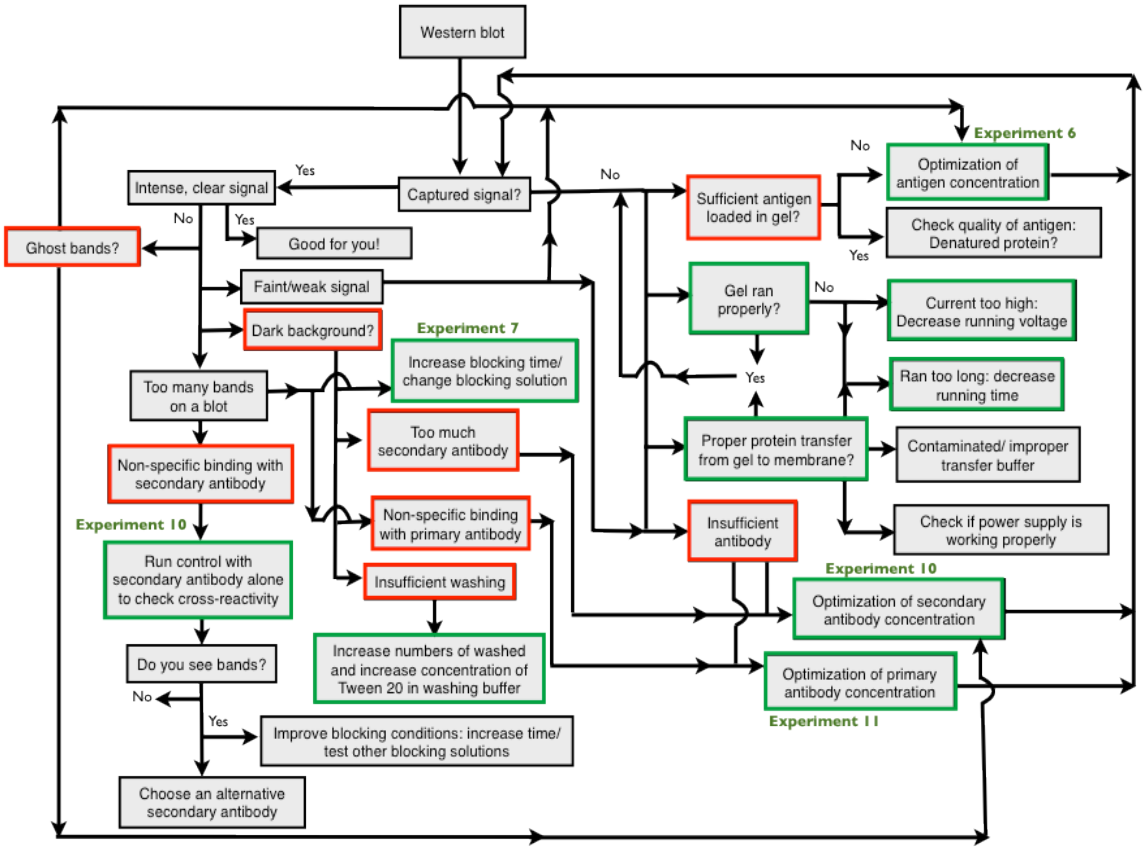


Figure 4.13. Flowchart of the Western blot technique with the possible causes for failure to capture of a signal using chemiluminescence detection method. The colours indicate a summary of the challenges encountered (in red), the troubleshooting trials (in green) and the experiments related in an attempt to capture a strong clear signal.

DISCUSSION

This study was the first to compare proteins in *D. albipictus* saliva to salivary proteins of *D. andersoni*, and to demonstrate differences in protein profile between these two species of ticks. Despite the similarities in salivary proteins from both tick species, one protein in particular, at 25 kDa, was present only in winter tick saliva, suggesting that there are salivary proteins differentiating the two tick species. While this was the first research to identify salivary proteins in winter tick saliva, there are previous studies reporting salivary proteins of wood ticks (Gordon and Allen, 1987; Bergman et al., 1995; Bergman et al.,

2000; Lysyk et al., 2009). Because of the relatively low concentration of proteins obtained from tick saliva, many researchers prefer to work with other antigens of tick origin such as salivary glands extracts in Western blot analysis (Almeida et al., 1994; Oleaga et al., 2007). However, not all antigens present in salivary glands are naturally injected into the host during blood feeding, thus the antibodies against these proteins are unlikely to be present in naturally infested hosts.

Proteins of host origin such as albumin, hemoglobin, and even host IgG may be found in saliva of many ixodid ticks species (Valenzuela et al., 2002; Madden et al., 2004). Similarly, in this study the protein 15 kDa was observed in saliva from both wood and winter ticks fed on cattle but it was not observed in saliva from winter tick fed on moose and reindeer, suggesting that this protein could be potentially of host origin; more specifically, from cattle. However, a more in depth analysis with molecular characterization of the salivary proteins of both tick species fed on the same host species is necessary to confirm these findings. In the past, the presence of these proteins in tick saliva was attributed to contamination with the host blood caused by tick regurgitation during saliva collection, but since these same proteins were found in tick hemolymph and salivary glands prior to saliva collection (Madden et al., 2004; Brossard and Wikel, 2005), it is now believed that the ingestion of these proteins of host origin may have some unknown physiological function, or may be one of the several mechanisms evolved by the tick to evade host immune response (Brossard and Wikel, 2005).

Western blot analysis of 2009 sera using colorimetric detection method

Access to archived sera was essential to gain technical expertise and to test the feasibility of using Western blot to investigate the presence of antibody response after exposure to ticks. Although the antibody reaction to the tick salivary protein at 40 kDa was only observed in sera of animals after exposure to ticks, there was no consistency in the results among animals i.e., this protein was present in 50% of the animals tested. In addition, there were no apparent pattern in animals producing more antibodies to tick salivary proteins based on the number of ticks cattle were exposed, nor were there differences whether animals were exposed to both male and female ticks as opposed to female ticks only. For example, the post-exposure sera of animal 929, that was exposed only to 25 female ticks reacted to numerous proteins in tick saliva, while the post-exposure sera of animal 920, exposed to 150 ticks, only reacted to the protein at 40 kDa at one and two months after tick exposure. No antibody reaction was observed after three months of exposure in the majority of animals tested, with the exception of animal 929. While this absence in response could be attributed to technical problems, the experiment was repeated once more and the same results were obtained. Cruz et al. (2008) also observed considerable variation in protein recognition between cattle experimentally infested with *Rhipicephalus microplus*, and the response not only varied among animals, but also between repeated exposure and between tick antigens (salivary gland, gut and larval extract) (Cruz et al., 2008). Given this variability, a larger sample size is needed to recognize patterns and to overcome these individual variations in immune response.

Western blot analysis of 2011 sera using colorimetric and chemiluminescence detection methods

No distinct antibody response was observed in bovine sera from Group 1 and Group 2 after exposure to ticks using either the colorimetric or the chemiluminescence detection methods. With regards to chemiluminescense, dark background was a problem in the majority of the experiments, obscuring completely, or at least, partially, the visualization of the antibody reaction to tick salivary proteins; however, despite these challenges, when the results from colorimetric and chemiluminescence methods were compared, both provided similar results. In both methods, there was not distinct antibody response after exposure to ticks, but the only common observation was that recognition of the protein at 35 kDa by animal 115 in both pre-and post-exposure sera. These results suggest that both techniques were working properly, but no bands were visualized because these animals had little or no immunological response to tick exposure. Nonetheless, technical problems are not completely out of the equation thus it is important to consider other factors that may have contributed to not detecting the antibody response in these animals. The quality and the quantity of the antigen are fundamental for the success in capturing a clear signal. That is, the viability and the antigenicity of the proteins in tick saliva are of utmost importance to obtain successful results in Western blot analysis.

Western blot is a powerful diagnostic tool, very sensitive, and commonly used in veterinary and human research. Chemiluminescence is more sensitive than the colorimetric detection method, requiring a small amount of antigen to capture a clear signal and is thus desirable when antigen availability is limited; however, it is technically more challenging and requires more expertise to perform and to troubleshoot than the colorimetric method.

The very limited amount of tick saliva, in particular from winter ticks, was the main reason why chemiluminescence was the method of choice in this study; however, the lack of a pre-existing validated protocol for this technique to investigate the antibody production of cattle after tick exposure lead to several optimization and troubleshooting trials.

Beyond Western blot - Insights about this experience and recommendations for future studies

Developing a diagnostic tool for tick surveillance goes beyond testing tick-challenged sera and identifying which protein bands occurs after tick exposure. Certainly this study was only the first step of a long and laborious process that will require a better understanding about origin, function, and antigenicity of the proteins in tick saliva, as well as a comprehension of host immunity against these antigens. I hope this study enhanced the curiosity of other researchers to study *D. albipictus* and *D. andersoni* salivary proteins, and to expand their interest in understanding the origin and the role of these proteins in stimulating and in modulating host immunity. Several studies have documented the challenges and limitations of using proteomic approaches to identify tick salivary secretions responsible to stimulate and modulate host immunity (Madden et al., 2004; Oleaga et al., 2007); however, examples of studies discovering potential markers specific to exposure to other arthropods such as to *Lutzomyia longipalpis* (Lutz & Neiva, 1912) (Diptera: Psychodidae) (Souza et al., 2010) highlight the potential use of similar techniques for ticks. Yet, it is important to keep in mind that there are substantial biological differences in feeding mechanisms and the time spent on host between these two arthropods, thus finding this marker to tick exposure could be a daunting task for several reasons. For example, proteins in tick saliva vary between tick species and developmental stages, and even

different weights of engorged females can influence protein composition in saliva (Gordon and Allen, 1987; Brown, 1988b; Oleaga et al., 2007).

