Dolphin and Union Caribou Health Assessment

Interim results, July 2020

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Executive Summary

This report includes the preliminary results of an ongoing collaborative health assessment of the Dolphin and Union (DU) caribou herd based on samples collected from 2015-2019. Samples were obtained through community-based sampling of hunted animals (Kugluktuk, Cambridge Bay and Ulukhaktok) and from caribou captured and collared for population monitoring purposes by the Government of Nunavut.

We found that DU caribou were commonly and continuously exposed to different pathogens that can cause reproductive or health problems. Through serology (blood tests), we detected an overall exposure to the bacteria *Brucella suis* biovar 4 of 14.4 % (CI95%, 10.2-20.1) and *Erysipelothrix rhusiopathiae* 22.3 % (CI95%, 17.0-28.8), as well as to the viruses α-herpesvirus 86.9 % (CI95%, 79.2-92.0) and pestivirus 21.2% (CI95%, 15.9-27.5). We also detected exposure to the protozoan parasites *Toxoplasma gondii* (ranging from 0.0% to 39.3%) and *Neospora caninum* (ranging from 0.0% to 33.3%), both of which varied quite substantially among years. Larvae from lung (*Varestrongylus eleguneniensis*) and muscle (*Parelaphostrongylus andersoni*) parasites were detected in 6.6% of the feces, and low numbers of the protozoan parasite *Besnoitia tarandi* (cause of 'sandpaper disease on lower legs') was detected in 43.5% of the metatarsal (lower back leg) skins examined (cysts/mm^2; min.:0.039, median:0.235, max.:1.036).

Brucella suis biovar 4 was isolated from the bone marrow, swollen joints and surrounding tissues, and/or testicles, of five caribou submitted because of disease concerns by hunters from Kugluktuk. Animals that had been exposed to *Brucella* were skinnier and less likely to be pregnant (63% pregnancy rate) compared to those that were not exposed (91% pregnancy rate). Preliminary analyses on collared caribou suggests that *Brucella* exposure may negatively affect adult survival. Brucellosis can also cause late-term abortions or reduced survival of newborn calves, but that was not possible to assess with our sampling approach.

We tested hair for trace mineral levels and stress hormones (cortisol) and feces for stress hormones as well. Trace mineral levels were in general lower than those found in healthy mountain caribou and captive reindeer that we have previously tested. This was especially true for selenium and molybdenum. Further analyses are required to understand the significance of these lower values for caribou health. Preliminary results of stress levels in feces and hair both indicated a decreasing trend in stress in the latest two years (2018-2019).

Overall (captured and harvested caribou combined), DU caribou had higher pregnancy rates in adult females (87.6%; 2015-2019) and better body condition based on kidney fat (2018-2019), than reported in previous studies from 1987-1991 and from 2001-2003. These findings of higher pregnancy rates, declining stress levels, and higher body condition suggest that the last few years may have been better for the herd than previous periods. Further and ongoing monitoring is critical to determine if these trends continue. Note that we did not assess late term abortions or stillborn calves, neonate survival or calf survival, all of which can impact the recruitment and, therefore, the population growth.

Hunter- and capture-based sampling of DU caribou over the last 5 years have provided an invaluable set of samples and data that allowed us to determine the current health status of the DU caribou and compare it to what was known in the past and what will happen in the future. This work provides an unprecedented health assessment of the DU caribou, identifying important threats to the herd and human health, such as brucellosis, but at the same time, providing some suggestion of improvement in body condition and pregnancy rates. Ongoing assessment, through the use of all possible sample and knowledge sources, is critical for tracking and understanding the health status and anticipating the trajectory of the DU caribou herd. Community-based monitoring, when implemented in a systematic and ongoing manner as has been done by the communities involved here, can provide early detection of shifts in population health and trends, as well as identification, and a broader understanding of, the impacts of current and emerging threats.

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1. Background

The Dolphin and Union (DU) herd (*Rangifer tarandus groenlandicus* x *pearyi*) is considered its own Designable Unit in Canada which means that it requires separate assessments and management plans from other caribou populations such as the barren-ground caribou or Peary caribou. There are three main Inuit communities in the Kitikmeot region (Nunavut; Kugluktuk and Cambridge Bay) and the southeastern part of the Inuvialuit region (Northwest Territories; Ulukhaktok) that partly rely on this caribou herd for food and other resources, and for whom this caribou herd forms part of their cultural heritage.

Local and Traditional knowledge documented in Cambridge Bay and Kugluktuk identified that the DU herd began declining in the early 2000s and associates the decline with increases in disease, poorer body condition, and changes in sea ice (Hanke et al., 2020; Tomaselli et al., 2018). Scientific surveys estimate the herd size based on fall aggregations on the southern edge of Victoria island before crossing towards mainland. The last population survey performed in 2018 estimated 4,105 ± 694.8 (Leclerc and Boulanger, 2020), which represents approximately an annual rate of decline of 62% since 2015 (18,413 ± 6,795) and a total drop of about 88% since 1997 (34558 ± 4283) (Dumond and Lee, 2013; Leclerc and Boulanger, 2018). In 2017, the Dolphin and Union caribou was recommended endangered by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) (COSEWIC, 2017).

Wildlife health is better conceived as the resilience of populations to changes, rather than the absence or presence of disease. Health is the outcome of cumulative biotic, abiotic and environmental factors that can interact at individual or population levels (Macbeth and Kutz, 2018; Stephen, 2014). To understand better the health of DU caribou and the possible drivers of population decline, we established a health assessment program that integrates both scientific and local knowledge for this herd in 2015. For the purposes of this research, we defined health broadly to include both infectious and non-infectious disease, and indicators of how well the population is doing such as body condition, stress or reproductive rates.

This program is a combined effort of different partners committed to understand better and safeguard the future of this caribou herd. The main partners include the local Hunter and Trapper Organizations and Committees (HTOs and HTCs), the Governments of Nunavut and Northwest

Territories, Polar Knowledge Canada (POLAR), Canada North Outfitting, and the Kutz Research group at the University of Calgary.

Work presented in this report has been funded by Polar Knowledge Canada, Irving Maritime Shipbuilding, Nunavut Arctic College, Canada North Outfitting, Crown-Indigenous Relations and Northern Affairs Canada, and NSERC. Additional in-kind and financial support has been provided by all partners.

2. Objectives

The aim of this program is to perform a comprehensive health assessment of the Dolphin and Union caribou herd, provide initial information on the effect of select infectious diseases and a health baseline for future assessments in the face of environmental and climate change. The specific objectives are to investigate health indicators such as body condition, pregnancy, stress and trace mineral levels and other factors like infectious diseases and parasite status. All these parameters are either informative on how well the population is doing (health indicators) or about possible threads that may affect population trends.

3. Approach

3.1. Sampling and data sources

For this program we used different sample sources including caribou hunted through both subsistence and sport-hunts (collections led by HTOs and HTCs), and caribou captured for collaring and monitoring purposes (collections led by Government of Nunavut). This mixed approach regarding sampling sources increased the sample size and allows us to obtain a better assessment of the herd status.

