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Liver Parasites and Body Condition in Relation to Environmental Contaminants in Caribou (*Rangifer tarandus*) from Labrador, Canada

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ABSTRACT. Over the last several decades, elders and hunters of the Innu Nation in Labrador, Canada, have expressed concerns over perceived declines in environmental health and the integrity of country food, including caribou. The primary objective of this study was to determine links between specific health parameters and contaminants found in caribou from the George River herd. Twenty-seven caribou killed by local Innu hunters between February and December 2001 were evaluated for gross and microscopic pathology, body condition, liver parasitology, and contaminant levels in kidney and fat. Overall, the sampled caribou appeared to be in adequate body condition for the time of year, and no clinically significant lesions were found. Concentrations of selenium, metals (Hg, Cd, and Pb), 20 organochlorine pesticides (HCB, α-HCH, γ-HCH, aldrin, dieldrin, methoxychlor, mirex, α- and β-endosulfan, heptachlor, heptachlor epoxide, γ-CHL, cis-CHL, trans-nonachlor, and *o,p′*- and *p,p′*-DDD, DDE, DDT), and 24 PCB congeners were within the ranges reported for caribou in Canada. In general, contaminant levels were relatively low, with the exception of cadmium in kidneys (geometric mean: 6.5 µg/g wet weight; range: 1.5–44.0 µg/g). Two types of liver parasites were found: the liver fluke *Fascioloides magna* (prevalence: 78%; geometric mean abundance: 4.2 flukes/caribou) and a tapeworm larva consistent with *Taenia hydatigena* (prevalence: 50%; geometric mean abundance: 0.6 larvae/caribou). Using multiple variable regression analysis, we found renal concentrations of cadmium to be positively associated, and selenium to be negatively associated, with *F. magna* abundance.

Key words: body condition, environmental contaminants, liver fluke, *Fascioloides magna*, parasites, *Rangifer tarandus*, cadmium, Innu people, Labrador, George River caribou herd

RÉSUMÉ. Ces dernières décennies, les aînés et les chasseurs de la nation montagnaise du Labrador, au Canada, ont exprimé des inquiétudes au sujet du déclin de la santé de l'environnement et de l'intégrité de la nourriture provenant de la campagne, telle que le caribou. L'objectif principal de cette étude consistait à déterminer les liens qui existent entre certains paramètres de santé précis et les contaminants se trouvant dans le caribou du troupeau de la rivière George. Vingt-sept caribous ayant été tués par les chasseurs montagnais de la région entre les mois de février et de décembre 2001 ont subi des examens pathologiques macroscopiques et microscopiques, en plus d'avoir été évalués pour en déterminer l'état du corps, la parasitologie du foie et les taux de contaminants dans le foie et le gras. Dans l'ensemble, l'état des corps de caribous échantillonnés semblait adéquat pour cette période de l'année et aucune lésion clinique importante n'a été signalée. Les concentrations de sélénium, de métaux (Hg, Cd et Pb), de 20 pesticides organochlorés (HCB, α-HCH, γ-HCH, aldrine, dieldrine, méthoxychlore, mirex, α- et β-endosulfane, heptachlore, heptachlorépoxyde, γ-CHL, cis-CHL, trans-nonachlore ainsi que *o,p′*- et *p,p′*-DDD, DDE, DDT) et de 24 congénères de PCB s'établissaient dans les étendues signalées pour le caribou au Canada. En général, les niveaux de contaminants étaient relativement faibles, à l'exception du cadmium se trouvant dans les reins (moyenne géometrique : 6,5 µg/g poids humide; étendue : 1,5–44,0 µg/g). Deux types de parasites du foie ont été trouvés : la douve *Fascioloides magna* (prévalence : 78 %; abondance moyenne géométrique : 4,2 douves/caribou) et un cestode du genre *Taenia hydatigena* (prévalence : 50 %; abondance moyenne géométrique : 0,6 larves/caribou). Nous avons également réalisé une analyse de

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régression à variables multiples qui nous a permis de constater que les concentrations de cadmium sont positivement associées et celles de sélénium sont négativement associées à l'abondance de *F. magna*.

Mots clés : état du corps, contaminants environnementaux, douve, *Fascioloides magna*, parasites, *Rangifer tarandus*, cadmium, Montagnais, Labrador, troupeau de caribous de la rivière George

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INTRODUCTION

Caribou (*Rangifer tarandus*) is the dominant large terrestrial herbivore in many northern ecosystems. The George River herd (GRH) is the largest caribou population in eastern Canada. The range of the GRH includes much of Labrador and extends across the Ungava Peninsula well into the province of Quebec (Fig. 1). The GRH population underwent rapid growth up to the mid-1990s, when it was estimated at approximately 700 000 animals, and has recently declined to approximately 400 000 animals (R. Otto, pers. comm. 2007).

Members of the Innu Nation in Labrador hunt caribou from the GRH on a seasonal basis. Caribou have important nutritional, cultural, and spiritual significance for the Innu people (Armitage, 1990; Loring, 1997), who are concerned for the health of Labrador caribou populations because of industrial development and other potential impacts on the ecosystem, such as environmental contaminants (Innes, 1998).

Research programs such as the Northern Contaminants Program and the Arctic Monitoring and Assessment Programme have recently brought attention to the issue of environmental contaminants such as metals, organochlorines (OCs) and radionuclides in northern ecosystems in Canada and worldwide (Fisk et al., 2003; AMAP, 2004, 2005). Many studies of environmental contaminants throughout northern Canada have consistently found elevated cadmium concentrations in the kidneys of moose and caribou (Muir et al., 1997; Gamberg et al., 2005), including the GRH caribou (Crête et al., 1989; Robillard et al., 2002).

The most important route of contaminant exposure for terrestrial animals is usually through food consumption (Muir et al., 1997). The diet of GRH caribou consists of a variety of plant types, including grasses and sedges, woody stems and twigs, leaves, lichens, and mosses (Crête et al., 1990). Lichens and mosses, which lack root systems, take up metals and minerals directly from the air and are often used to monitor atmospheric deposition of metals (Dietz et al., 1998). While tissue contaminant levels have been well reported, the potential biological effects of contaminants in cervids, including caribou, are not well understood. Several published studies investigating the biology and ecology of the GRH have included health parameters such as parasite burdens and body condition (e.g., Parker, 1981; Huot and Beaulieu, 1985; Lankester and Luttich, 1988); however, the relationship between health parameters and contaminant levels has not been examined in this caribou population.