Western blot is still the method of choice to identify the antigenic proteins responsible for inducing host immune response after exposure to ticks; however, for future studies, the recommendation is to use this technique, combined with ELISA, to obtain quantitative and qualitative results of antibody production after exposure to ticks. Many studies have used ELISA (and many variations of this technique) to first quantify antibody response after tick exposure and then used Western blot for further identification of proteins inducting such antibody response (Wozniak et al., 1996; Ogden et al., 2002; Pruett et al., 2006; Cruz et al., 2008). In this study, ELISA would have been beneficial to quantify the levels of antibody response of the experimentally infested cattle (*2011 sera*) to understand whether these animals produced an antibody response or not after exposure to ticks, and to compare these results with those obtained in Western blot to investigate if they are compatible.

CONCLUSION

This was the first study to compare protein bands in *D. andersoni* and *D. albipictus* saliva and to investigate cattle antibody response after exposure to winter ticks. The recognition of the tick salivary protein at 40 kDa by archived sera (2009 sera) after exposure to ticks suggests that this protein could be responsible for inducing antibody response to wood ticks, but these results were not consistent among all animals. Sera from animals experiment infested in 2011 did not react to this 40 kDa. While the lack of protein recognition in sera of experimentally infested animal (*2011 sera*) could be partially

attributed to technical challenges with chemiluminescence detection, the consistency in the results from both colorimetric and chemiluminescense suggests that these animals did not respond immunologically as expected to the tick exposure, or produced a very limited response that was not detectable. The combined use of Western blot with ELISA may be beneficial to quantify this immune response and then, with Western blot, identify which of these proteins are responsible to induce such response.

Cattle have been used as model for host-tick immunity research for many decades but several aspects of this host-tick interaction are still not completely understood (Kemp et al., 1986; Dipeolu et al., 1992; de la Fuente et al., 1998; Bishop et al., 2002; Kashino et al., 2005). It is important to acknowledge the challenges involved in developing a diagnostic tool for tick surveillance, especially in considering the complexity of this host-tick system. However, it is also important to reiterate the relevance of having a diagnostic tool to detect tick exposure, in particular for wildlife research. The currently available methods for tick surveillance are time consuming, costly and logistically challenging, thus such tools would improve tick surveillance by providing rapid results and allowing considerable cost reduction in logistics and transportation.

CHAPTER FIVE

WINTER TICKS IN THE SAHTU

ONGOING MONITORING FOR WILDLIFE HEALTH ASSESSMENT AND FOOD SECURITY IN NORTHERN COMMUNITIES

Northern residents from the Sahtu Settlement Area (the Sahtu), Northwest Territories, rely heavily on moose and caribou for subsistence. In recent years, increased numbers of observations of moose clinically affected with winter ticks have been reported by local residents from the Sahtu (Kutz et al., 2009). The aim of this research was to investigate these reports and to determine where winter ticks were occurring in the Sahtu by collecting hides and digesting them to search for ticks. This research also aimed to test the feasibility of using a serodiagnostic tool as an alternative method for tick surveillance by investigating antibody response to tick exposure, using cattle as model species. With this study, I provide a glimpse of the current distribution of winter ticks in the Sahtu, address the apparent range expansion of this parasite in the region, and highlight the importance of ongoing monitoring of *D. albipictus* for wildlife health assessment and food security in these communities under current climate change scenarios.

This study provides the first definitive evidence, based on recovery and identification of winter ticks that the range of *D. albipictus* reaches as far as 66° N. This confirms anecdotal observations from the Sahtu, NT (Kutz et al., 2009). The occurrence of *D. albipictus* at 66° N significantly exceeds the northern limit of winter tick distribution suggested by Wilkinson (1967) which lies approximately at 64° N (Chapter 2, Figure 2.3), and the findings of clinically affected moose by Samuel (1967), which were south of 62° N. Kutz et al. (2009) suggested that this range expansion of winter tick could be a result of climate change.

The effects of climate change can be noticed worldwide, especially in Arctic and Sub-Arctic regions (Stenseth et al., 2002; Parmesan and Yohe, 2003; Hinzman et al., 2005).

The Sahtu, in particular, has already experienced the effects of this climate change with an increase of over 1.5°C in mean annual temperature since 1950 (Woo et al., 2007). There is strong evidence that climate change is altering biological and ecological processes in the Arctic at all trophic levels (Hinzman et al., 2005; Post et al., 2009). Under current and predicted climate change scenarios, alterations in host-parasite dynamics, such as an increase in survival and development rate of parasites and longer transmission periods, are anticipated to occur (Bradley et al., 2005; Hoberg et al., 2008; Kutz et al., 2009; Hoberg et al., 2012; Kutz et al., 2012). For example, the recent range expansion of deer keds (*Lipoptena cervi* Linnaeus, 1758) (Diptera: Hippoboscidae) in Fennoscandia appear to be caused by changes in climate and by increase host density in the region (Valimaki et al., 2010). Deer keds are blood-sucking flies that have moose as the main host (Madslien et al., 2011), and similar to *D. albipictus,* deer keds can cause severe alopecia and deleterious consequences to moose health. Mild winters observed in the recent years appear to be facilitating development and survival of deer keds in their pupal stage, thereby increasing the transmission risk to hosts (Valimaki et al., 2010; Madslien et al., 2011).

While not all parasites seem to benefit from warming temperatures caused by climate change (Hoar and Kutz, 2011; Molnar et al., 2013), ticks in particular, appear to be positively affected. Survival and development of numerous species of ixodid ticks are positively affected by warmer temperatures (Ogden et al., 2006; Danielová et al., 2008; Eisen, 2008; Estrada-Pena et al., 2008; Gage et al., 2008; Materna et al., 2008; Ogden et al., 2008; Knap et al., 2009). Climate change is considered one of the main driving forces for range expansion of *Ixodes ricinus* (Linnaeus, 1758) in several European countries (Medlock et al., 2013) and the predictions are that, by 2100, habitat in Africa and in the rest of the

world will become more suitable for several African tick species even under the most conservative climate change scenarios (Cummins and Van Vuuren, 2006). Similarly in North America, the geographic range of the tick *Ixodes scapularis* Say, 1821 is projected to increase considerably by 2080 as the habitat in northern regions of Canada becomes more suitable for ticks (Ogden et al., 2006).

More specifically for *D. albipictus*, there are various reasons why climate change may be influencing winter tick ecology in the Sahtu and influencing the range expansion of this parasite. Environment conditions such as temperature and snow cover have significant impact on survival and oviposition of engorged females and viability of larvae (Drew and Samuel, 1986a; Drew and Samuel, 1986b). Engorged female ticks exposed to cold stress under laboratory conditions produced less viable eggs than engorged females maintained at constant temperature (25°C) (Drew and Samuel, 1986b). Similarly, in field conditions, fluctuating temperatures affected negatively the survival of engorged females, oviposition and egg incubation period (the time from when eggs were laid until hatching), as well as the ability of larvae to successfully infest a host (Drew and Samuel, 1986a; Samuel and Welch, 1991; Aalangdong and Samuel, 2001). Under projected climate scenarios for the Sahtu, engorged female ticks may encounter better conditions in late spring, produce more eggs, and larvae may persist longer in the environment, which may increase considerably the risk of transmission to new hosts.

In the Sahtu, few alternative ungulate hosts species are available for winter ticks, i.e., white-tailed deer and mule deer are extremely rare and elk are absent from the region (Veitch, 2001; Kutz et al., 2012). Currently, moose appear to be the main host maintaining winter tick populations in the region. This ungulate species occurs at much lower densities

(0.16 moose/ km^2) in the Northwest Territories than, for instance, in central Alberta, where moose density is 7.5 times higher (1.20 moose/ km^2) (Stenhouse et al., 1995). Thus, it is likely that this low moose density and climate conditions may have previously limited survival and establishment of winter ticks in northern latitudes. Under current climate change scenarios, moose habitat is projected to expand 19-64% due to an increase in frequency of forest fire regimes (Joly et al., 2012). As habitat becomes more suitable for moose, an increase in moose density is expected, and as a result, increase in winter tick transmission to new hosts.

Winter ticks in the Sahtu and the potential risks of transmission among hosts

To date, it is unknown if boreal woodland caribou (*Rangifer tarandus caribou* (Gmelin, 1788)) from the Sahtu, which are sympatric with moose year round, are infested with winter ticks because this species has not been examined. *Rangifer* subspecies are highly susceptible to winter tick infestations in captive situations (Welch et al., 1990b) and reports of woodland caribou infested with winter ticks in southern regions of the Northwest Territories also indicate that these ungulate species are susceptible to this parasite in the wild (Welch et al., 1990b; Kutz et al., 2009). However, the role of woodland caribou in maintaining winter tick populations in the Sahtu is unknown. Boreal woodland caribou populations in the region are currently listed as *Threatened* species by the Federal Species at Risk Act (SARA) and across most locations of Canada their populations are declining due to habitat loss and human disturbance, thus it is essential to increase efforts for winter tick surveillance in this species.