The hunter-based sampling was done in collaboration with the Hunters and Trapper Organizations (HTOs) and Committees (HTCs) of the communities of Cambridge Bay, Kugluktuk and Ulukhaktok, Canada North Outfitting, the governments of Nunavut and NWT, and Polar Knowledge Canada. The hunters or local outfitter guides were provided with a sampling kit to collect a set of samples (feces, hair from the neck, blood on filter paper, the left hind leg, the left kidney with the surrounding fat – note that there were some variations among communities), basic information of the animal and any observed abnormalities (Tomaselli and Curry, 2019). Samples from live-captured caribou were collected in collaring activities organized by the Government of Nunavut. The sera, neck hair and feces from captured adult female DU caribou were collected by GN biologists while the animal was physically immobilized under standardized capture-release protocols.

Dolphin and Union caribou on its current winter range in mainland Kitikmeot overlap with the range of other barren-ground caribou herds and it is known that intermix of individuals from different herds may happen (Bergerud et al., 2008). For this program, we requested Dolphin and Union caribou samples from hunters. To confirm the hunter identification, when possible, all animals were also tested by microsatellite genotyping at Wildlife Genetics International (Nelson, BC). The genetic ancestry of the individuals was assessed using 18 microsatellite markers that relate to known variability between the different herds (Serrouya et al., 2012). The individual herd identity was assessed based on their genetic ancestry and calving location (for capture-collared), or, in the absence of genetic data, on sampling location and morphological traits described by harvesters (Leclerc and Boulanger, 2018). Animals were assigned to the DU herd if their genetic background was greater than 50% DU based on consistent results from genetics and calving location from collared animals (results not shown).

3.2. Laboratory Analyses

The age of harvested animals was determined through the cementum annuli from the incisors (i1 when available, n= 90) at Matson's lab (Manhattan, MT, USA) (McEwan, 1963), or by teeth eruption pattern in animals less than two years old (Wu et al., 2012). Animals were assigned to the adult age class if determined to be 22 months old or older, based on dental examination or assessment by biologist on capture.

Blood samples (n=197) were analyzed to assess the exposure of the animals to pathogens (i.e. *Brucella* spp., pestivirus, herspesvirus, *Erysipelothrix rhusiopathiae*, *Toxplasma gondii*, *Neospora caninum*) using different commercial and in-house ELISAs (enzyme-linked immunosorbent assay) that detect specific antibodies (Appendix 1, Supplementary table).

The pregnancy status of the females was assessed by measuring the concentrations of the progesterone hormone in fecal samples. Physiological stress was measured indirectly (cortisol and its metabolites) in hair and feces of the animals. The feces were analyzed at the Endocrinology lab in Toronto Zoo, the hair collected in 2015-2017 at the University of Saskatchewan (Faculty of Veterinary Medicine), and the hair collected in 2018-2019 at the Endocrinology Lab at the Toronto Zoo (Ashley et al., 2011; Morden et al., 2011). Fecal stress hormone levels reflect physiological status from approximately the last 24-48 hours before sample collection, representing the recent period, whereas hair values reflect the hair growth period from late spring to fall, representing conditions over several months during the previous summer/fall (Macbeth and Kutz, 2018).

Trace mineral concentrations in hair from the neck were assessed with high-resolution inductively coupled plasma mass spectrometry at the Alberta Centre for Toxicology. This method has been successfully applied to assess trace minerals in hair from muskoxen (Mosbacher et al., unpublished results), mule deer (*Odocoileus hemionus*) (Roug et al., 2015), moose (*Alces alces*) (O'Hara et al., 2001) or cattle (O'Mary et al., 1970). The application of this method for caribou hair analyses has great potential for broader scale analyses of trace mineral levels across animals, years and herds.

Parasitological examinations (Baermann technique) of the feces were performed to estimate the presence of parasite larvae (Kafle et al., 2017). The detection of *Besnoitia tarandi* was done with histological (microscopic view of tissue) examinations of the skin of the front of the lower left leg (metatarsus). A cyst density in this location was also estimated according to previously described methods (Ducrocq et al., 2012).

Body condition (fat stores) was recorded by the hunter by measuring the rump fat thickness, and then in the lab using the Riney Kidney Fat index (using the weight of the fat surrounding the kidneys to its poles and the weight of the kidney, see (Adams et al., 2008)) and calculating the percentage of fat in the bone marrow of the metatarsus (leg provided by the hunters), following standardized CARMA protocols (Adams et al., 2008).

4. Results and discussion

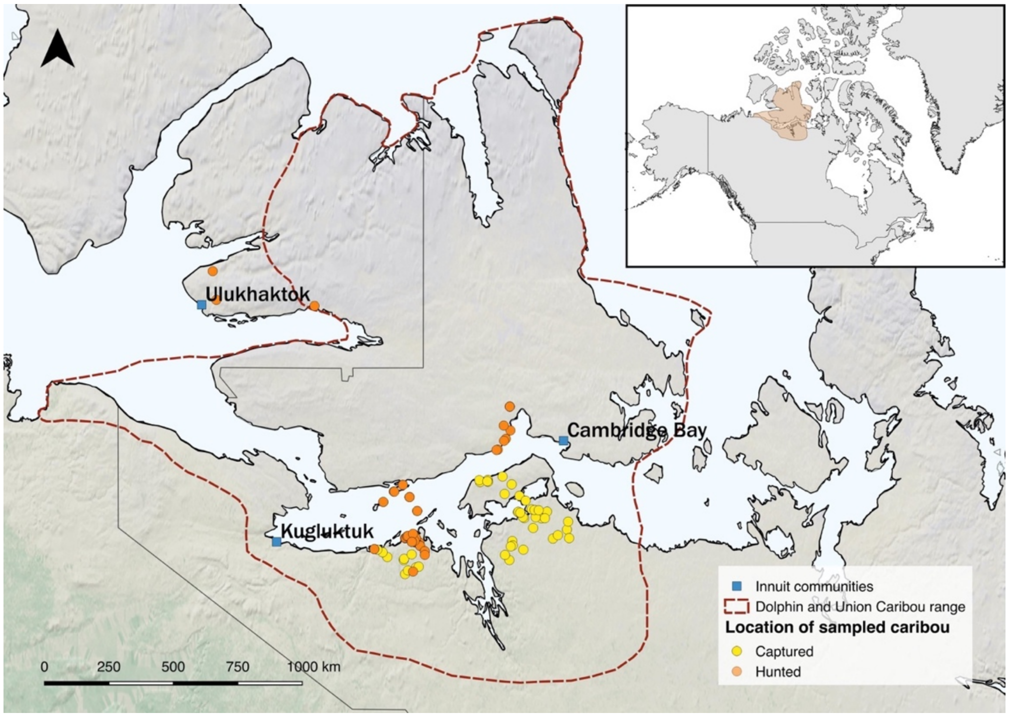
We received samples from a total of 245 caribou sampled from 2015 to 2019. Among these, 218 were analyzed for their genetic profiles and 17 animals (9 captured and 8 harvested) were excluded from the program as they were assigned as barren-ground animals from neighboring herds.