The present study, carried out in collaboration with the Innu Nation, evaluates health parameters of caribou harvested by Innu hunters in Labrador in relation to tissue concentrations of environmental contaminants. The specific objectives were 1) to determine body condition and hepatic parasite burdens of caribou killed during the regular subsistence hunt and examine selected tissues for gross and microscopic pathological abnormalities; 2) to determine the tissue concentrations of selenium, metals, and a wide range of OC contaminants in these caribou; and 3) to examine the relationship between the specific health parameters and tissue contaminant levels in the caribou through multiple regression analyses.

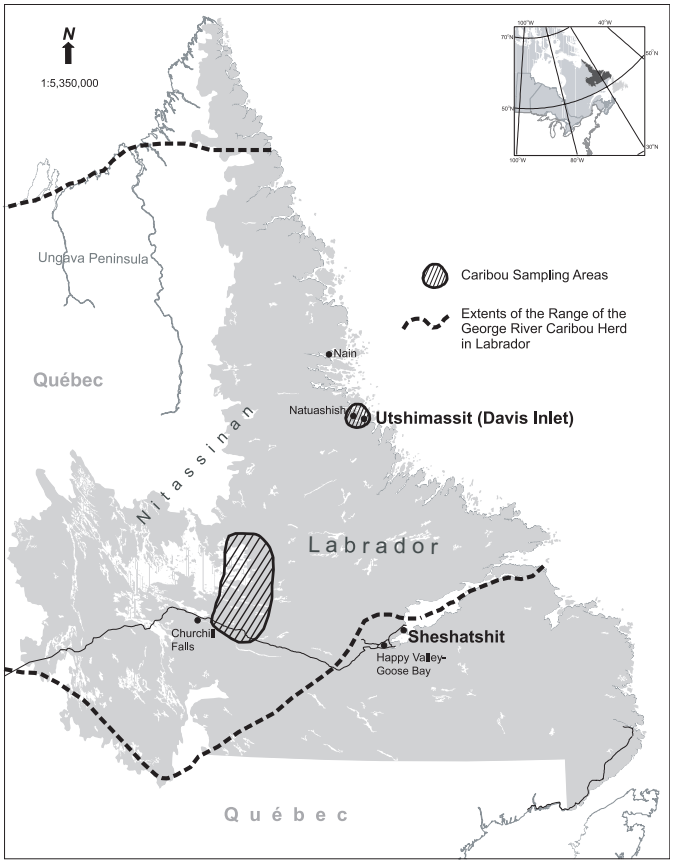

FIG. 1. Map of Labrador indicating caribou sampling areas and the northern and southern extents of the range of the George River caribou herd.

MATERIALS AND METHODS

Caribou Locations and Sample Collection

To ensure that the results were clearly representative of the animals that make up the Innu harvest, we chose the

sample population of 27 GRH caribou from those that were shot during the regular subsistence hunt by residents of the Labrador Innu communities of Sheshatshit and Utshimassit (Davis Inlet) (Fig. 1). The caribou were killed in south-central and coastal-northern Labrador (approximately 61˚ W – 64˚ W and 53˚30′ N – 56˚ N). Samples were collected from caribou killed in February 2001 (winter, n = 13) and in October and December 2001 (fall, n = 14).

All methods for collection, handling, processing and disposal of samples were agreed upon by the Innu Nation co-researchers, community elders, hunters and their family members, and the researchers from the Atlantic Veterinary College (AVC), University of Prince Edward Island. In order to retain strong community acceptance of the research and its findings, researchers placed a high degree of importance on maintaining the traditions surrounding the hunt and ensuring that the caribou were still considered to be suitable for community distribution and consumption after sampling.

Once killed, each caribou was cleaned by removing the gastrointestinal tract, leaving the thoracic cavity intact, and comments from the hunter regarding the perceived health of the animal were recorded.

Gross examination of the carcass and organs was limited to the exterior of the animal and to the abdominal organs (stomachs, intestines, liver, kidneys, uterus, fetus if present, and bladder), testes, and thyroid glands. The kidneys, thyroid glands, testes, and liver were collected and put on snow or ice for up to 12 hours until they could be frozen. Kidneys with attached fat were wrapped in aluminum foil and placed in a plastic bag for subsequent contaminant analyses.

All caribou, with the exception of one adult, were aged either by size (calf, immature) (n = 4) or by cementum annular count of the primary (middle) incisor (n = 22) (Matson's Laboratory, Milltown, Montana, USA).

After examination and sample collection were complete, all caribou were taken back to the communities for distribution, while study samples were frozen at -20˚C and transported to the AVC for further examination and sampling.

At the AVC, the tissues and organs were thawed and examined in more detail for gross abnormalities. Peri-renal fat collected for OC analysis was manipulated using stainless steel instruments rinsed three times in hexane and then wrapped in aluminum foil triple-rinsed in hexane. Fat was kept frozen at -20˚C. One half of one kidney was submitted for metal analysis. Medial sections of all kidneys and sections of grossly abnormal hepatic tissue were taken from thawed samples, and then fixed and processed routinely for light microscopic examination.

Body condition was evaluated by determining the kidney fat index (KFI), a recognized index of body fat in caribou throughout a wide range of body conditions (Huot, 1988; Chan-McLeod et al., 1995). For caribou from which both kidneys were collected (no injury to either kidney, n = 14), average KFI was calculated.

For parasite recovery, the livers were examined at the AVC for the presence of the large American liver fluke (*Fascioloides magna*) as per Lankester and Luttich (1988). Hepatic cysts (ca. 25 mm in diameter) that were consistent with cestode (tapeworm) larvae were also noted. Parasitological parameters (prevalence, abundance, mean abundance, and mean intensity of infection) were determined for liver fluke and tapeworm infections, following Bush et al. (1997).

Analysis of Tissue Contaminants

The kidney was chosen for metal analysis because results from numerous previous studies had shown moderate to high levels of cadmium in caribou kidneys (e.g., Larter and Nagy, 2000). Representative portions of both cortex and medulla were sampled and homogenized prior to analysis, so that results would reflect total kidney concentrations. All kidney samples were analyzed for cadmium, lead, and selenium at the AVC Diagnostic Toxicology Laboratory. Sample digestion for analysis followed EPA (Environmental Protection Agency) Method 200.2 Revision 2.8. Analytical methods for selenium followed Feuerstein and Schlemmer (2000). An atomic absorption spectrophotometer (AAS) (Perkin Elmer Analyst 800) equipped with a transversely heated graphite atomizer was used for selenium and lead analysis, and an AAS equipped with a flame apparatus fuelled by acetylene and air with a manual sampling system was used for cadmium (Pesce and Kaplan, 1987). A sample of the tissue was dried to determine percentage moisture. Sample batches were run with blanks, spikes, and duplicates. Samples of standard reference materials (at least one for each 10 caribou samples) were also analyzed. We used National Institute of Standards and Technology (NIST) pine needles for lead; National Research Council lobster hepatopancreas (NRC TORT-2) for selenium; and Environment Canada, National Water Research Institute TMDA54.2 and NRC TORT-2 for cadmium. Recoveries from these reference samples were within ± 15% of previously established values.