While moose and barrenground caribou (*Rangifer tarandus groenlandicus* (Borowski, 1780)) are seasonally sympatric in the Sahtu during winter, the lack in temporal and spatial synchronicity between caribou migration and winter tick transmission period (fall) may have prevented the invasion of barrenground caribou populations by winter ticks. That is, for the barrenground caribou to be infested with *D. albipictus*, they need to be exposed to tick questing larvae during the fall. A disruption of this synchronicity such as earlier migration of caribou during the fall in areas of winter tick occurrence, or even persistence of the host-seeking winter tick larvae due to favourable climate for a longer period on the may facilitate transmission of winter ticks to barrenground caribou populations. With the exception of this research, no other studies have been conducted to assess presence of winter ticks in barrenground caribou populations. All barrenground caribou hides analyzed in this study were negative for ticks; however, due to the small sample size from a single area of the Sahtu, it is difficult to affirm if this reflects the situation of the herd, and therefore, more research is needed in this regard.

Insights about winter tick surveillance methods

Hunter-based sample collection in the Sahtu and chemical digestion of hides

Surveying winter ticks in a vast and remote area such as the Sahtu was possible in this study because of the collaboration with local hunters, with local Renewable Resource Councils and with the interest and effort of Wildlife Resource Officers and Wildlife Managers. The collaboration with the biologists from the Government of the Northwest Territories was essential to facilitate the logistics for this study; without their assistance the challenge of transporting these hides would have been much bigger. Many challenges were

encountered in hunter-based collections for winter tick surveillance in the Sahtu, but without the hunters' involvement, the access to moose and barrenground caribou hides would have been considerably more difficult, if not impossible. Inconsistency in data and sample collection was the major limitation experienced with hunter-based collections in the Sahtu. However, this inconsistency was probably caused by the hunters' lack of understanding of how to properly collect the samples and how to record the information due to inadequate training and clarification of the sample collection techniques. Thus, there is a considerable room for improvement in hunter-based collection, which can be achieved either by ensuring enhanced training of the participants and by adjusting the sample kit so it is more intuitive and easy to use, especially considering that many of the hunters have no scientific background. Notwithstanding some of the challenges, hunter-based sample collection remains the best approach to continue monitoring the expansion of *D. albipictus* in the Sahtu because it provided easier accessibility to biological samples of the harvested animals in a vast and remote area, and therefore, it is strongly recommended for future studies.

Chemical digestion of moose and caribou hides in KOH was very effective to detect the occurrence of winter ticks in the Sahtu. The sampling efforts of this study were concentrated in seasons when winter ticks are commonly found parasitizing the host, i.e., from fall through spring, which coincided with the main moose and caribou hunting seasons in the Sahtu. Digestion of hides provided accurate information of *D. albipictus* occurrence in the Sahtu because it allowed recovery and identification of the tick specimens; however, this methodology was time-consuming, labour-intensive and above all, finding storage space for all moose and barrenground caribou hides was logistically

challenging. The partnership with biologists of the Government of the Northwest Territories was fundamental to facilitate storage and transportation of such large number of moose and barrenground caribou hides until laboratorial analysis; however, such support may not be the available at all times for many studies. Thus, if there were an alternative method for winter tick surveillance that would allow rapid assessment with biological samples that could be easily stored and transported, it would be more helpful.

Alternative methods for winter tick surveillance

Cattle sera were analyzed using Western blot to test the feasibility of developing a serodiagnostic tool for winter tick surveillance. This study was the first to attempt to investigate antibody production in cattle after experimental infestation with winter ticks, and to compare different proteins in *D. albipictus* and *D. andersoni* saliva. The challenges encountered in this study could be partially attributed to limited technical expertise, but also to biological difficulties. It is known that hosts acquire immunity to ticks (Brown, 1985; Brown, 1988a; Wikel, 1996) and despite the vast literature available, many aspects of this complex host-tick immune interaction are not well understood. Several variables such as the degree of host susceptibility, tick species, and recurrent host exposure to tick infestations are factors that could influence in the immune response (Ogden et al., 2002; Cruz et al., 2008). Future studies should aim to gain a better understanding of the host-tick interactions in order to develop a diagnostic tool for tick surveillance. It is important to acknowledge that there are challenges and limitations involved in developing such a tool and that this research was just the beginning of this long process; however, it is also necessary to emphasize the importance of having such a diagnostic tool that can be used to detect exposure to ticks, especially in wildlife research. Despite the differences in

susceptibility and immunity between domestic and wild host species, studies such as this with cattle are valuable to understand the challenges and limitations in developing a diagnostic tool for tick surveillance and transferring this knowledge to wild species.

A diagnostic tool that would allow detecting exposure to ticks through sera could greatly reduce the costs involved in transportation and logistics when surveying winter ticks in remote northern latitudes. Such a methodology could also facilitate sample collections for hunters interested in collaborating with the research and even ease the transportation for them while harvesting for subsistence, which consequently, would probably contribute for a better and a more standardized sample collection, and possibly use archived sera to investigate historical distribution.

CONCLUSION

Through this study I have contributed with new geographical records of *D. albipictus* distribution in northwestern Canada. Yet, there remain many gaps in knowledge about winter tick ecology and host-tick dynamics under a changing climate. I hope to have contributed with this study to enhance the curiosity of other researchers to further investigate *D. albipictus* in the Sahtu and elsewhere across the Northwest Territories to monitor changes in sample prevalence and in intensity of tick infestations over the years. I also hope to have contributed to encourage other researchers to keep pursuing the development of a serodiagnostic tool for tick surveillance that would be rapid, less time consuming, and that could be performed with samples that are easily transportable.

I recommend that future studies continue to use hunter-based collections, encouraging participation of hunters, building local capacity, and bringing the results of the

research back to the community. Hunters were very receptive and interested in participating in the research because moose and caribou are important subsistence species and they understand that monitoring parasites and diseases in wildlife is essential to ensure food safety and food security for future generations. More studies encompassing caribou, moose, and winter tick ecology are necessary to increase the understanding of host-parasite interactions in the Sahtu and the response to the rapidly changing climatic conditions. Understanding how parasites adapt and survive in the environment, and how these parasites interact with their hosts is the core of a solid model to predict the potential impacts of climate change in biological systems.

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APPENDICES

APPENDIX A. Louse species found on barrenground caribou hide digests from the Sahtu Settlement Area, Northwest Territories.

Table A.1. Numbers of nymphs, adult male and adult females of *Bovicola tarandi* (Mjöberg, 1910) (Phthiraptera: Trichodectidae) and *Solenopotes tarandi* (Mjöberg, 1915) (Phthiraptera: Linognathidae) observed on barrenground caribou hides according to sample ID and hunting locations. Thanks to Dr. Lance Durden from the Department of Biological Sciences at the Georgia Southern University for identifying the species.

Location Coordinates	**Sample ID**	**Louse species**	**Nymphs**	**Adult male**	**Adult females**
Hottah Lake 65° 2' 24" N 118° 17' 59" W	WHM203	*Bovicola tarandi*	0	0	3
	WHM204	*Bovicola tarandi*	0	0	2
		Solenopotes tarandi	1	0	0
	WHM206	*Bovicola tarandi*	17	0	6
	WHM210	*Bovicola tarandi*	0	0	5
	WHM211	*Bovicola tarandi*	2	0	0
	WHM212	*Bovicola tarandi*	1	0	1
Fish Lake 66° 1' 00" N 118° 1' 59" W	WT1030	*Bovicola tarandi*	0	0	2
		Solenopotes tarandi	0	1	0
	WT1031	*Solenopotes tarandi*	0	0	1
	WT1032	*Bovicola tarandi*	15	0	9
	WT1033	*Bovicola tarandi*	17	0	14
	WT1037	*Bovicola tarandi*	0	0	1
Winter Road 66° 52' 08" N 126° 53' 24" W	WRoad	*Bovicola tarandi*	14	0	8
	Total		67	1	52

APPENDIX B. Protocol of tick infestation for cattle (Lysyk & Majak 2003) and reindeer

PRE-INFESTATION

1- Treatment of the animals with subcutaneous injection of 200µg/kg Doramectin (Pfizer Animal Health, Pfizer Canada Inc, Kirkland, QC) in the mid-cervical area, three (3) weeks before transferring animals from the Wildlife Facility

2- Confinement of the animal two (2) weeks before infestation to allow acclimatization of animals and house them in individual pens (or in pairs) allowing visual contact to each other to minimize stress.

Blood collection

Materials:

- Latex gloves
- Alcohol 70%
- Vacutainer needle 21G 1 ½"
- Vacutainer adapter
- Cotton balls
- 10 ml tubes for blood collection (serum)
- 5 ml tubes for with Heparin for blood collection (CBC)

Procedure:

1- Prepare material for blood collection: Vacutainer adapter and needle, cotton balls and alcohol 70%

2- Restrain the animal manually and shave the hair in the blood collection area using an automatic shaver

3- Collect blood samples by jugular venipuncture before exposure to ticks (Pre-exposure sera) in three (3) Vacutainer red top glass tubes (10 ml) for sera, and in one (1) Vacutainer purple top plastic tube (4 ml) containing

Ethylenediaminetetraacetic acid (EDTA) for Complete Blood Counts (CBC) analysis

4- For the purple top plastic tube (CBC), homogenize gently the purple top plastic tube for about a minute to avoid blood from clotting, and place the red top glass tubes on a rack after collection and let it rest until blood is coagulated.