We have analyzed 202 of 228 DU caribou sampled to date (Table 1; Figure 1). Samples collected in the fall 2019 (7 Kugluktuk, 18 Cambridge Bay, 1 Ulukhaktok) could not be analyzed because research labs were closed in the COVID-19 crisis, and their results are not included in this report.

Table 1. Dolphin and Union caribou sampled since 2015 by hunters and guides from different communities and by the Government of Nunavut (GN) during caribou captures.

	Source	**2015**	**2016**	**2017**	**2018**	**2019**	**Total**
Cambridge Bay	Subsistence and sport hunt	15	7	7	2	18	49
Kugluktuk	Subsistence hunt	0	0	0	40	49	89
Ulukhaktok	Subsistence hunt	0	0	0	2	2	4
Government of Nunavut	Captures	17	18	0	51	0	86
Total		**32**	**25**	**7**	**95**	**69**	**228**

Figure 1. Locations of the Dolphin and Union (DU) caribou sampled in this study. The distribution range of the DU herd as it appears in the COSEWIC assessment and Species at Risk act of DU caribou herd 2017 is indicated with a dashed red line (COSEWIC, 2017; Environment and Climate Change Canada, 2018).



4.1. Health indicators

Body condition (energy stores), pregnancy rates and stress hormones (cortisol and metabolites) are useful to understand the health status of the DU caribou herd and can be directly or indirectly associated with population growth (Macbeth and Kutz, 2018). We compared the results obtained in this project for body condition and pregnancy rates with historic data on the same herd collected by Anne Gunn as a territorial biologist from Northwest Territories in 1987-1991 (n=99) (Gunn et al., 1991), and by a previous study performed by the University of Oxford from 2001 to 2003 (n=82) (Hughes et al., 2009). This historical information was gathered and kindly provided by Anne Gunn and Don Russell.

Body condition: we assessed body condition of caribou through different metrics depending on the origin of the sample. Based on Kidney Fat Index and animals harvested in early Spring (April-

May), DU caribou were in better body condition in 2018 and 2019 compared to the time period of 1987-1991 (Figure 2). Local knowledge from Cambridge Bay collected in 2014 indicated caribou were in lower body condition since around mid 90s-2000s (Tomaselli et al., 2018), but no measured kidney fat information is available for that period. Thus, although body condition is higher recently compared to 1987-1991, it is not possible to confirm if this represents a sustained trend or just a couple of good years for the herd.

Pregnancy: the average pregnancy rate from adult females in Spring (mid-April to beginning of May) from 2015 to 2019 was 87.6%, with relatively high values each year (2015=88.2%, 2016=87.5%, 2018=85.5% and 2019=92.9%). These rates were higher than those recorded in 1987-1991 and 2001-2003 (Figure 2). The lower pregnancy rates in the early 2000s is consistent with the beginning of the declining period of DU caribou herd as identified by Traditional Knowledge in Cambridge Bay and Kugluktuk (Hanke et al., 2020; Tomaselli et al., 2018). Low pregnancy rates will translate to lower productivity and recruitment of the herd. However, high pregnancy rates do not necessarily translate to higher recruitment as recruitment is also affected by late term abortions, and neonate and calf survival.

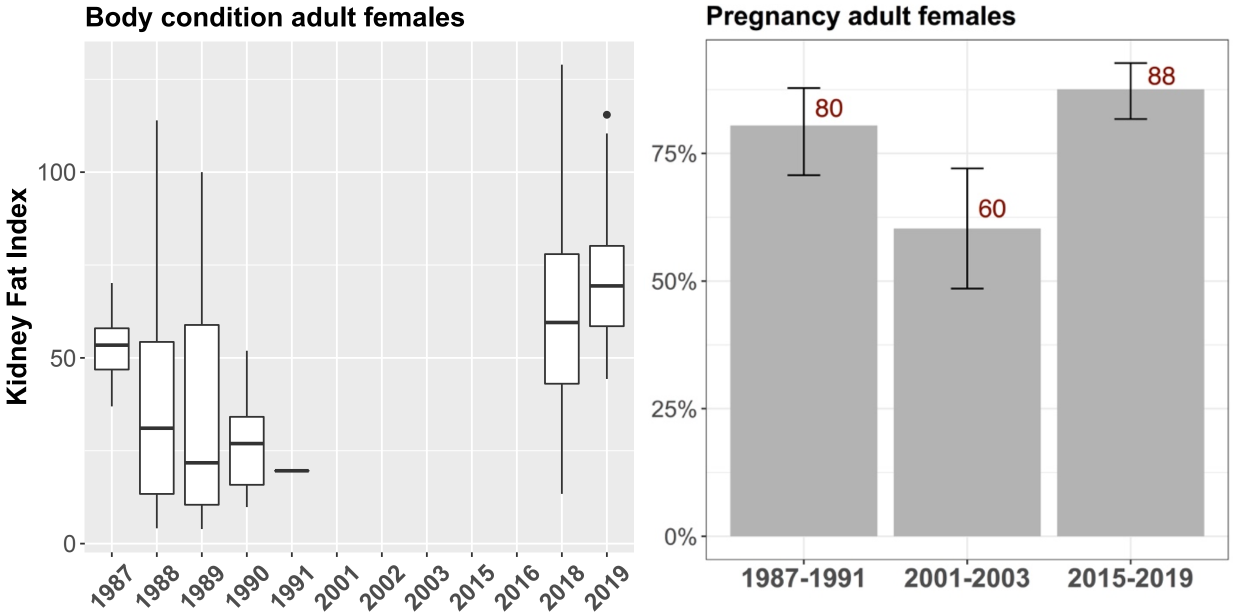


Figure 2. Comparison of body condition (left, n=136) and pregnancy rates (right, n= 287) in Spring (mid-April to the beginning of May) in adult females of the Dolphin and Union caribou herd, based on the current study (2015-2019) and historic data (Gunn et al., 1991). For the pregnancy rates, information from

several years were pooled by years indicated on the horizontal axis. No comparable body condition information was available for 2001-2003.

Stress hormones: levels of glucocorticoids and metabolites are indicative of a physiological response to stressors that can be related to a caribou's life history (rut, migration, sex), disease or to challenges associated with environmental, ecological or human disturbance (Macbeth and Kutz, 2018). Our fecal sample results suggest lower stress levels in the last few years compared to 2015. Similarly, hair stress levels representing the summer-fall of 2018 were lower than those from previous summer-fall periods 2014-2017 (Figure 3). These findings of lower stress levels in recent years are consistent with the observations in Kugluktuk of cooler summers, low insect abundance, and overall perceived better conditions for caribou in these two last years (Hanke et al., 2020).