Analysis of kidney samples for total mercury was carried out at Philip Analytical Services (Halifax, Nova Scotia), following EPA method 245.6. Approximately 0.3 g of homogenized tissue sample was digested and analyzed for total mercury using cold vapour atomic absorption spectrophotometry (Leeman PS200 Mercury Analyzer). Reagent blanks, duplicates, reference materials (e.g., dogfish liver DOLT-2 or dogfish muscle DORM-2), and method spikes were prepared and analyzed in the same manner as the samples. One reagent blank, one duplicate, one spike, and one reference material were analyzed for every 20 samples, with a minimum of one per batch. A total quality control effort of recovery within 10% was maintained.

Peri-renal fat samples were analyzed for OC pesticides and polychlorinated biphenyls (PCBs) at the Environmental Quality Laboratory (Environment Canada, Moncton, New Brunswick, Canada). Analyses included 20 OC pesticides: hexachlorobenzene (HCB), α-hexachlorocyclohexane (α-HCH), lindane (γ-HCH), aldrin, dieldrin, methoxychlor, mirex, sum of endosulfans (α- and β-endosulfan), sum of chlordanes (heptachlor, heptachlor epoxide, γ-chlordane

[γ-CHL], cis-chlordane, trans-nonachlor), and sum of dichlorodiphenyltrichloroethanes (DDT) and metabolites (*o,p′*- and *p,p′*-DDD, DDE, DDT), and 24 PCB congeners (8, 18, 28, 29, 44, 50, 52, 66, 77, 87, 101, 104, 105, 118, 126, 128, 138, 153, 170, 180, 187, 195, 206, 209). A description of the full analytical protocol for OC pesticides and PCBs is found in Jones et al. (1998). Analyses were performed using capillary column gas chromatography (GC) with an electron capture detector (ECD) (Agilent 5890 GC with dual ECD detectors). Tissue samples containing approximately 2 g of fat with surrogate solution added were blended with sodium sulfate and methylene chloride using an ultrasonic extractor. A subsample of the extract was used for lipid concentration determination by gravimetric analysis. The remaining extract was cleaned through a gel permeation chromatograph followed by a small silica-gel micro column. Fractionation was completed on a silica gel column using hexane and 50/50 hexane/methylene chloride prior to GC/ECD analysis. Results were reported in ng/g dry weight with detection limits ranging from 3 ng/g (PCBs) to 20 ng/g (methoxychlor). Lipid weight concentrations were calculated by dividing the concentration of the contaminant in dry weight by the percent lipid of each sample. A sample of the tissue was dried to determine percent moisture. Wet weight concentrations were determined by multiplying the dry weight concentration by the percent dry matter of the sample (% dry matter = 100% - % moisture) of the sample. All sample batches were run with procedural blanks, spikes, and duplicates along with certified reference materials (NIST 1974a mussel tissue). Recoveries for certified reference materials were all within the certified ranges. Analytical duplicates were within 9%.

Statistical Analysis

Means and 95% confidence intervals were calculated for the contaminants detected in over 50% of samples analyzed. Arithmetic means were calculated for normally distributed contaminant levels and geometric means for the log-normally distributed ones. For the calculation of means, contaminant values less than minimum detection limits (MDL) were replaced with one-half of the MDL.

For univariate statistical analyses, health parameters and contaminant concentrations that were continuous in nature but not normally distributed (KFI, hepatic parasite abundance, renal cadmium and lead concentrations) were transformed using the natural log (ln) to make them normally distributed. Simple associations among and between individual health parameters and contaminants and confounding variables (season, age, gender) were examined using chi-square (χ^2) statistics for categorical variables and pairwise Pearson's correlation coefficients for continuous variables. Differences in continuous variables between groups were assessed using Student's t-test. Only those contaminants detected in 50% or more of the samples were analyzed using univariate statistics. Results of variables with $p \leq 0.05$ were reported as statistically significant, but statistical trends ($0.05 < p \leq 0.2$) were also reported to indicate which variables were offered as potential predictor variables in subsequent multiple variable regression analyses (Dohoo et al., 2003).

Multiple variable regression analyses were used to determine associations between specific health parameters and contaminant levels simultaneously, controlling for possible confounding factors. Only KFI, tapeworm, and *F. magna* prevalence and abundance were used as health parameters for regression analyses because gross and microscopic lesions were rare, severely limiting the power to find significant risk factors. Those variables found to be significant at $p \leq 0.2$ upon univariate analysis with health parameters were offered to the regression models in a manual, forward stepwise manner. Two-way interaction variables between significant predictors were assessed, where applicable. The final models included those variables that were significant at $p \leq 0.1$ in order to ensure that all potential associations were considered and to account in part for the relatively small sample sizes.

Goodness of fit of the final models was determined using standard regression diagnostic procedures. Statistical analyses were completed using STATA (Statistical Package, v. 8.0; Stata Press, College Station, Texas, USA).

RESULTS

Descriptive Results

Of the 27 caribou examined, 16 (59%) were female. Adults (≥ 2 years old) represented 74% of caribou sampled. Ages ranged from 0 (calf) to 12 years, and the mean age was 3 years (95% CI: 2.0–4.4). No significant seasonal differences were found in the age distribution ($\chi^2 = 12.3$, df = 8, $p = 0.14$) or sex distribution ($\chi^2 = 0.3$, df = 1, $p = 0.58$).

Although KFI was measured in only 25 caribou (93%) because of gunshot wounds or damage to the kidneys during cleaning, all caribou examined had conspicuous fat stores and appeared to be in adequate body condition for the time of year (geometric mean KFI: 47.9; 95% CI: 38.2–60.1). All caribou examined were considered to be suitable for consumption by the hunters.