Tick cage placement

Materials:

- Clippers
- Flat paintbrush (1 x 25 mm)
- Contact cement (Helmetin, Toronto, Ontario, Canada)
- Stockinet sleeve 20 cm long (QMD Medical, Montreal, Quebec, Canada)
- Permanent marker
- A piece of PVC pipe (10 cm diameter with 5 cm wide)

Procedure:

Note: Place tick cages a week before the infestation to allow animals to become used to the cages.

1- Choose the areas on the back of the animal where the ticks cages will be placed

2- Shave the hair of the animal using an automatic shaver 20 cm length (cranio-caudal) and 20 cm wide (latero-lateral) at the location where tick cage will be placed (Figure B1.A)

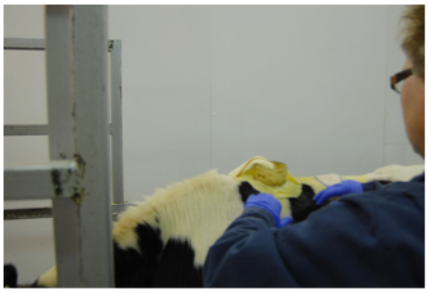
3- Place the piece of the PVC pipe in the shaved area and then use the markers to delineate the area ticks will be placed

4- Use the flat paintbrush to spread the contact cement externally around that marked area (Figure B1.B)

5- Surround the PVC pipe with the stockinet sleeve and glue the stockinet outside the marked area (Figure B1.C and Figure B1.D)

6- Wait for about three (3) minutes with the stockinet and the PVC pipe in place to allow the glue to dry (Figure B1.E) and verify if the stockinet was properly glued on the animal with no loose spaces.

(A)  (B) 

(C)  (D) 

(E) 

Figure B1. Demonstration of the procedure for tick cage placement on the back of the animal. (A) Shaving the hair of the animal at the location ticks cages will be placed; (B) and (C) Application of the contact cement around the marked circle; (D) Attachment of the stockinet sleeve on the back of the animal; (E) Stockinet sleeve glued on the back of the animal.

INFESTATION (Day 0)

Materials:

- Ticks
- Soft covered wires
- Fibreglass window screen (30 cm diameter)

Procedure:

1- Place the ticks inside the aperture of the stockinet sleeve, twist it securely and close the aperture soft covered wires (Figure B2.A and Figure B2.B)

2- Place the fibreglass window screen over the stockinet sleeve to protect the ticks from animal's grooming (optional) (Figure B2.C and figure B2.D)

(A)  (B) 

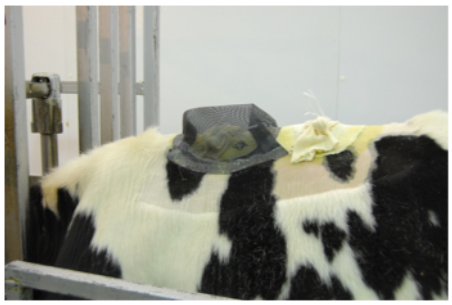
(C)  (D) 

Figure B2. Demonstration of the infestation procedure. (A) Ticks are placed inside the stockinet sleeve; (B) Sleeves are twisted and tied with soft covered wires; (C) and (D) Attachment of the fibreglass window cover to protect the ticks from animal's grooming.

POST-INFESTATION PROCEDURE

1- Monitor weekly tick attachment success and developmental stage; count attached ticks, classify according to development instars, and record information on data sheet.

2- Blood collection of the experimental animals after exposure to ticks at 7^{th}, 14^{th}, 21^{st} days post-infestation and then monthly until the end of the experiment.

3- At the end of the experiment, visually inspect animals for any remaining ticks and treat them with subcutaneous injection of 200μg/kg Doramectin (Pfizer Animal Health, Pfizer Canada Inc, Kirkland, QC) in the mid-cervical area

4- Keep animals in quarantine and then, transfer experimental animals together with other animals.

APPENDIX C. Results of the Complete Blood Count (CBC) analysis of the captive reindeer experimentally infested with *Dermacentor albipictus*.

Table C1. Hematological values of the captive reindeer experimentally infested with *Dermacentor albipictus* according to Animal ID and days post-infestation.

Date	Days post-infestation	Animal ID	RBC	Hemoglobin	Hematocrit	Mean Corp Vol.	Mean Corp HGB	MCHC	RDW	Platelets
		Reindeer 1	11	167	0.47	42.7	15.2	357	18.4	485
24-Dec-10	27	Reindeer 2	10.4	164	0.46	44	15.7	356	17.8	550
		Reindeer 3	11.9	182	0.52	43.4	15.3	352	19.2	595
		Reindeer 4	13.4	179	0.51	37.7	13.3	354	24.8	INV
		Reindeer 1	11.2	172	0.46	40.8	15.4	377	18.8	605
21-Jan-11	55	Reindeer 2	10.6	172	0.45	42.1	16.2	385	18.5	656
		Reindeer 3	11.6	183	0.48	41	15.8	384	20.4	703
		Reindeer 4	12.8	180	0.48	37.2	14	377	24.8	773
		Reindeer 1	10.8	166	0.44	40.6	15.3	378	18.6	580
18-Feb-11	83	Reindeer 2	9.9	157	0.42	42.2	15.8	376	17.7	578
		Reindeer 3	11.4	176	0.47	40.8	15.4	378	20.8	539
		Reindeer 4	12.7	176	0.47	36.7	13.8	377	22.2	781
		Reindeer 1	10.4	163	0.43	40.8	15.6	383	18.1	560
18-Mar-11	111	Reindeer 2	9.8	155	0.41	41.7	15.8	380	17.2	593
		Reindeer 3	10.9	171	0.45	40.7	15.7	385	20.8	552
		Reindeer 4	12.2	171	0.45	36.8	14	381	20.7	702
		Reindeer 1	4.5	73	0.2	44.7	16.3	365	16.5	INV
15-Apr-11	139	Reindeer 2	10.1	160	0.43	42.6	15.9	372	16	341
		Reindeer 3	11.3	177	0.49	43.4	15.7	361	19.8	552
		Reindeer 4	11.4	162	0.47	24.3	14.3	347	20	916

Table C1. cont.

Date	Days post-infestation	Animal ID	RBC	Hemoglobin	Hematocrit	Mean Corp Vol.	Mean Corp HGB	MCHC	RDW	Platelets
		Reindeer 1	9.4	147	0.39	41.4	15.7	379	17.6	586
13-May-11	168	Reindeer 2	8.5	134	0.35	41.6	15.8	380	15.7	493
		Reindeer 3	11.2	176	0.47	41.9	15.7	374	23.1	554
		Reindeer 4	10.2	149	0.39	37.7	14.6	387	18	541
		Reindeer 1	8.9	137	0.37	41.4	15.4	372	15.9	449
9-Jun-11	195	Reindeer 2	9.4	147	0.4	42.5	15.7	369	18.3	510
		Reindeer 3	10.7	166	0.45	42	15.5	370	21.1	350
		Reindeer 4	10.1	149	0.39	38.5	14.6	380	19.5	427
		Reindeer 1	11.8	173	0.47	39.5	14.7	372	22.6	525
4-Jul-11	220	Reindeer 2	10.7	162	0.46	43.3	15.2	351	20.3	551
		Reindeer 3	11.9	183	0.5	41.7	15.4	368	22.8	417
		Reindeer 4	11.1	153	0.42	38.1	13.8	362	22.8	484
		Reindeer 1	10.2	158	0.43	41.9	15.5	370	23.1	674
5-Aug-11	252	Reindeer 2	9.6	150	0.43	45.1	15.7	348	20.8	589
		Reindeer 3	10.6	165	0.47	44	15.6	355	22.6	581
		Reindeer 4	10	141	0.4	39.7	14.1	354	24.4	560
		Reindeer 1	10.7	167	0.45	42.1	15.6	369	22.9	638
1-Sep-11	279	Reindeer 2	10.2	162	0.46	45.2	15.6	352	22.2	462
		Reindeer 3	11	174	0.48	43.6	15.9	364	23.2	391
		Reindeer 4	10.9	154	0.43	39	14.1	362	24	580