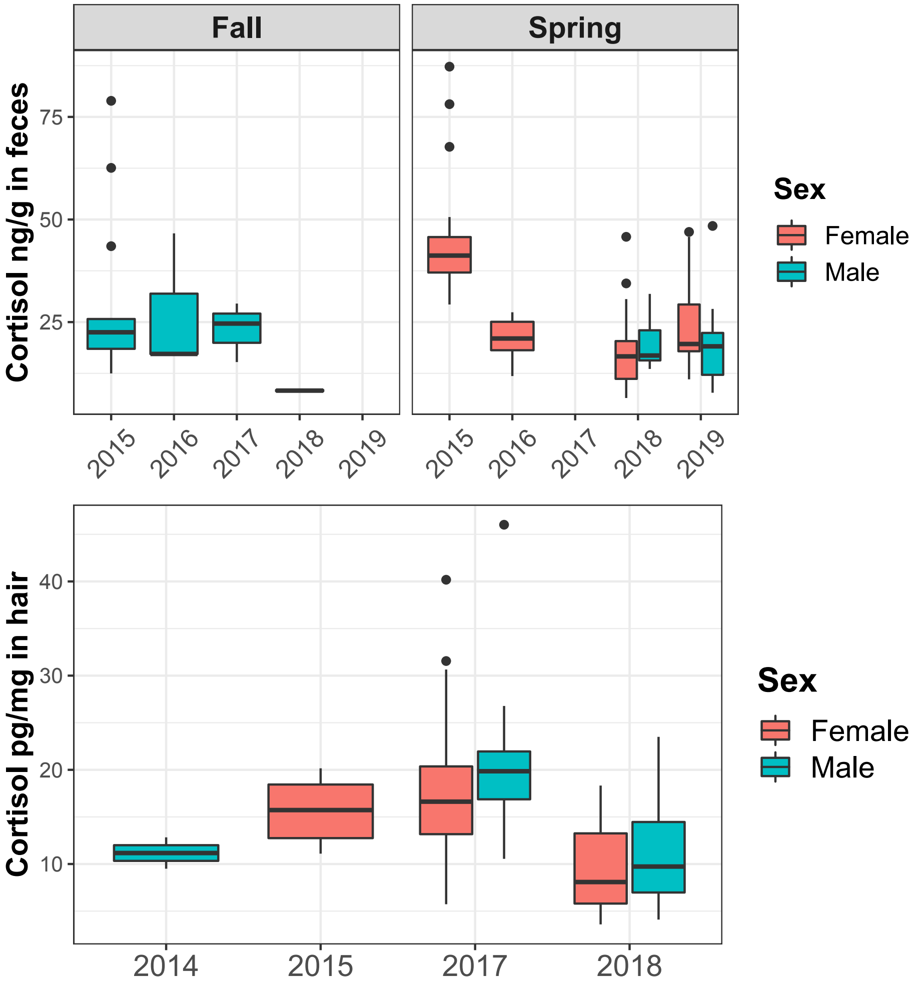


Figure 3. Cortisol and/or its metabolite concentrations measured in feces (top, n= 179) and hair (bottom, n= 150). Note that hair samples reflect the growing period of the previous summer-fall and are identified as such, even if they were collected in the subsequent year (e.g., winter hair samples collected Jan-May 2015 are labelled as 2014 as they reflect stressors from the 2014 summer).

4.2. Exposure to pathogens

We found that the Dolphin and Union herd is commonly and constantly exposed to different pathogens that may affect the health or the reproductive performance of the caribou. In particular, the overall exposure of this herd to *Brucella suis* biovar 4 (BS4) was 14.4 % (CI95%, 10.2-20.1), *Erysipelothrix rhusiopathiae* 22.3 % (CI95%, 17.0-28.8), α-herpesvirus 86.9 % (CI95%, 79.2-92.0) and pestivirus 21.2% (CI95%, 15.9-27.5). Annual fluctuations were detected for antibodies against the protozoan parasites *Toxoplasma gondii* (ranging from 0.0% to 39.3%) and *Neospora caninum* (ranging from 0.0% to 33.3%). Figure 4 shows the annual percentages of the animals sampled that tested seropositive for exposure to these pathogens. These include some of the results that were already published in Carlsson et al., 2019 (Carlsson et al., 2019). Please note that, with the exception of *Brucella,* being positive for exposure does not necessarily mean that the animals were sick with the disease at the time of testing.

Dolphin and Union caribou have a higher exposure to *Brucella* and α-herpesvirus than most of the other caribou herds from the Arctic (Carlsson et al., 2019). Although all pathogens tested are known to cause reproductive or health problems in *Rangifer* (reindeer and caribou) (Tryland and Kutz, 2018), their population effects in the herd are still largely unknown. See Appendix 2 for a brief description of known individual health effects in *Rangifer* or other ruminants.