On gross and laboratory examination of livers, two types of parasites were found: a fluke (*F. magna*) and a tapeworm larva (Table 1). *Fascioloides magna*, found in the majority of livers, was present as encapsulated and migrating flukes. Most capsules contained two flukes, and a single capsule contained four flukes. The most heavily infected caribou, a seven-year-old female, had a total of 30 capsules in the liver and 67 encapsulated and migrating *F. magna*. Gross and microscopic hepatic lesions associated with *F. magna* parasitism varied among animals depending on the intensity of the infection; however, none were considered clinically significant. Infected livers had between 1% and 40% of their parenchyma affected. Even the most severely infected liver (30 capsules) had numerous areas that appeared normal

TABLE 1. Prevalence and geometric mean abundance and intensity of liver parasites (*Fascioloides magna* flukes and tapeworm larvae) in caribou from the George River herd, Labrador, in 2001.

	Prevalence (95% confidence interval) *n*	Mean abundance (95% confidence interval) [range, *n*]	Mean intensity (95% confidence interval) [range, *n*]
Flukes	78% (62–94) 27	4.2 flukes/caribou (2.0–8.2) [0–67, 26]	8.3 flukes/infected caribou (4.9–14.0) [1–67, 20]
Tapeworm larvae	50% (48–52) 27	0.6 larvae/caribou (0.3–1.1) [0–9, 26]	1.9 larvae/infected caribou (1.3–3.0) [1–9, 13]

TABLE 2. Concentrations of selenium, metals (µg/g wet weight) and organochlorine contaminants (ng/g wet weight) in caribou from the George River herd, Labrador, in 2001.

Contaminant (*n*)	Number (%) above MDL[1]	Mean concentration[2] (95% confidence interval) and [range]	MDL (95% confidence interval)
Kidney (27)			
% Moisture		77.8 (77.2–78.4)[3]	
Selenium	27 (100)	1.2 (1.2–1.3)[3] [0.9–1.5]	0.05
Mercury	27 (100)	0.66 (0.58–0.75)[3] 0.36–1.10]	0.01
Cadmium	27 (100)	6.5 (4.7–8.9)[4] [1.5–44.0]	0.4
Lead	22 (82)	0.09 (0.06–0.13)[4] [nd–0.75]	0.05
Peri-renal fat (27)			
% Lipid		65.2 (60.3–70.2)[3]	
HCB[5]	27 (100)	24.2 (21.5–26.9)[3] [11.8–36.2]	2.3 (2.2–2.4)[3]
α-HCH[5]	6 (22)	nc [nd–4.8]	2.3 (2.2–2.4)[3]
γ-Chlordane	3 (11)	nc [nd–4.7]	3.0 (2.9–3.2)[3]
SumPCB[5]	2 (7)	nc [nd–10.3]	2.3 (2.2–2.4)[3]

[1] MDL = Minimum Detection Limit.
[2] nd = not detected, nc = not calculated.
[3] Arithmetic mean.
[4] Geometric mean.
[5] HCB = hexachlorobenzene, α-HCH = α-hexachlorocyclohexane, sumPCB = sum 24 polychlorinated biphenyl congeners.

microscopically. The tapeworm larvae were less common than the flukes and fewer in number.

Other noteworthy gross or microscopic lesions found included a marked unilateral interstitial nephritis with multiple cyst formation in a 12-year-old female, possibly the result of a previous bacterial infection, and a mild multifocal interstitial nephritis in another animal. All other kidneys examined contained only very small numbers of lymphocyte aggregates. None of these lesions was considered to be clinically significant.

Contaminants detected in caribou tissues are shown in Table 2. All caribou had detectable levels of Hg, Cd, and Se in kidney and HCB in peri-renal fat. Only three of the 20 individual OC pesticides measured (HCB, α-HCH, and γ-CHL) and four of the 24 PCB congeners (87, 101, 118, and 209, reported as sumPCB) were detected in peri-renal fat samples.

Univariate Analyses

Some specific health parameters and tissue contaminants appeared to be influenced by season. KFI and liver tapeworm larvae abundance were greater ($p \leq 0.05$) in winter than in fall. Mercury concentrations in kidney were higher in fall, whereas HCB concentrations in fat were higher in winter (Table 3).

TABLE 3. Seasonal comparisons of specific health parameters and mean concentrations of tissue contaminants (selenium and metals in kidney, in µg/g wet weight, and hexachlorobenzene [HCB] in peri-renal fat, in ng/g wet weight) in caribou from the George River herd, Labrador, in 2001. Bold indicates significance at $p \leq 0.05$.

	Fall	Winter
Health Parameter		
Kidney Fat Index[1]	**40.2 (29.8–54.4)**	**59.9 (42.1–85.1)**
Fluke prevalence[2]	71.4 (47.7–95.1)	84.6 (64.9–104.2)
Fluke abundance[1]	3.9 (2.3–10.6)	5.9 (2.1–14.6)
Tapeworm larvae prevalence[2]	38.5 (12.0–65.0)	61.5 (35.1–87.9)
Tapeworm larvae abundance[1]	**0.3 (0.0–0.7)**	**1.1 (0.3–2.4)**
Contaminant		
Selenium[3]	1.15 (1.08–1.23)	1.23 (1.16–1.32)
Mercury[3]	**0.80 (0.68–0.91)**	**0.52 (0.43–0.60)**
Cadmium[1]	6.2 (3.8–10.0)	6.9 (4.3–10.9)
Lead[1]	0.11 (0.08–0.15)	0.07 (0.04–0.15)
HCB[3]	**21.3 (17.4–25.3)**	**27.4 (24.1–30.6)**

[1] Geometric means (95% confidence interval).
[2] Percentages (95% confidence interval).
[3] Arithmetic means (95% confidence interval).

Some interesting associations were found between liver parasite infection and age and specific health parameters. The mean age of infected caribou was greater than that of

TABLE 4. Mean age (years) and specific health parameters in caribou from the George River herd, Labrador, that were infected and not infected with liver parasites in 2001. Means (95% confidence intervals) are shown for flukes (*Fascioloides magna*) and tapeworm larvae. Bold indicates significance at $p \leq 0.05$, and italics, at $0.05 < p \leq 0.2$.

	Flukes		Tapeworm Larvae	
	Infected	Not Infected	Infected	Not Infected
Age[1]	**3.9 (2.4–5.3)**	**1.2 (0–2.4)**	*2.4 (0.6–4.1)*	*3.4 (2.4–4.4)*
KFI[2]	49.3 (38.8–62.5)	42.8 (16.8–108.9)	*57.4 (43.4–75.9)*	*43.8 (31.6–60.6)*
Fluke abundance[2]	na	na	*7.8 (2.6–19.4)*	*2.3 (0.7–6.0)*
Tapeworm larvae abundance[2]	1.2 (0.8–1.8)	1.0 (0 3–2.8)	na	na

[1] Arithmetic mean.

[2] Geometric mean.