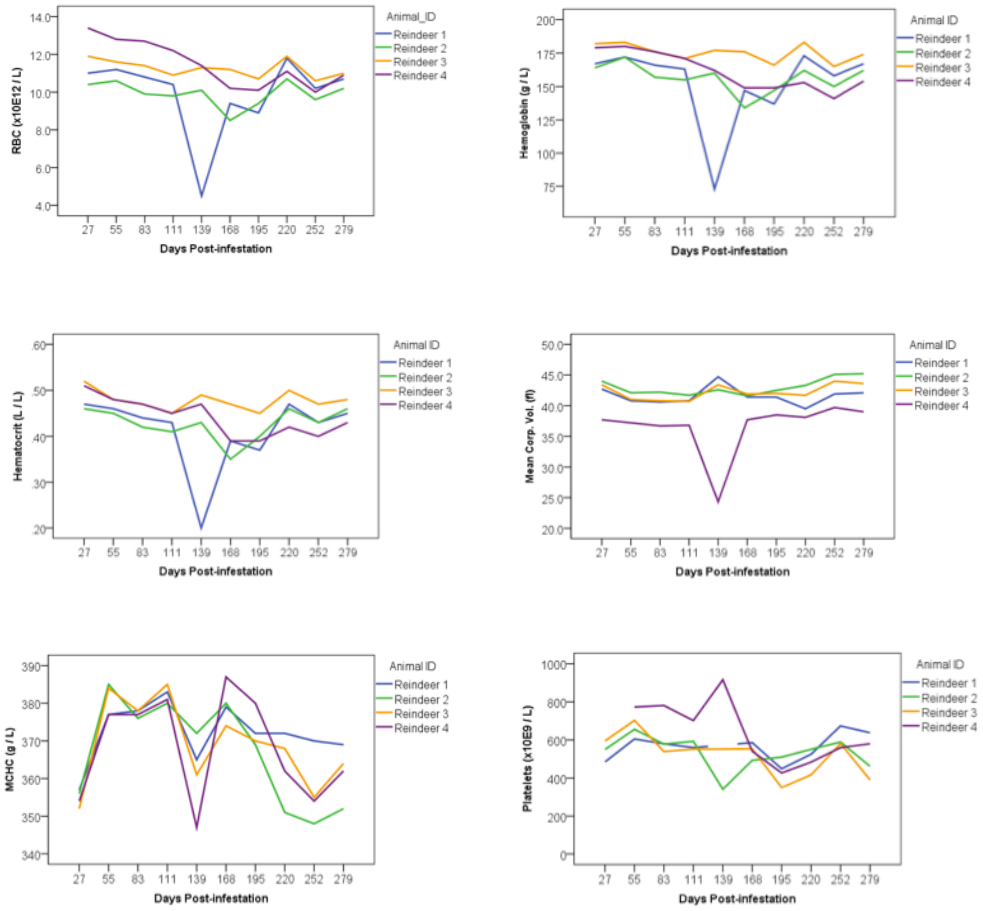


Figure C1. Hematological values of captive reindeer experimentally infested with *Dermacentor albipictus* according to Animal ID and days post-infestation.

Table C2. White blood cells and leucocytes counts of the captive reindeer experimentally infested with *Dermacentor albipictus* according to Animal ID and days post-infestation.

Date	Days post-infestation	Animal ID	White Cell	Neutrophils %	Lymphocytes %	Monocytes %	Eosinophils %	Basophils %
		Reindeer 1	7.8	46	40	11	3	0
24-Dec-10	27	Reindeer 2	6.1	58	36	5	1	0
		Reindeer 3	7.6	52	37	8	3	0
		Reindeer 4	6.9	42	48	9	1	0
		Reindeer 1	5.3	42	49	6	3	0
21-Jan-11	55	Reindeer 2	7.3	44	45	9	2	0
		Reindeer 3	6.9	49	47	4	0	0
		Reindeer 4	6.3	46	43	7	4	0
		Reindeer 1	3.8	53	41	4	2	0
18-Feb-11	83	Reindeer 2	5.7	47	49	1	3	0
		Reindeer 3	4.9	45	46	4	5	0
		Reindeer 4	5.3	44	50	2	3	1
		Reindeer 1	4.6	45	40	6	8	1
18-Mar-11	111	Reindeer 2	7.3	45	47	3	5	0
		Reindeer 3	6.9	43	50	6	1	0
		Reindeer 4	5.8	50	45	4	1	0
		Reindeer 1	1.7	62	31	2	5	0
15-Apr-11	139	Reindeer 2	6.5	41	49	0	10	0
		Reindeer 3	5.9	35	59	2	4	0
		Reindeer 4	6.3	45	43	6	6	0
		Reindeer 1	6.3	45	39	2	13	1
13-May-11	168	Reindeer 2	6.7	38	48	1	11	2
		Reindeer 3	5.5	44	43	2	11	0
		Reindeer 4	6.8	49	40	1	10	0
		Reindeer 1	10	41	35	6	15	3
9-Jun-11	195	Reindeer 2	9.2	33	54	6	7	0
		Reindeer 3	7.4	31	61	5	2	1
		Reindeer 4	10	43	49	4	3	1

Table C2. cont.

Date	Days post-infestation	Animal ID	White Cell	Neutrophils %	Lymphocytes %	Monocytes %	Eosinophils %	Basophils %
4-Jul-11	220	Reindeer 1	8.5	38	50	1	10	1
		Reindeer 2	12.8	40	52	5	3	0
		Reindeer 3	8.8	28	60	2	10	0
		Reindeer 4	10.4	46	47	4	2	1
5-Aug-11	252	Reindeer 1	9.4	37	49	5	8	1
		Reindeer 2	12.2	21	58	4	16	1
		Reindeer 3	10.9	27	54	7	12	0
		Reindeer 4	9.5	39	46	2	12	1
1-Sep-11	279	Reindeer 1	10.7	43	49	6	2	0
		Reindeer 2	16.8	32	59	6	3	0
		Reindeer 3	10	32	50	6	3	0
		Reindeer 4	13.2	35	56	4	5	0

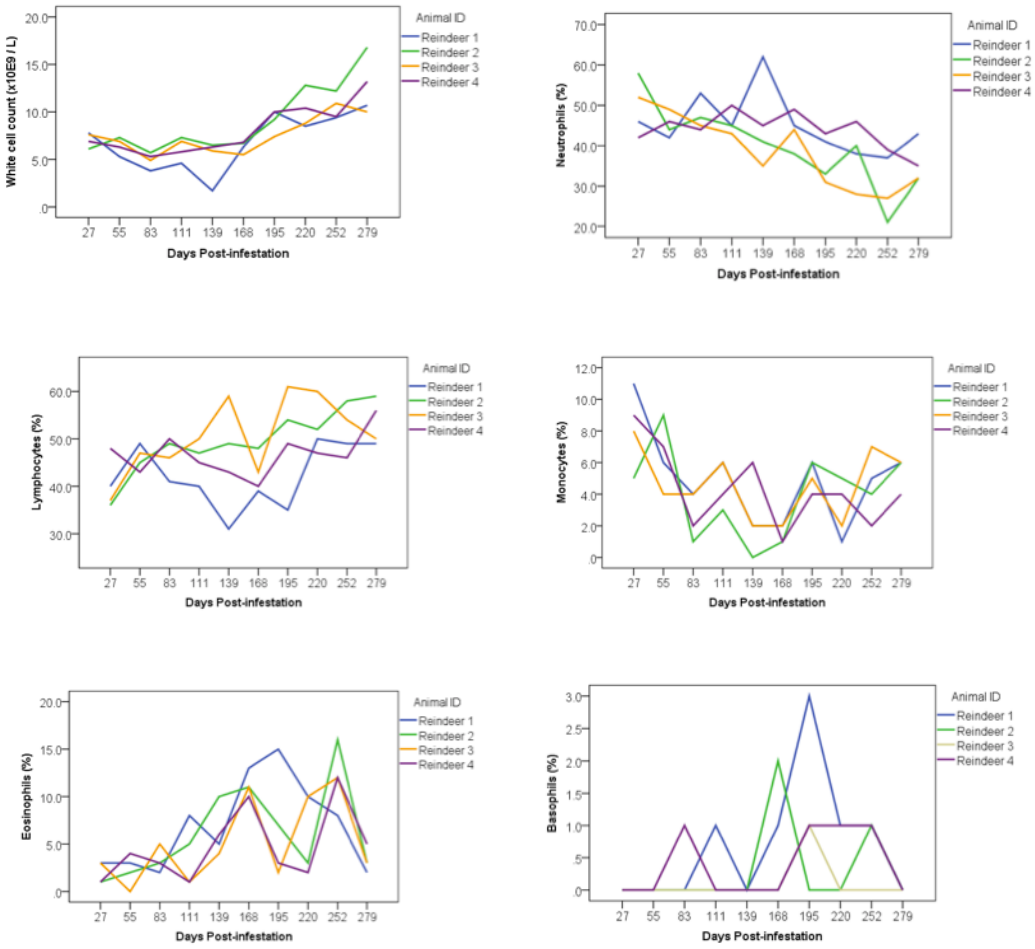


Figure C2. White blood cells and leucocytes counts of the captive reindeer experimentally infested with *Dermacentor albipictus* according to Animal ID and days post-infestation.

APPENDIX III

Muskox and Caribou Health Monitoring Program

ACTIVITY UPDATE SEPTEMBER 2018

UNIVERSITY OF CALGARY
FACULTY OF VETERINARY MEDICINE

MUSKOX HEALTH

Muskox and Caribou Health Monitoring Program

Activity Update September 2018

Introduction

The Muskox and Caribou Health Research Program is a collaborative program among universities, communities, industry and territorial and federal government agencies. The program was initiated in 2008 in response to the apparently changing health status of muskoxen. At that time, the muskox lungworm, *Umingmakstrongylus pallikuukensis,* was detected in muskox samples submitted from a community hunt on southwest Victoria Island; this suggests a range expansion of the parasite towards the north. In addition, the bacterium *Erysipelothrix rhusiopathiae* was identified for the first time in muskoxen and was implicated in multiple severe die-offs in muskox populations from Banks and Victoria Island in 2009-2013.