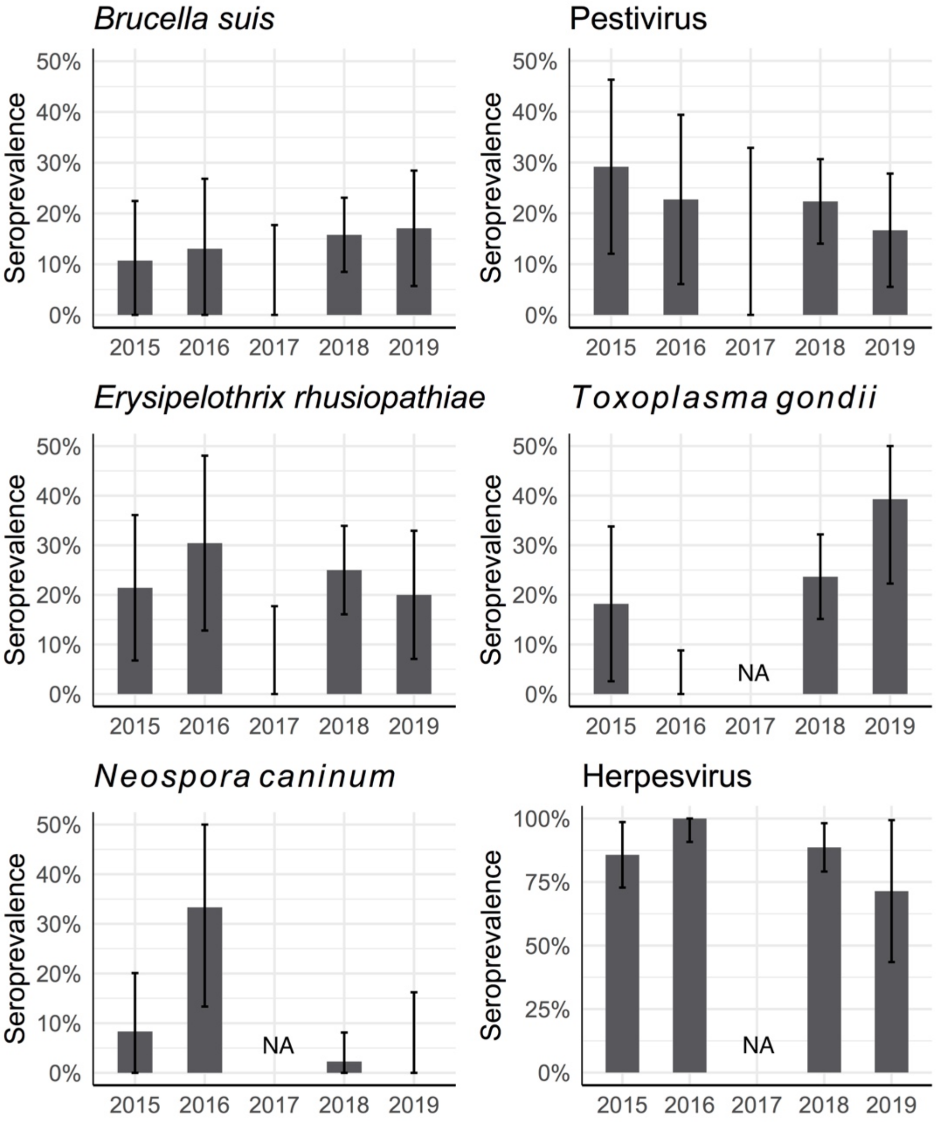


Figure 4. Percentage of animals tested that show exposure based on blood testing (seroprevalence) to bacteria, viruses and parasites (sample size varies from 170 to 197, except for Herpesvirus which was 97). The smaller bars show the confidence intervals (95%) for each year estimate and the initials NA indicate that no samples were analyzed for that year. Please note that in 2017 very few animals were sampled and only from Cambridge Bay. All age categories were included in these estimates.

In April-May, non-pregnant females were more likely to be seropositive (have antibodies) for *Brucella* (38.9%, 7/18, CI95% 20.3-61.38) compared to pregnant females (10.3%, 12/117; CI95% 6.0-17.0). Caribou that had antibodies against *Brucella* were in lower body condition and adult females were less likely to be pregnant (63% pregnancy rates of *Brucella*-exposed caribou versus 91% for *Brucella*-non exposed caribou) (Figure 5). *Brucella* biovar 4 causes reproductive disease mostly in the first gestation after exposure, which can explain part of the variability observed in the results. Stillbirths and weak calves with limited survival are also common brucellosis outcomes which occur in later stages of pregnancy and, therefore, would not be detected based on our sampling design and times. For all the other pathogens, we did not detect any association between the caribou being exposed to the pathogen (had antibodies) and an effect on body condition or pregnancy. This is not surprising, as antibodies only indicate exposure to these pathogens and not necessarily that they are still infected. Therefore, the effect of the infection on health parameters

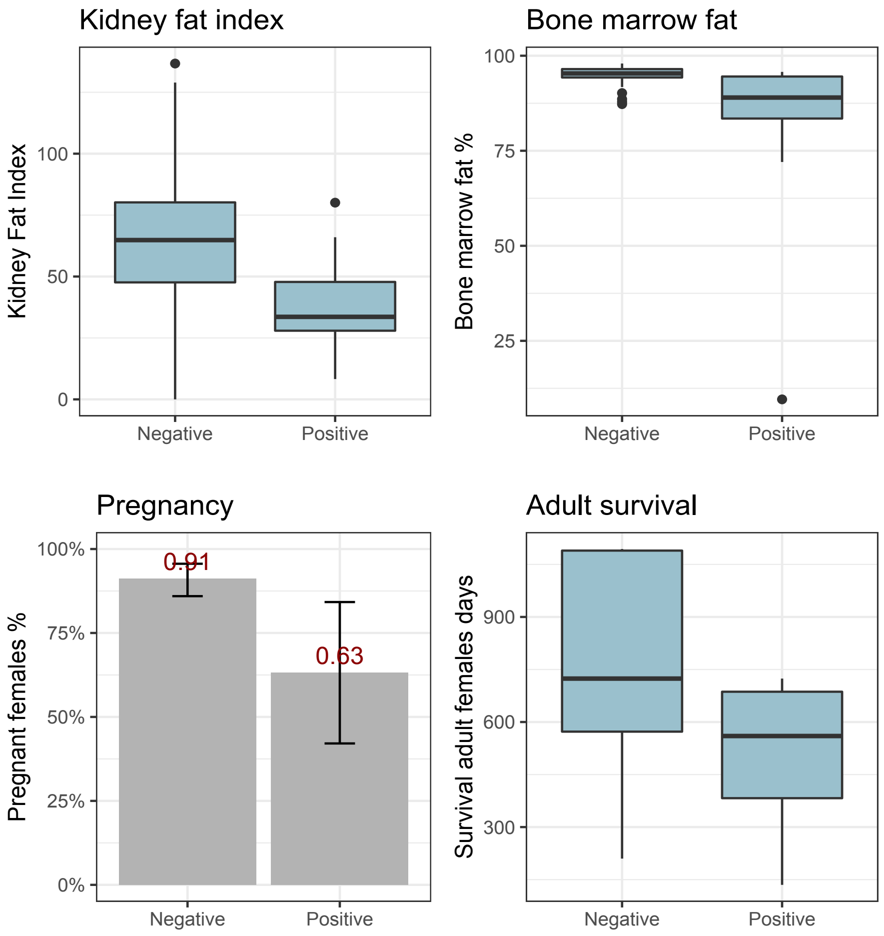


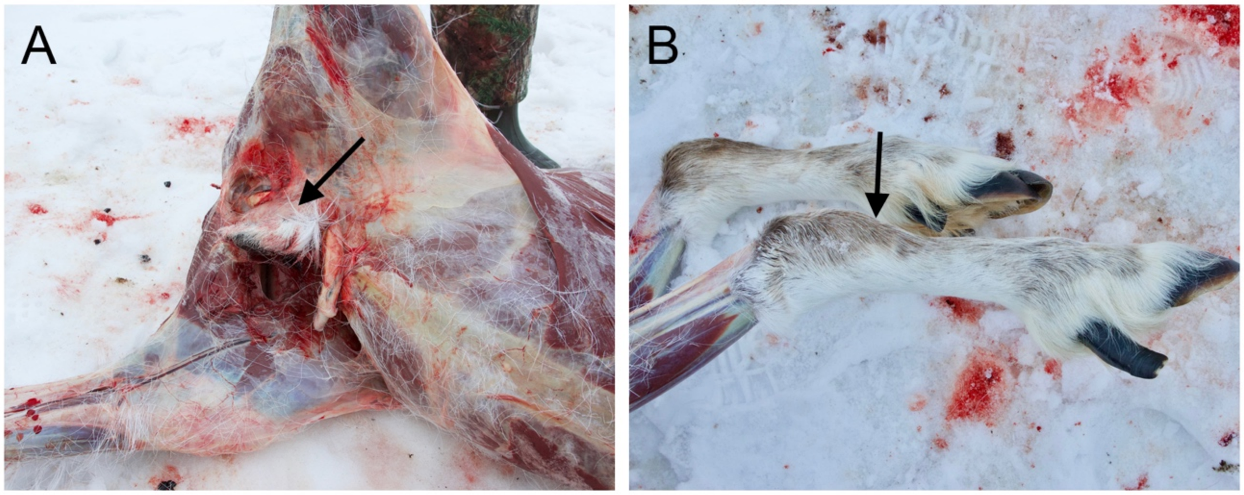
Figure 5. Effects of exposure to *Brucella* on health indicators of Dolphin and Union caribou in early Spring (April-May, n=87). Top: boxplots showing significantly (p<0.05) higher Kidney Fat and Bone Marrow fat in caribou negative to *Brucella* (no antibodies) compared to those that were positive (*Brucella* antibodies detected). Bottom left: Comparison of pregnancy status for adult female caribou that exposed (positive) or not (negative) to *Brucella* (n=66). Caribou that were seronegative for *Brucella* were significantly more likely to be pregnant than those that were positive (Fisher's exact test p=0.02, OR=0.18). Bottom right: preliminary data on adult female caribou survival (days post-capture) of positive and negative animals to *Brucella*. These results are not statistically significant but shows a tendency for shorter survival time of animals that are exposed to *Brucella* (n=44). Further analyses are needed to properly assess the effects of BS4