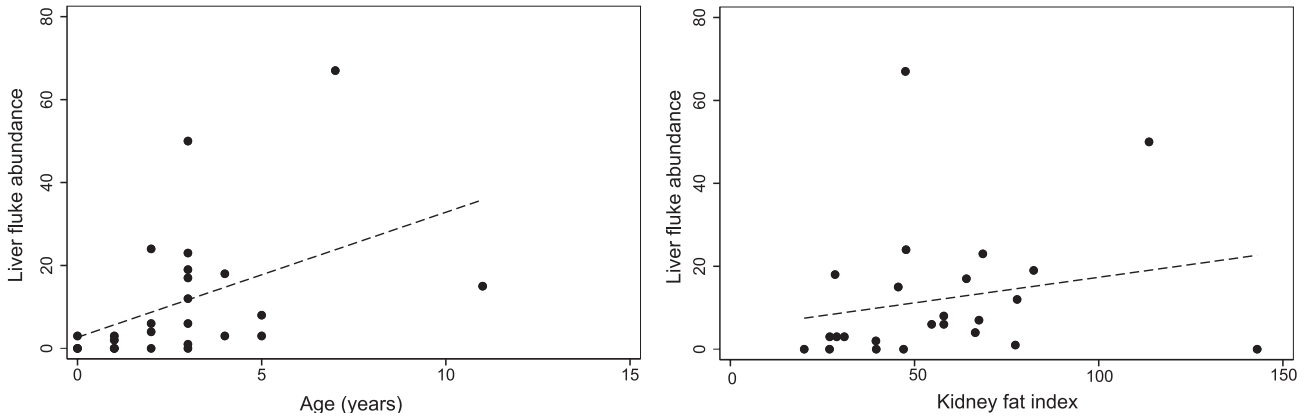

FIG. 2. Liver fluke (*Fascioloides magna*) abundance in relation to age (r = 0.53, $p < 0.05$) and kidney fat index (r = 0.29, $p < 0.2$) in caribou from the George River herd, Labrador, in 2001.

non-infected caribou for liver fluke infections, and an opposite trend was found for liver tapeworm larva infections (Table 4). There was also a trend toward KFI and liver fluke abundance being greater in caribou infected with tapeworm larvae than in non-infected caribou (Table 4). Liver fluke abundance increased with increasing age and with increasing KFI (Fig. 2). No significant associations were found between health parameters and gender.

Several noteworthy associations were found between contaminant levels and specific health parameters. In particular, significant associations ($p \leq 0.05$) were found between renal Cd concentrations and liver fluke prevalence and abundance, as well as tapeworm larva prevalence (Table 5). Positive associations were found between KFI and the contaminants HCB in fat and Se in kidney (Fig. 3). Cadmium concentrations in kidney increased with increasing age (r = 0.73, $p \leq 0.05$) and were positively correlated with kidney Se concentrations (r = 0.47, $p \leq 0.05$).

Multiple Variable Regression Results

Those variables found to have associations ($p < 0.20$) with KFI and liver parasites were offered to the multiple variable regression analyses. Significant final multiple variable models ($p \leq 0.10$) are shown in Table 6 and are interpreted below.

For KFI, although a linear association was not found between KFI and age, a quadratic form of age was negatively associated with KFI and was offered to the model. Multiple linear regression analysis results showed that the only significant predictor of KFI was age (Table 6). Figure 4 shows the relationship between KFI and age, including the predicted values for KFI based on the regression model presented in Table 6. Predicted values indicate that KFI increased with age until five years of age and then decreased, with the highest KFIs occurring between three and seven years of age.

For *F. magna* abundance, multiple linear regression analysis showed renal cadmium and selenium levels were both significant independent variables in the final model (Table 6). Kidney selenium was offered as an independent variable because it was significantly correlated with kidney cadmium, and it was suspected to be a potentially confounding variable. For this sample of caribou, as cadmium levels increased and selenium levels decreased, *F. magna* abundance increased. Selenium levels had an apparent protective effect on *F. magna* infection when cadmium levels were considered. Increasing cadmium concentrations from 3.7 µg/g ww to 9.1 µg/g ww (25th to 75th percentile) increased the predicted *F. magna* abundance from 3 flukes to 12 flukes when selenium concentrations were low (1.12 µg/g ww, 25th percentile) and from 1 fluke to 6 flukes when selenium concentrations were high (1.31 µg/g ww, 75th percentile). The relationship was apparent at relatively low levels of cadmium (< 10 µg/g ww) (Fig. 5).

TABLE 5. Mean tissue-contaminant concentrations in caribou from the George River herd, Labrador, infected or not infected with liver parasites in 2001. Contaminants are selenium and metals in kidney (µg/g wet weight) and hexachlorobenzene in peri-renal fat (ng/g wet weight). Liver parasites are flukes (*Fascioloides magna*) and tapeworm larvae. Pearson correlations between contaminant concentrations and liver parasite abundances are also shown. Significance at $p \leq 0.05$ is shown in bold and at $0.05 < p \leq 0.2$ in italics.

Contaminant	Concentrations in Relation to Fluke Infection Mean (95% CI)		Correlation Coefficient with Fluke Abundance	Concentrations in Relation to Tapeworm Larva Infection Mean (95% CI)		Correlation Coefficient with Tapeworm Larvae Abundance
	Infected	Not Infected		Infected	Not Infected	
Selenium[1]	1.21 (1.15–1.27)	1.16 (1.02–1.31)	0.08	*1.24 (1.17–1.31)*	*1.16 (1.08–1.24)*	*0.39*
Mercury[1]	*0.64 (0.54–0.75)*	*0.72 (0.57–0.87)*	-0.14	0.63 (0.50–0.77)	0.68 (0.54–0.82)	-0.08
Cadmium[2]	**8.0 (5.7–11.1)**	**3.2 (1.7–6.0)**	**0.68**	**8.4 (5.7–12.5)**	**4.6 (2.8–7.5)**	0.23
Lead[2]	*0.1 (0.06–0.15)*	*0.07 (0.03–0.16)*	*0.37*	0.08 (0.05–0.13)	0.10 (0.05–0.19)	*-0.26*
HCB[2,3]	23.2 (20.2–26.5)	23.5 (16.5–33.5)	-0.03	24.8 (21.4–28.7)	22.8 (18.8–27.7)	*0.27*

[1] Arithmetic mean.
[2] Geometric mean.
[3] HCB = hexachlorobenzene.