In response to these health changes, we launched a collaborative, multifaceted research program with the aim of understanding the general health of muskoxen in this region. The program has grown and evolved over the years and strives to bring traditional, local and scientific knowledge together to better understand the health of muskoxen and caribou.

The research that we've accomplished to date has only been possible because of the amazing collaboration among communities, governments, universities and the qiviut and sport hunting industries. We thank all the individuals and organizations that have contributed to this work and look forward to working with you further. In the following pages, you will see a brief overview of the various projects that are currently underway as well as contact information for the researchers involved.

Please feel free to contact me about the overall project and with any questions or concerns you may have.

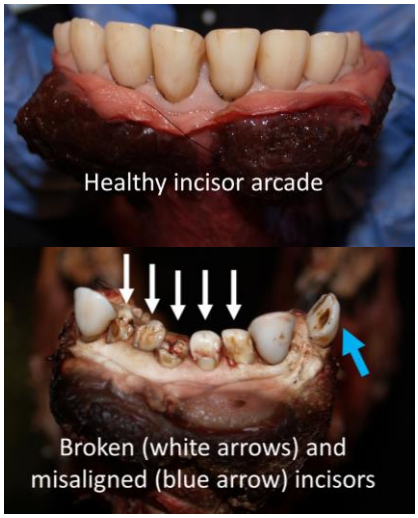
Best,

Susan Kutz,
Professor of Ecosystem and Public Health, Faculty of Veterinary Medicine, University of Calgary
Ph: 403 210-3824
Email: skutz@ucalgary.ca

Incisor breakage in muskoxen

What's the issue?

Good teeth are essential for the health and survival of muskoxen. Animals that cannot feed correctly are more susceptible to diseases, predation or starvation. As part of our muskox health monitoring program, we have collected and examined **the lower jaws of 162 harvested muskoxen**. We found that the most frequent issue is **breakage of the front teeth** (incisors) and that muskoxen on Victoria Island have more broken incisors than the animals on the mainland.



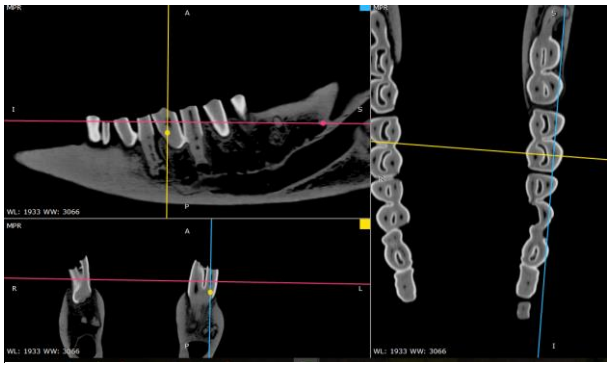
What are the causes?

We don't know yet why there is such a high occurrence of incisor breakage in Victoria Island muskoxen. Possible causes are:

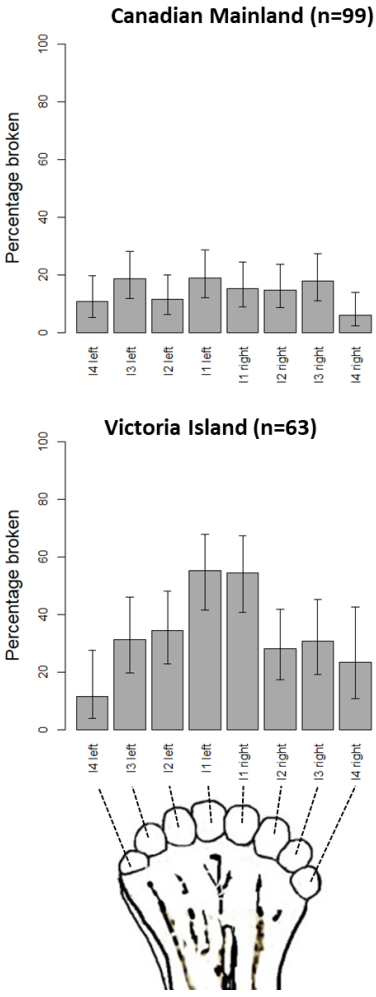
- vitamin/mineral deficiencies or imbalances
- changes in vegetation resulting in mechanical breakage (especially during the winter – if there is less snow in winter to insulate the plants, they might be frozen particularly hard resulting in damage to the incisors)
- genetic: e.g., increased occurrence of animals with misaligned teeth which are more likely to break

What's next?

We did CT-scans on 80 jaws (a technique similar to X-ray) and are currently examining the scans to try to find what could be the cause of incisor breakage. We also plan to do other tests such as trace mineral and micro-hardness analyses.



CT-Scan of a muskox jaw. This technique allows to examine the jaw from all angles.



Difference in incisor breakage in muskoxen from the mainland and Victoria Island.

Research conducted by Fabien Mavrot (fabien.mavrot@ucalgary.ca)

Measuring stress in muskoxen

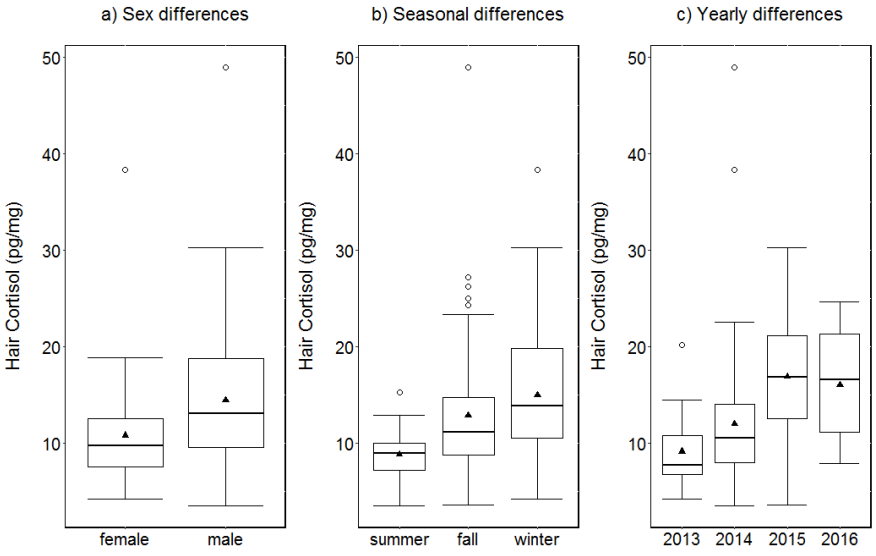
What are we doing?

When an animal is stressed, it releases stress hormones, also known as glucocorticoids. The short-term release of these hormones enables the animal to escape from life-threatening situations and is essential for survival. Conversely, repeated or long-term release of these hormones, over weeks to months, may have negative effects on reproduction, survival, and immunity. Thus, stressed animals are more likely to get sick and may have reduced reproductive success. Measuring the stress levels of muskoxen can consequently give us information about the general health status of the animals or the populations. Stress hormones, such as cortisol, are incorporated into the feces, guard hairs and qiviut, and the levels measured represent, respectively, the stress experienced by the animal during a few days and months.

Results from **150 qiviut samples** collected in the communities of Cambridge Bay, Sachs Harbour, Kugluktuk, Ulukhaktok and Paulatuk between 2013 and 2016 showed a high variability in stress levels among individuals with cortisol levels ranging from 3.51 to 48.92 pg/mg. The sex of the animals, along with the season and year the samples were collected, all had an effect on qiviut cortisol levels. Concentrations were higher in males than females, summer levels were lower than fall and winter, and levels increased from 2013 to 2015 (see figure).

What's next?

An additional 138 qiviut samples and 195 fecal samples collected in 2016-2018 by hunters from Kugluktuk, Ulukhaktok, and Cambridge Bay are currently being analyzed. We will then begin to evaluate how qiviut and fecal stress hormone levels are related to other measures of the health of individual animals, such as body condition or infection intensity of both gastro-intestinal parasites and lungworms. We will also further study the hormonal response to stress in an experimental trial on captive muskoxen in collaboration with the University of Alaska, Fairbanks. Finally, traditional, local, and scientific knowledge will be gathered to gain a better understanding of the stressors affecting muskoxen and their health. For this, group interviews will be conducted in with local hunters and community members in Kugluktuk. The ultimate goal of this project is to determine if qiviut cortisol levels can be used as an indicator of individual and/or population health.



Differences in muskox stress level between by sex, season and year.