may not be reflected at the time of sampling for most of the other pathogens. In contrast, *Brucella* causes chronic infections and disease that can persist for long-time periods.

Brucella suis biovar 4 (BS4) causes chronic infections in caribou, mostly evident with swollen joints or testicles of the affected animals (Figure 6). This bacterium was isolated from the bone marrow and lesions of five disease cases (four swollen joints, one swollen testicle) submitted by hunters from Kugluktuk in 2018 and 2019. Notably, in two out of three of these cases BS4 was isolated from the bone marrow of non-affected hind legs.

Evidence of clinical disease due to brucellosis (hygromas-swollen joints and orchitis) were recorded by hunters or field sampling in 3.0% of all the caribou sampled (6 among 202 caribou sampled), which is a smaller proportion of the caribou that had antibodies (14.4%) against BS4. Among the caribou that were exposed to BS4 (antibody detection), 21.4% had visible signs of brucellosis (6 caribou within 28 *Brucella*-seropositive). These findings indicate that animals with clinical brucellosis, detectable by the hunters, is significant but still a smaller proportion of the BS4-exposed animals. In other words, hunters may be exposed to BS4 from some caribou even though they do not observe abnormalities. Note that for all these sample estimates are not controlled by age, which influences exposure to BS4 (Cotterill et al., 2018).

Figure 6. Lesions from Dolphin and Union caribou harvested by Kugluktuk hunters from which Brucella suis biovar 4 was isolated. A: Swollen testicle (bigger than normal); B: Swollen carpal joint ‘front knee’ and claw overgrowth indicating an impaired movement in that leg.



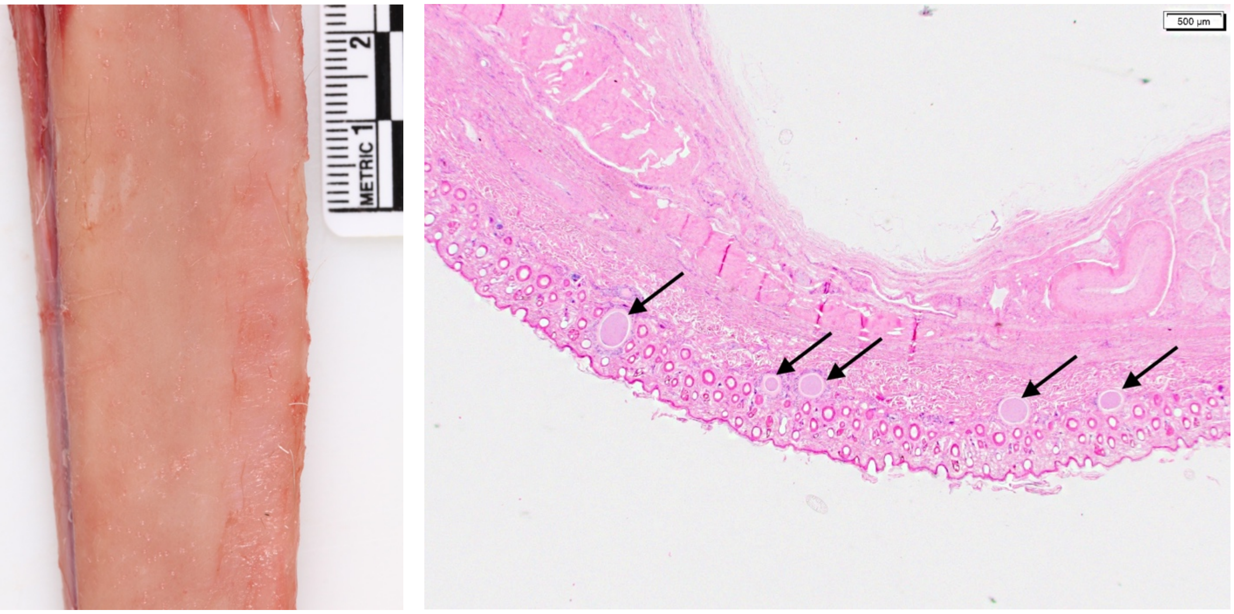
Brucellosis primarily affects the reproductive performance of the animals but also causes abscesses in different tissues or granulomatous lesions in internal organs (Neiland et al., 1968; Rausch and Huntley, 1978). No gross lesions or antibodies against *Brucella* were detected in 62 DU caribou from southeastern Victoria island between 1986-1990 (Gunn et al., 1991). However, local knowledge from Cambridge Bay indicates that signs of brucellosis, such as swollen joints, may have been first noticed in the 1980s in DU caribou, and these signs were increasingly observed in the subsequent years concurrent with the decline of the herd (Tomaselli et al., 2018). Interviews of Kugluktukmiut from 2003 also indicated increases in signs consistent with brucellosis (swollen joints) in DU caribou at that tie (Hanke et al., 2020). It is possible that brucellosis was historically absent or rare in the DU caribou. This is in stark contrast to today where this herd has one of the highest burdens of brucellosis among migratory tundra caribou (Carlsson et al., 2019).

Based on data from radio-collared caribou, our preliminary analyses suggest that *Brucella* may also affect caribou survival. This is not unexpected considering the severe lesions that BS4 can cause in caribou (Neiland et al., 1968). Brucellosis has been associated with reproductive problems in barren-ground caribou herds in Alaska (Neiland et al., 1968), and with population declines of the Southampton Island herd, Nunavut (Campbell, 2013). Given the high percentage of DU caribou that have been exposed to *Brucella* (Figure 5), it is likely that this bacteria is significantly affecting the reproductive performance of the herd and population dynamics, as demonstrated in other wildlife with enzootic brucellosis (Cotterill et al., 2018; Fuller et al., 2007).