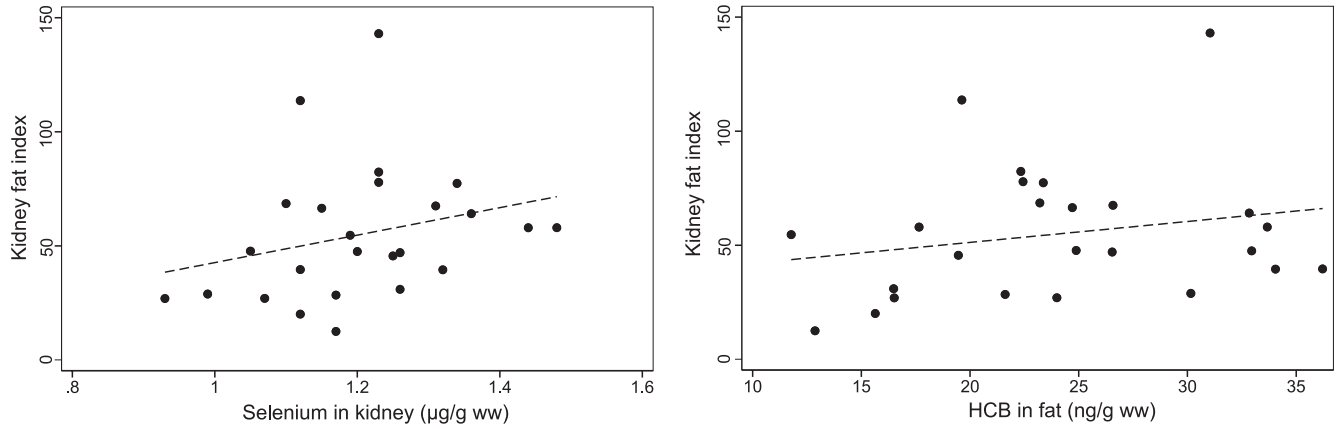

FIG. 3. Kidney fat index in relation to selenium concentration in kidney (µg/g wet weight [ww]) ($r = 0.36$, $p < 0.2$) and hexachlorobenzene (HCB) concentrations in fat (ng/g ww) ($r = 0.31$, $p < 0.2$) in caribou from the George River herd, Labrador, in 2001.

Although age was positively associated with both renal cadmium and *F. magna* infection (older caribou had higher levels of cadmium and a greater abundance of *F. magna*), controlling for age did not significantly alter the final model of predictors for *F. magna* abundance. Age is clearly related to both cadmium levels and *F. magna* infection. However, it is unclear from the small number of GRH animals examined whether cadmium is associated with *F. magna* infection independent of age (within age cohorts). For the three-year-old cohort, cadmium and *F. magna* abundance had a significant positive correlation ($r = 0.68$, $p < 0.01$, $n = 8$). For the remaining age cohorts, the small number of animals examined (range: 1–4) precluded separate analyses within age groups.

For *F. magna* prevalence, renal selenium concentration was again offered to the final model as a potential confounding variable, but did not appear in the final models. Depending on which variable was entered into the final model first, two separate significant final models were produced, each with a single significant predictor. Renal cadmium concentration and age were each found to be significant risk factors in separate models for *F. magna* prevalence on multiple variable logistic regression analysis, and both predictors had a positive relationship with *F. magna* prevalence (Table 6). For this sample of caribou, animals with high levels of renal cadmium and older animals had increased probability of *F. magna* infection.

Multivariable logistic (prevalence) and linear (abundance) regression analyses for liver tapeworm infection did not result in significant final models that fit the data and therefore are not presented. Standard regression diagnostic procedures confirmed a reasonable fit of all final models reported above.

DISCUSSION

The GRH is the only caribou herd in North America endemically infected with *F. magna* (Wobeser et al., 1985).

TABLE 6. Results of multiple regression analyses for kidney fat index (KFI) and liver fluke (*Fascioloides magna*) infection in caribou from the George River herd, Labrador, in 2001.

Health Parameter	Regression Analysis	Coefficient (SE)	Significant Parameters	Intercept	F value	Model *p*-value	*n*	Adjusted R-squared
Ln (KFI)	linear	0.12 (0.05) 0.03 (0.01)	Age (Age – mean Age) Squared	3.69 (0.16)	6.81	0.005	24	0.34
Ln(*F. magna* abundance)	linear	1.69 (0.32) 4.02 (1.83)	Ln(cadmium) (µg/g ww) selenium (µg/g ww)	3.28 (1.99)	14.2	< 0.0001	26	0.51
F. magna prevalence	logistic logistic	2.05 (0.88) 1.00 (0.47)	Ln(cadmium) (µg/g ww) Age	-2.03 (1.38) 0.89 (0.95)	7.24[1] 7.90[1]	< 0.01 < 0.01	27 26	0.26[2] 0.28[2]

[1] LR test (chi-square).
[2] Pseudo R^2.

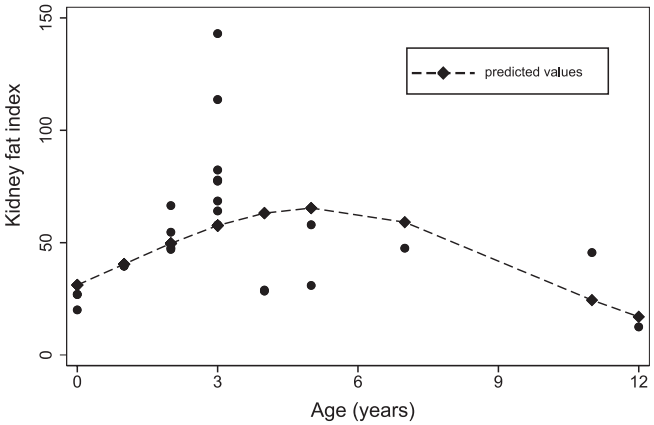

FIG. 4. The predicted relationship between kidney fat index (KFI) and age in caribou from the George River herd, Labrador, in 2001.

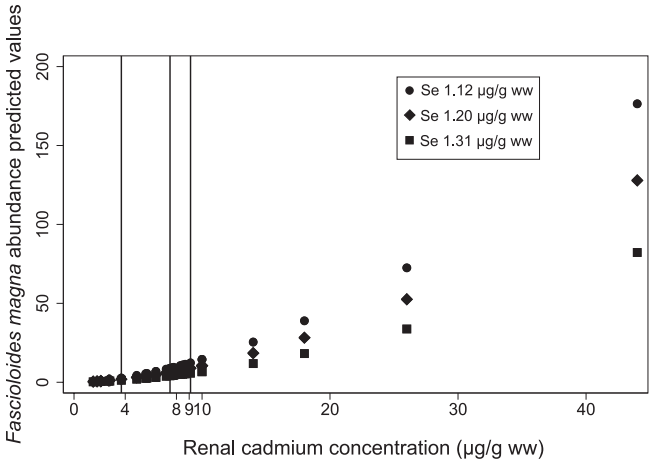

FIG. 5. The predicted relationship between liver fluke (*Fascioloides magna*) abundance and cadmium concentration in kidney (µg/g wet weight) at low (1.12 µg/g ww, 25th percentile), median (1.20 µg/g ww) and high (1.31 µg/g ww, 75th percentile) concentrations of selenium in kidney in caribou from the George River herd, Labrador, in 2001. The solid lines indicate the 25th, median, and 75th percentile concentrations of cadmium.