Research conducted by Juliette Di Francesco (juliette.difrancesco@ucalgary.ca)

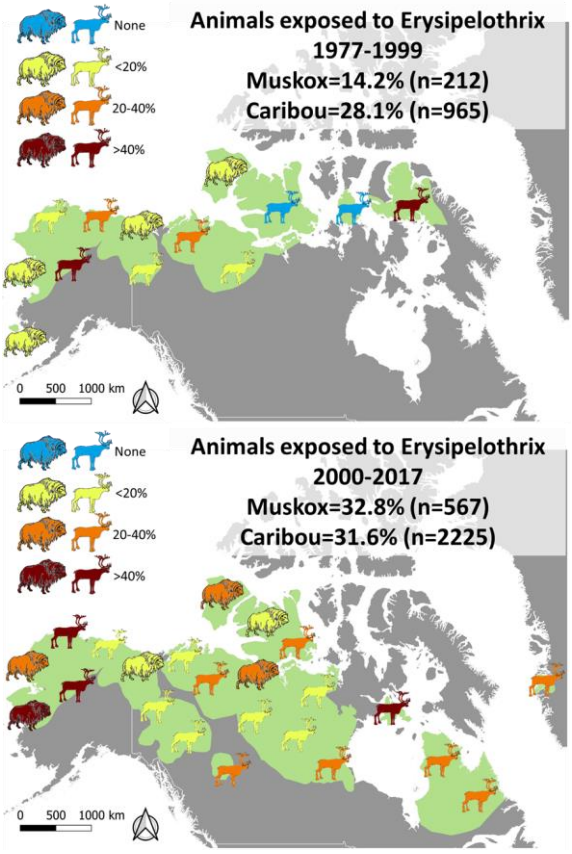
Erysipelothrix in Arctic Wildlife

What's the issue?

Erysipelothrix rhusiopathiae is a bacterium that is suspected to have caused multiple sudden deaths of muskoxen on Banks and Victoria Islands and Alaska from 2009-2013. Caribou are also affected by this bacterium. Our goal is to better understand how animals get *Erysipelothrix* and what it does to muskox and caribou populations.

How widespread is it? Since when is it here?

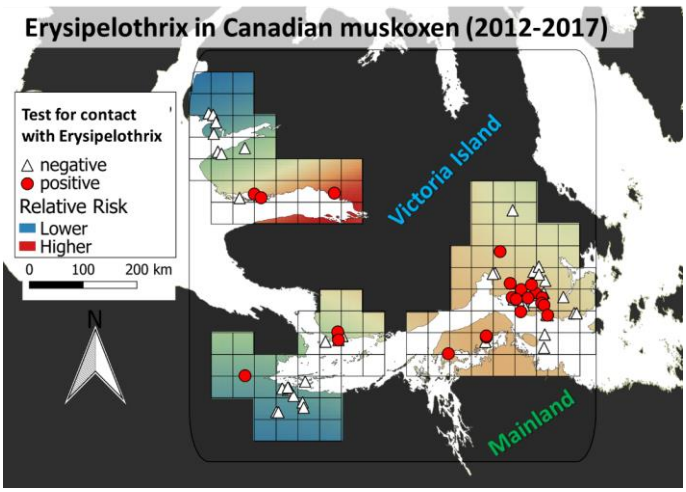
To answer those questions, we analyzed blood samples of caribou and muskoxen collected over 40 years across North America to see if the animals were exposed to *Erysipelothrix*. We have tested **779 muskoxen** and **3,190 caribou.**



What did we find?

We found that *Erysipelothrix* was already present in muskoxen and caribou since at least the 1970's.

It is widespread in North America: almost every herd had positive animals. But there were great differences in how many animals were in contact with the bacterium from year-to-year and between different regions. In general, *Erysipelothrix* seems to have increased in recent years and we think it may have effects on caribou and muskox populations.



What's next?

With the help of the samples collected by local harvesters, we are continuing to monitor the bacterium in the Arctic. In our future research, we hope to be able to understand why *Erysipelothrix* is more common now than it was in the past and to better assess its impact on caribou and muskox populations.

Research conducted by Michele Anholt (rmanholt@gmail.com) **and Fabien Mavrot** (fabien.mavrot@ucalgary.ca)

Muskoxen, caribou, and a fox found dead on Prince Patrick Island, summer 2017

Carcasses of three muskoxen and one Peary caribou investigated on Prince Patrick Island. Erysipelothrix *was found in all animals.*

What's the issue?

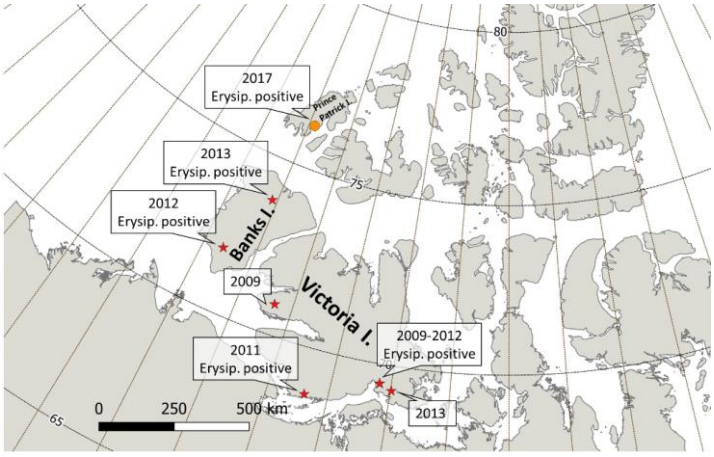
In July 2017, three adult muskoxen were found dead at the same location on Prince Patrick Island.

We investigated the carcasses and concluded that the animals died from **an infection with the bacterium *Erysipelothrix rhusiopathiae***. We also found *Erysipelothrix* in nine additional nearby carcasses (2 other muskoxen, six Peary caribou and one Arctic fox).

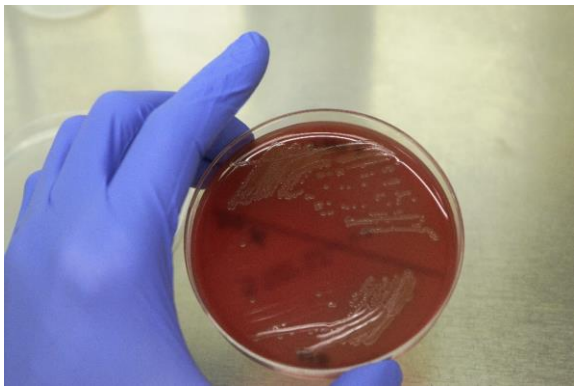
Molecular analyses of the isolated *Erysipelothrix* bacteria showed that all animals were infected **with the same strain of the bacterium**. In addition, the bacterium isolated during the 2017 die-off on Prince Patrick Island is very closely related to the *Erysipelothrix* strain found in muskox carcasses on Banks and Victoria Islands in 2009-2013.

Why is it important?

Erysipelothrix has been linked to both muskox and caribou mortalities in past years. In particular, the multiple die-offs on Banks and Victoria Islands in 2009-2013 raised concern about the possible impact of *Erysipelothrix* on muskox populations. The results from this investigation show that *Erysipelothrix* is still circulating in the Arctic and causing disease in caribou and muskoxen.



Locations of known muskox die-offs in the Canadian Arctic since 2009. "Erysip. positive" indicates die-offs where Erysipelothrix *was detected in the carcasses.*



Isolation of the bacterium Erysipelothrix on a culture plate. The brown-yellowish spots are colonies of Erysipelothrix *growing on the red culture medium.*

Investigation conducted by Fabien Mavrot (fabien.mavrot@ucalgary.ca)

Muskox qiviut as a tool to monitor population health and trends

What's the issue?

Monitoring populations is time-consuming, expensive and difficult in remote areas of the Arctic. Because of this, it is important to find new ways to monitor wildlife populations in collaboration with hunters and others. The aim of this project is to see if mineral levels in hair of muskoxen can predict the future population trends: such a tool will have great value to guide co-management of muskoxen.

What's the rationale?

The use of hunter-based sampling can provide important information on population health and trends that may not always be available from direct population surveys. Trace minerals are things like copper, zinc, calcium and other elements that animals get from their food. These minerals are critical for an animal's health, affecting their resistance to disease, growth and reproduction. We are testing if the mineral status in hair can serve as an indicator of population health and if it can be used to predict future population trends.

How do we do it?

This project is a collaboration of researchers, wildlife biologists and First Nations, Inuit and Metis community members. A key piece of our work is using hunter-based collected samples from the Community-Based Wildlife Health Monitoring Program in the Inuvialuit and Kitikmeot regions. Through this program, hunters collect hair, blood and fecal samples, which will be analyzed to provide information on hair mineral levels, overall health, pregnancy, parasites and diseases and more. This will allow us to evaluate the relationship between trace mineral concentrations in hair samples and individual and population health and trends.

What's next?

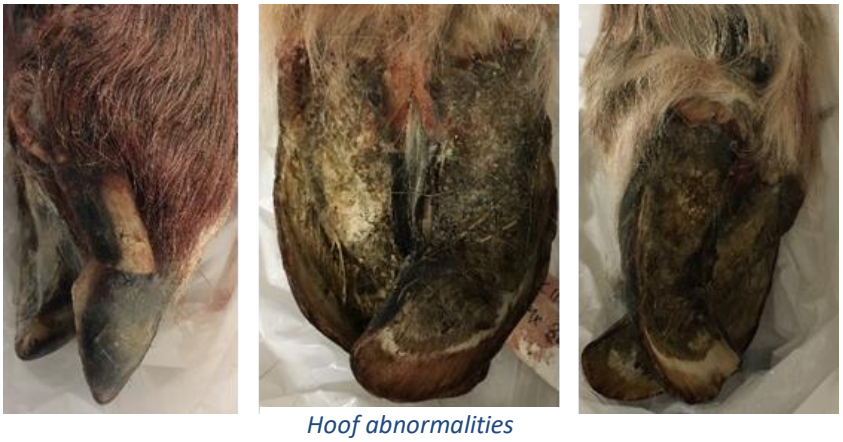
This is a new project that started in April 2018. We have started to analyze samples during the fall and winter of 2018 and will, hopefully, have results to report soon. Once validated, we anticipate that hair mineral content will be a useful tool for estimating population health and trends.