4.3. Parasite detection

Besnoitia tarandi is the cause of 'sandpaper' disease, or rough skin commonly observed on the lower legs, the muzzle, the whites of the eyes, and sometimes elsewhere under the skin of caribou by hunters. *Besnoitia* cysts were detected in the skin of the metatarsus (lower hind leg bone) in 43.5% of caribou that we tested (n=81) (Figure 7). This is a similar prevalence as reported in 2007-2008 for the closest barren-ground herds Bathurst (40%), Bluenose-West (59%), and higher than Southampton (12%), Leaf River (28%) and George River (15.8%) herds (Ducrocq et al., 2011). The cyst density (cysts/mm^2; min.:0.039, median:0.235, max.:1.036) of the DU caribou was lower than in barren-ground herds (George River - min.:0.1, median:0.8, max.:3.6; and Leaf River min.:0, median:0.8, max.:4.5) (Ducrocq et al., 2013). Although *Besnoitia* is common in barren ground caribou it can occasionally cause a significant disease (Ducrocq et al., 2011; Glover et al., 1990). We did not detect any association of *Besnoitia* cyst density with health indicators (body condition, pregnancy, cortisol) in the DU caribou, and no signs of disease were reported by the hunters of the tested caribou. *Besnoitia* was first noticed in DU caribou in six out of 82 animals examined (7.3%) in 1987-1990 by gross observation of the cysts. Unfortunately, direct comparison with our more recent data is not possible because the sensitivity of the different methods used differ considerably. Local knowledge from Cambridge Bay suggests that signs of *Besnoitia* were occasionally noticed in caribou in the 1980s and may have slightly increased since the 1990s and early 2000s (Tomaselli et al., 2018). It is though that *B. tarandi* may be spread by flying biting insects, and that current warming conditions in the Arctic may favor its spread and increase in. Further standardized monitoring of this parasite will allow us to better understand its trends and effects on DU caribou.

Figure 7. Left: Multiple *Besnoitia tarandi* cysts (shiny dots that look like water droplets or salt grains) in the periosteum (bone surface tissue) of the lower left leg bone. Right: microscopic section of the metatarsal skin with *B. tarandi* cysts indicated with arrows (right photo: Jamie Rothenburger, Faculty of Veterinary Medicine, University of Calgary).



Dorsal spine larvae from the parasites *Parelaphostrongylus andersoni* (muscle worm) and *Varestrongylus eleguneniensis* (caribou lungworm) were found in 9.0% of the fecal samples analyzed (n=145), most of them with low numbers of larvae per gram of feces (min.: 0.20, median: 3.75, max.: 148.32). These results show that only a few individuals have a high number of parasites (e.g, 148 larvae per gram); this is the typical pattern observed in situations where parasites have been present in a population for a long time (Dobson, 1995). Neither of these parasites had a clear negative effect on the health indicators assessed (pregnancy, body condition, stress levels), and both are within the ranges reported in other tundra caribou populations (Kutz et al., 2012).

4.4. Trace minerals in hair

Trace minerals are essential in low levels growth, successful reproduction and immunity. We determined the concentrations of some key trace minerals, Calcium (Ca), Manganese (Mn), Iron (Fe), Copper (Cu), Zinc (Zn), Selenium (Se) and Molybdenum (Mo), in parts per million (ppm), in the neck hair of caribou (Table 2). Outliers associated with the methodological approach, above or below 1.5 times the interquartile range, were removed to calculate ranges and mean values.

Table 2. Concentration of some trace minerals in ppm (parts per million) measured in hair from Dolphin and Union Caribou, 2018 and 2019.

Mineral	**Females (n=111)**			**Males (n=22)**		
	minimum	**mean**	**maximum**	**minimum**	**mean**	**maximum**
Ca	178.7	363.3	546.1	213.9	361.6	527.7
Mn	0.150	0.480	0.900	0.290	0.489	0.870
Fe	5.560	9.592	16.35	6.710	10.36	17.26
Cu	4.410	6.464	8.450	4.970	6.164	7.530
Zn	60.91	85.79	116.14	67.93	85.41	117.32
Se	0.100	0.234	0.380	0.160	0.230	0.340
Mo	0.050	0.064	0.080	0.050	0.069	0.080

The concentrations of trace minerals in the hair, from animals collected in winter or spring, corresponds to the hair growing period mostly from the previous summer and fall. Assessment of hair minerals in caribou is a new field of study and reference values for ‘normal’ concentrations in hair have not yet been generated. We, therefore, cannot comment whether the levels observed suggest deficiencies with health implications. However, values for Se and Mo were significantly lower compared to those in northern mountain caribou in British Columbia and from captive reindeer from the University of Calgary (Kutz lab, unpubl. data). We didn’t find a direct association between trace mineral concentrations in DU caribou and pregnancy status, nor did we detect any association with body condition, disease or seroprevalence. Further data in this and other caribou herds will help clarify whether these mineral levels are adequate or deficient for proper growth, reproduction, immunity or survival.

5. General conclusions and discussion

We analyzed samples from a total of 202 Dolphin and Union caribou collected by hunters, guides and biologists in the Kitikmeot and Inuvialuit regions from 2015-2019. The analyses of samples from 26 animals submitted in fall 2019 has been delayed because research labs have been shut down during the COVID-19 crisis. Since 2015, Dolphin and Union caribou have had relatively high pregnancy rates, and in the most recent years, body condition was higher than in 1987-1991 and 2001-2003. Preliminary results of stress levels in this herd also indicate a decreasing trend in stress in the latest two years (2018-2019). These findings (good body condition, pregnancy rates and lower stress) indicate a few good years for the DU caribou but ongoing monitoring is needed to determine if this is a positive trend. We were not able to assess late term abortions, stillborn calves, neonate survival or calf survival, all of which can be outcomes of infection with the pathogens detected in these caribou, and all of which impact the recruitment and population dynamics.

We also found a common and continued exposure of this herd to different pathogens that may cause reproductive or health problems. The effects of these pathogens in the health of the herd are not easy to assess based only on serology (blood tests), yet we identified a negative impact of brucellosis on body condition, pregnancy and possibly survival of adult females. *Brucella* causes a chronic, debilitating disease in caribou and a reduced reproductive performance (Neiland et al., 1968). Brucellosis is also associated with the birth of weak offspring and high neonatal death rates in other ruminants (Grilló et al., 1999; Rhyan, 2013); the overall impact on caribou population dynamics, even with a moderate seroprevalence, may be considerable and deserves further investigation. It is noteworthy that the epidemiology of virulent and chronic diseases like brucellosis are typically shaped by predator-prey interactions (Packer et al., 2003), and management decisions on predator control (e.g., wolf culling or hunting incentives) may increase the spread and impact of the disease in the herd.

DU caribou did have hair trace mineral levels lower than other caribou or reindeer that we have tested previously. We don't currently know what are 'normal levels' of trace minerals in caribou hair, nor do we understand how or if these lower trace mineral levels in the DU caribou may reflect deficiencies that could impact their growth, reproduction, immunity or survival. We do, however, anticipate that the changing arctic climate may affect the nutritional quality, and trace mineral status, of caribou forage plants (Oster et al., 2018).