Other definitive hosts (species in which the parasite exists in its adult form) include wapiti (*Cervus elaphus*) and white-tailed deer (*Odocoileus virginianus*) (Pybus, 2001). A variety of snail species act as intermediate hosts for the liver fluke (Pybus, 2001). The prevalence of *F. magna* infection in the caribou sampled (78%) was significantly higher ($p \leq 0.05$, based on χ^2 statistic) than the prevalence found in other studies of the GRH conducted in the 1980s. Lankester and Luttich (1988) found a prevalence of 58%, Huot and Beaulieu (1985) found 49% infected, and Parker (1981) found a prevalence of 15% (based on the presence of capsules only). However, the mean intensity of infection in the current study was similar to that reported by Lankester and Luttich (1988). Increased prevalence of *F. magna* may occur with increased definitive host density. Other factors, some of which can influence intermediate host density, may also play a role: habitat use and movements by ungulates, seasonal variations in temperature and moisture, and local conditions of wetland habitats (Pybus, 2001).

In this study, regression analysis resulted in two significant predictors of *F. magna* abundance: kidney Cd and Se concentrations. Our results showed that selenium levels were not by themselves associated with *F. magna* infection (Table 5). However, when cadmium and selenium levels were considered together, as selenium levels decreased, abundance of *F. magna* increased (Table 6 and Fig. 5). It may be that any immunotoxic effects of Cd, as demonstrated by increasing *F. magna* abundance, are more pronounced when Se levels are lower. This is the first time that this relationship has been found, and therefore supportive evidence for this relationship was not found in the literature, making confirmatory research essential.

The positive role of selenium in host immunity is well documented in experimental and domestic animals (Dhur et al., 1990; Arthur et al., 2003). However, in relation to parasite infection, the protective role of selenium has not been convincingly demonstrated. Investigations into the association between selenium and cadmium levels in horses (Junnila et al., 1987) and rats (Jamall and Smith, 1985) have been equivocal. In addition, there is inconsistent evidence in the literature of immunotoxic effects in experimental animals from chronic cadmium exposure (Scheuhammer, 1987; Taylor et al., 1999). Although we found a significant positive correlation between renal cadmium and selenium levels, the biological significance of this relationship is unclear.

An alternative explanation for the association seen between renal cadmium concentrations and *F. magna* infection could relate to concurrent exposure of caribou to both cadmium and *F. magna* larvae while foraging. Pybus (2001) suggested that the variation in prevalence and intensity of *F. magna* infection within host populations may reflect individual differences in the use of emergent vegetation (and thus exposure to infective stages of the parasite). However, there is no evidence that this foraging behaviour would also increase an animal's exposure to cadmium.

Although evidence for immunotoxicity with cadmium exposure in animals is scant, the potential for renal toxicity has been well established. The earliest light microscopic change in this organ in mammals and birds is proximal tubular necrosis (Alden and Frith, 1991). There is also evidence that exposure to cadmium can result in disturbances in calcium balance and decreases in bone density (Taylor et al., 1999). The threshold for significant renal tubular damage in mammals is generally reported as 100 µg/g ww (Cooke and Johnson, 1996; AMAP, 2005). However, lower threshold concentrations have also been published depending on the species. On the basis of a review of the literature on experimental and domestic species, Outridge et al. (1994) reported a threshold concentration of 30 µg/g ww for kidney damage in mammals. Generally, these published toxicity thresholds are based on controlled exposure under laboratory conditions. Extrapolating these thresholds to free-ranging species introduces a degree of uncertainty because of the many factors that influence toxicity in the wild, such as dietary intake of other metals and nutrients, environmental factors, and species differences (Cooke and Johnson, 1996; Froslie et al., 2001; AMAP, 2005). Thus, the thresholds for cadmium toxicity in caribou cannot be accurately predicted given the current lack of knowledge (Froslie et al., 2001). However, these published thresholds do provide general guidance in identifying species and regions of potential concern (AMAP, 2005; Fisk et al., 2005).

Although large mammals such as ungulates and seals have been shown to have some of the highest levels of cadmium recorded in wildlife (Muir et al., 1997), relatively few studies of wildlife populations have documented biological effects associated with high tissue cadmium concentrations.

Recent studies of free-ranging wildlife species that have evaluated kidney cadmium concentrations in relation to renal histopathology have shown varied results. Beiglböck et al. (2002) found that roe deer (*Capreolus capreolus*) in Austria with kidney cadmium residues ranging from 0.01 to 22.08 µg/g ww had histopathologic alterations of kidney proximal tubular epithelial cells that were related to increased renal cadmium levels. Larison et al. (2000) examined white-tailed ptarmigan (*Lagopus leucurus*) from the Colorado ore belt and found that those with renal cadmium concentrations over 100 µg/g ww had histopathological evidence of renal injury. Ptarmigan with renal cadmium concentrations above this level also had reduced concentrations of skeletal calcium compared to controls.

However, other studies of free-ranging ungulates and seals found little evidence for biological effects in relation to cadmium concentrations. Paré et al. (1999) found no lesions characteristic of renal disease in 33 kidney samples from moose with mean cadmium concentrations of 123.1 (± 17.98) µg/g dry weight (approximately 35.2 ± 5.1 µg/g ww) submitted for histopathological examination. O'Hara et al. (2003) found no histopathologic evidence of renal lesions in caribou kidneys with cadmium concentrations of 0.54–33.0 µg/g ww from northern Alaska. Sonne-Hansen et al. (2002) found no evidence of cadmium-induced renal toxicity or skeletal demineralization in a study conducted on ringed seals (*Phoca hispida*) from Greenland. Renal cadmium concentrations of the seals examined ranged from 0–248 µg/g ww.

The highest concentration of renal cadmium in our caribou was 44 µg/g ww. On microscopic examination of kidneys from all caribou sampled, no evidence of proximal tubular injury was found, although some degree of autolysis and freezing artifacts may have masked subtle changes.