Research conducted by Jesper Bruun Mosbacher (jesper.mosbacher@ucalgary.ca)

Hoof abnormalities in muskoxen

What's the issue?

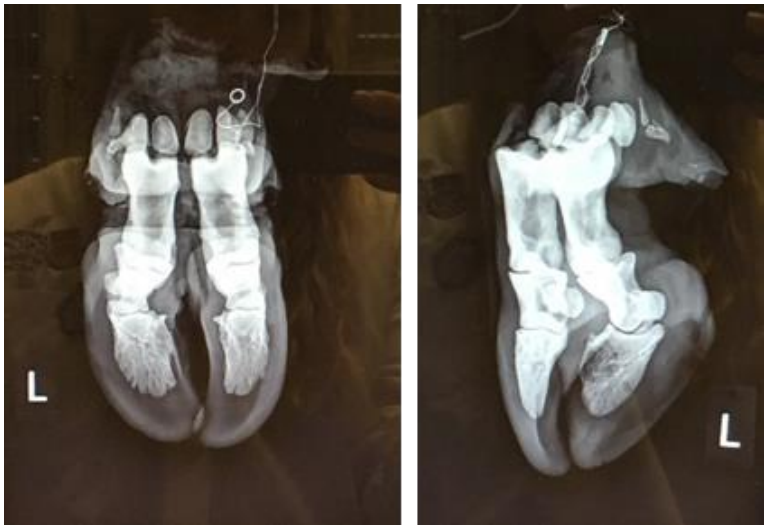
We are seeing abnormalities on the hooves of the animals collected by hunters Kugluktuk, Ulukhaktok, and Cambridge Bay (for example overgrowth or lesions). We know from other species that lameness can have a big impact on the animal's health, fertility, predation risk and survival in general, which all potentially influence the population abundance. Hoof abnormalities may also be an indicator of other abnormalities, stressors, or imbalances in the animal. In this study, we will examine muskox hooves collected by local hunters and identify, describe and evaluate hoof abnormalities in the different study areas.



Hoof abnormalities

What's next?

This is a new project, and still in its early beginnings. We have recently X-rayed all the hooves to identify and evaluate any bone or joint related abnormalities. The next step is a close examination of all the hooves using microscopic and other types of techniques. We hope to determine, what hoof abnormalities are present in the muskox populations. Ultimately, the hoof health will be compared to other health parameters measured on the same individuals, like mineral and stress levels or parasite infection, to see if they are related. We hope the results will tell us more about hoof health in muskoxen and its impact on individuals and populations.



X-rays of muskox hooves.

Research conducted by Tina Steiner Petersen (tina.petersen1@ucalgary.ca)

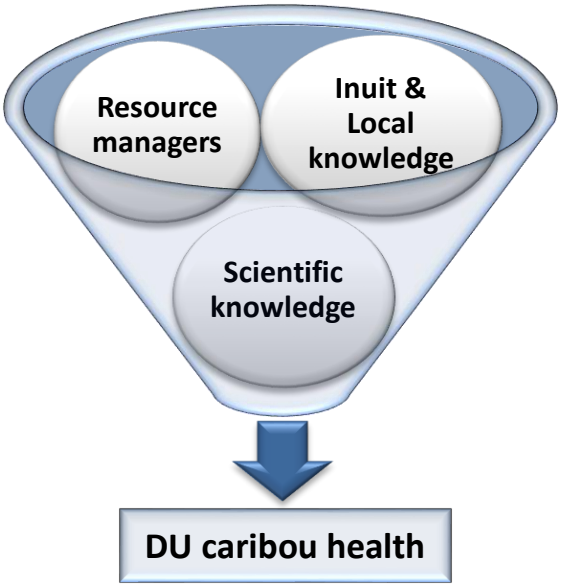
Expanding health research to the Dolphin and Union caribou herd

What's the issue?

The Dolphin and Union caribou herd is integral to Inuit life, both for culture and subsistence. This herd migrates in the fall and spring back-and-forth between Victoria Island and the mainland and is dependent on sea ice for this biannual journey. Preliminary local and scientific knowledge both indicate that this caribou herd is declining and in poorer health than before. Our goal is to expand on this knowledge to better understand the health of the Dolphin and Union caribou herd and understand what factors may be causing it to decline.

What's the rationale?

To help protect this herd, we need to bring everyone together and use everything we know about Dolphin and Union caribou, the environment and the other animals to help protect and care for these animals. To start this process, we need to understand how the Dolphin and Union caribou are doing now and develop better and quicker ways to measure changes in the populations.



How do we do it?

We start conversations between local Dolphin and Union caribou experts in the communities, veterinary medicine specialists and resource managers. Through adaptive co-management processes, we can incorporate information from all these different knowledge sources as we learn new things to constantly adapt the way we protect the Dolphin and Union caribou. The difficulties with working in any large team is being able to communicate well and trust each other. To help overcome this, we are acknowledging and trying to respect all the different cultures involved in a project like this, and we are starting small to build a good foundation for the future of this program.

What's next?

With the help of the Kugluktuk Hunters' and Trappers' Organization, we'll start by interviewing and working with Dolphin and Union hunters in Kugluktuk. Through individual and group interviews, the goal to is create a collective account of how Kugluktuk hunters see the Dolphin and Union caribou doing. *Are they sick? Are they fat? Are they surviving the winter? Are they surviving the migration?* This September-November 2018, I'll be in Kugluktuk, listening and learning from the people who know about Dolphin and Union caribou.

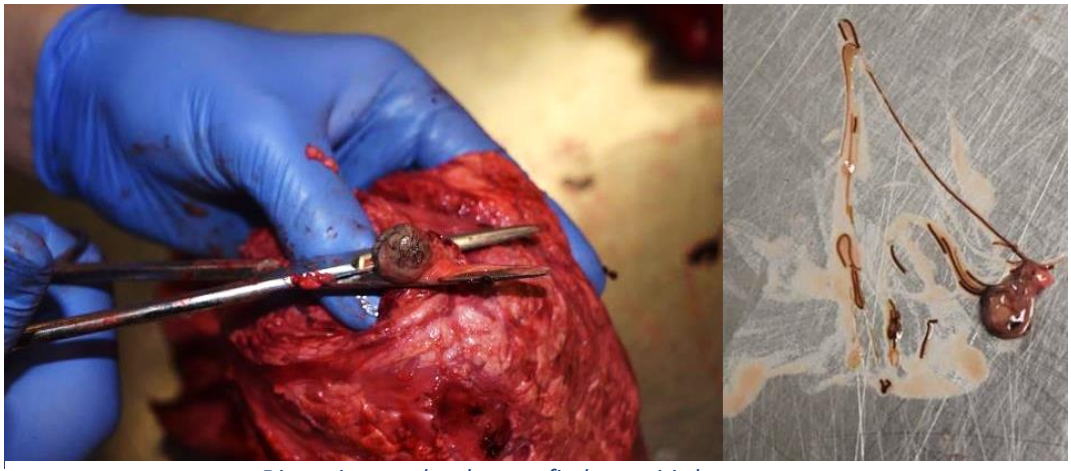
Research conducted by Andrea Hanke (andrea.hanke1@ucalgary.ca)

Educational activities in Arctic communities

Thanks to the NSERC: Promoscience Program, we have been able to offer educational activities in the Arctic communities of Kugluktuk (Nunavut) and Ulukhaktok (Northwest Territories). Those activities were organized in parallel with our field work and in collaboration with the local schools. We have delivered presentations on different topics related to wildlife health monitoring but also on ecology and career opportunities in science. We also organized "hands-on" workshops such as animal dissection, looking for parasites in muskox droppings, bone anatomy etc.

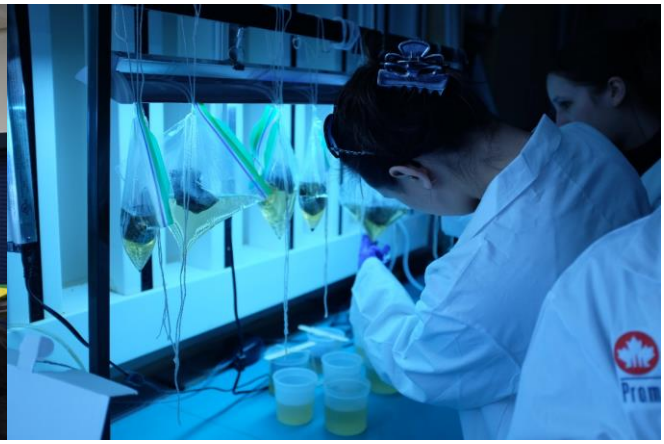
With these activities, we hope to directly engage the next generation of community members in science and, ultimately, inspire them to go further and develop the knowledge and skills for monitoring and management of wildlife populations in their rapidly changing environment. So far, the feedback has been extremely positive, and we are looking forward to continuing to bring science in the classrooms!

Many thanks to NSERC PromoScience and the school personnel in Kugluktuk and Ulukhaktok for making this possible.



Dissecting muskox lung to find parasitic lungworms

Learning caribou anatomy



Experiment to extract lungworm larvae from muskox feces

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