The isolation of *Brucella suis* biovar 4 from the bone marrow of diseased animals (swollen joints) highlight the importance for the hunters to carefully handle these cases separately and thoroughly cook any part of caribou showing these signs if the animal is consumed. It is important to note that, in addition to isolating *Brucella* from legs with obvious lesions, we also isolated it from the bone marrow of legs that had no obvious abnormalities on those same animals. While further research is needed to assess the distribution of *Brucella* in organs, muscles and tissues of infected caribou, these findings suggest that the removal of caribou parts affected by brucellosis may not be sufficient to eliminate the exposure risk to hunters. It is important to follow the recommendations of territorial public health divisions on food safety when signs of brucellosis are observed in harvested caribou.

Important note:

This program is based on cross-sectional observations - animals were sampled at a single point in time. With the exception of the captured caribou, their subsequent performance (reproduction and survival rates) could not be assessed in relation to pathogen exposure, stress or mineral levels. With the cross-sectional type of approach, it is frequently difficult to detect effects of pathogens that may affect wildlife populations. Mortality events due to disease are also unlikely to be captured with this sampling design. Continued surveillance, including focused efforts to sample sick or dead animals, as well as assessing the relationship between health indicators and survival and reproduction of captured animals will help to better assess trends and the impact of these pathogens that are consistently detected in DU caribou.

Appendix 1. Supplementary table

Tests used for detection of antibodies against selected pathogens in caribou samples and references of previous usage in *Rangifer*.

Target	**Test (Manufacturer)**	**Type of test**	**References**
Pestivirus	IDEXX BVDV/MD/BDV p80 Ab Test (IDEXX Laboratories Inc., Maine, USA)	bELISA	(Carlsson et al., 2019)
Alpha-herspesvirus	SERELISA IBR/IPV gB Ab Mono Blocking (Synbiotics, Europe SAS, France)	bELISA	(Carlsson et al., 2019; Das Neves et al., 2009)
Brucella	In-house competitive ELISA*	cELISA	(Carlsson et al., 2019)
Erysipelothrix rhusiopathiae	In-house indirect ELISA	iELISA	(Bondo et al., 2019)
Toxoplasma gondii	ID Screen Toxoplasmosis Indirect Multispecies (IDvet., Grables, France)	iELISA	(Bondo et al., 2019; Carlsson et al., 2019)
Neospora caninum	*Neospora caninum* Antibody Test Kit, cELISA (VMRD Inc., Pullman, WA, USA)	bELISA	(Carlsson et al., 2019; Curry et al., 2014)

bELISA: blocking Enzyme-linked immunoabsorbent assay; iELISA: indirect ELISA; cELISA: competitive ELISA.

*These analyses were performed at the Canadian Food Inspection Agency (CFIA), Brucellosis Centre of Expertise, Ottawa, ON, Canada.

Appendix 2. Information about diseases and pathogens included in this report

Erysipelas: *Erysipelothrix rhusiopathiae* is an opportunistic and zoonotic bacterium that can infect a wide range of animals and can survive for long periods of time in the environment. The clinical signs widely vary depending on the animal species and strains involved and can range from subclinical infections (no disease), to arthritis, endocarditis or sudden death. In humans, *E. rhusipathiae* can cause a mild cutaneous infection after skin penetration. These local skin infections are known as erysipeloids and are characterized by erythematous edema with defined raised borders, usually localized to the hands or fingers. This pathogen is believed to be associated with muskox mortalities in Alaska, Banks and Victoria island (Kutz et al., 2015), and woodland caribou in northern British Columbia (Forde et al., 2016).

Brucellosis: Brucellosis is a disease caused by bacteria *Brucella* spp., that can infect different kinds of animals – including caribou, muskoxen or even people. *Brucella suis* biovar 4 is the bacteria present in the Arctic (Forbes, 1991). It is commonly known from caribou, but also more recently has been detected as an emerging disease syndrome in muskoxen from Victoria island (Gunn et al., 1991; Tomaselli et al., 2019). Typical abnormalities associated with brucellosis in caribou are swollen joints, abscesses or altered reproductive tract (e.g. orchitis in males) (Forbes, 1991; Gunn et al., 1991). However, it is a disease that is also commonly associated with reproductive problems such as metritis, abortions, stillborn calves or retention of the placenta (Neiland et al., 1968). Contaminated parts of the animals can infect humans through the contact with eyes, nose, and mouth or through wounds and scratches in the skin. Insufficiently cooked meat or animal products can also be a source of infection.

Pestivirus: This virus belongs to the Flaviridae family and causes major economic losses in livestock industry. It is directly transmitted between animals and mainly associated with reproductive problems in ruminants, both domestic and wild. It can reduce the herd fertility with the death of the embryo, cause abortions or birth defects if the cows are infected throughout gestation (Larska, 2015). Infected animals may develop a febrile syndrome that typically last for around two weeks and that can be accompanied with digestive or respiratory problems. Under good conditions, most of the animals may recover without major health implications but the reproductive success can be severely affected. Importantly, these viruses can mutate and cause severe disease and eventually the death of the animals (Carman et al., 1998; Marco et al., 2009).

Toxoplasmosis and Neosporosis: Toxoplasmosis and Neosporosis are caused by the single-celled protozoan *Toxoplasma gondii* and *Neospora caninum*. These parasites reside in the intestinal tract of felines or canines and in the tissues of many rodents, wildlife, and animals raised for human food. Although felids (cat, lynx, etc) and canids (dogs, wolves, etc) are the only animals that can shed the parasite's eggs (oocysts) in their feces, *T. gondii* and *N. caninum* also reside in animals' tissues and are released when other animals or humans consume that tissue. In most cases do not cause disease but may cause flu-like symptoms including fever, enlarged lymph nodes, fatigue, headache, and sore throat depending on the strains and the infection dose. Pregnant animals who become infected with these parasites for the first time have a risk infecting their fetus, which can result in stillbirths, spontaneous abortions, and birth defects. Once inside the body, the parasite never leaves it and may reactivate under certain conditions.

α-herpesvirus: These types of viruses establish life-long latent infections in the trigeminal ganglia (like the cold sore viruses in humans). Animals usually get it early in life without major known problems. However, under stressors the infection can be reactivated and in *Rangifer* can cause different health issues that can range from mild symptoms such as oral ulcers to ocular problems (white eyes and secretion)(Tryland et al., 2009), respiratory problems and possibly abortion (das Neves et al., 2009b). It is a common virus historically found in caribou but its potential population impact is not known and may be exacerbated under environmental stressors (das Neves et al., 2009a).

Besnoitia tarandi: This is a protozoan parasite which life cycles is not fully understood, caribou and reindeer are the primarily intermediate hosts and it is believed that it can be transmitted by biting insects and/or carnivores. *Besnoitia* infection results in tissue cysts in and under the skin, on the whites of the eyes, on the bone surface, and in severe cases in the reproductive tract. Depending on its abundance it may cause inflammation, hair loss, thickening of the skin and ulceration. *Besnoitia* parasites are sometimes associated with infertility in males from other species and it is believed that may affect caribou in a similar way (Ayroud et al., 1995; Tryland and Kutz, 2018). In extreme cases, these parasites may cause systemic disease and death (Glover et al., 1990).

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