Except for a few cases of renal inflammatory changes, pathological findings in the caribou were limited to grossly evident hepatic lesions due to *F. magna* infection. The most severe infection found was 67 flukes (30 capsules). While hepatic lesions were extensive, they were felt to be clinically insignificant considering the large functional reserve and regenerative capacity of the mammalian liver (Cullen and MacLachlan, 2001). Although the presence of flukes in the liver causes noticeable damage, clinically significant pathological findings have not been reported in definitive hosts except with severe infections. Mortality due to infection was reported in wapiti with more than 500 flukes (Pybus, 2001). Mulvey and Aho (1993) reported subtle negative health effects such as decreased weight gains in young male white-tailed deer with moderate to heavy infections of *F. magna* (> 10 flukes) compared to uninfected or lightly infected deer (0–10 flukes). No reports of morbidity or mortality due to *F. magna* infection in caribou have been published (Pybus, 2001). No association was found in the present study between *F. magna* infection and body condition as measured by the KFI. Similarly, Lankester and Luttich (1988) found no association between *F. magna* infection and back fat depth in the GRH. Because of its cross-sectional nature, the present study could not examine differences in weight gain.

Fifty percent of the caribou that were examined were infected with cestode cysts in the liver that were consistent with the larval form of the tapeworm *Taenia hydatigena*. *Taenia hydatigena* has a wide range of intermediate hosts, including domestic livestock and wild cervids, and prevalence and intensity differ among different host species (Jones and Pybus, 2001). Definitive hosts for *T. hydatigena* include domestic and wild carnivores such as wolves (*Canis lupus*) (Jones and Pybus, 2001), which are present in Labrador. Accounts of clinically significant lesions due to *T. hydatigena* in wild intermediate hosts are rare. Addison et al. (1979) found no association between physical condition of moose in Ontario and intensity of *T. hydatigena* infection. Similarly, no significant lesions were found in relation

to *T. hydatigena* cysts in the liver of our sampled caribou, and there was no association between indices of infection and KFI in our final model.

On regression analysis, the only significant predictor of KFI in caribou was age, with young adults (3 to 7 years old) attaining the highest levels of KFI relative to younger and older animals. A similar parabolic relationship between KFI and age was seen in female caribou from the GRH by Parker (1981), and Thomas et al. (1989) noted that KFI decreased with increasing age in adult female barren-ground caribou.

We found seasonal differences in KFI that may be due, in part, to a variation in ages of caribou killed in the two seasons, although the differences seen in the age distribution between fall and winter were not statistically significant ($p = 0.14$). Another consideration is that the fall and winter seasons during which caribou were examined were also in different yearly cycles: animals sampled in winter 2001 (as opposed to those sampled in fall 2001) were subjected to environmental conditions and food availability of summer and fall of 2000. Similar factors may also account for the seasonal difference seen in tapeworm abundance.

Winter caribou had higher HCB concentrations in fat than did fall caribou. This finding may be related to the relatively higher KFI seen in winter caribou. As with all OC contaminants, HCB is highly lipid-soluble and thus an animal's body burden is stored almost exclusively in the lipid component of a tissue. We did find a positive trend between HCB concentrations in fat and KFI ($r = 0.31$, $p < 0.2$), which could partially explain the higher HCB concentrations seen in winter caribou compared to fall caribou.

A seasonal difference was also found in kidney Hg concentration, with higher levels in the fall compared to winter. The primary uptake route of Hg is diet and, once ingested, Hg is primarily stored in the liver and kidney (AMAP, 2005). The biological half-life for Hg compounds in mammals is relatively short (40–70 days) (Goyer, 1995). Thus, seasonal dietary differences could account for seasonal differences seen in kidney Hg concentrations.

The geometric mean renal Cd concentration in our study, 6.5 µg/g ww (95% CI: 4.7–8.9), was consistent with levels found in earlier studies on the GRH. Crête et al. (1989) and Robillard et al. (2002) found means [SE] ranging from 1.2 [0.1] µg/g ww to 10.7 [0.2] µg/g ww, converted from dry weight, depending on age and season. Similarly Elkin and Bethke (1995) and Larter and Nagy (2000) found means [SE] in caribou in the Northwest Territories and Nunavut, Canada, ranging from 2.0 [0.3] µg/g ww to 8.9 [1.0] µg/g ww, converted from dry weight assuming mean percent moisture of 79.3, depending on region. Gamberg et al. (2005) reported geometric means ranging from 8.4 to 83.8 µg/g ww, depending on region.

Concentrations of mercury, lead, and selenium in kidney of caribou in our study were low and fell within the ranges previously reported in the GRH (Robillard et al., 2002) and other herds in Canada (Elkin and Bethke, 1995). The threshold residue levels in kidney for systemic biological effects of Hg (30 µg/g ww) (Thompson, 1996) and Pb (90 µg/g dry weight; approximately 26 µg/g ww) (Ma, 1996) far exceed even the maximum kidney concentrations found in caribou in our study. Thus, the levels of Hg and Pb found were unlikely to have caused health problems in the animals examined.

Concentrations of OCs in fat were also low and within the ranges reported in caribou elsewhere in North America (Elkin and Bethke, 1995). As reported in Arctic terrestrial herbivores (Braune et al., 1999), hexachlorobenzene was the most abundant OC contaminant detected in our study. The levels of all OCs found were well below those reported to be associated with adverse health effects in mammals (AMAP, 2004) and were unlikely to have resulted in health problems in the caribou sampled.

CONCLUSIONS

The sampled caribou appeared to have sufficient fat reserves for the time of year, with the greatest fat reserves found in young adults (3 years old) compared to younger and older caribou. Only age was a significant ($p \leq 0.05$) predictor of KFI. Lesions identified in carcasses included two cases of chronic interstitial nephritis, as well as chronic hepatitis in those caribou infected with *F. magna*. None of these lesions were felt to have been of clinical significance.

The levels of detected contaminants fell within the reported ranges for caribou in Canada and elsewhere (Elkin and Bethke, 1995). All contaminants were well below the reported thresholds for toxic effects in other species, (AMAP, 2004, 2005) and were unlikely to have caused health problems in the caribou tested, although renal cadmium levels approached the reported toxic threshold in one case, which was not unexpected. There was no evidence of renal tubular toxicity due to chronic cadmium exposure; however, most caribou examined were young (< 5 years), with relatively low levels of renal cadmium.

Age was positively associated with renal cadmium levels and *F. magna* infection (older caribou had increased levels of both), and on multiple regression analysis, *F. magna* abundance was best predicted by renal cadmium and selenium levels. It is unclear, on the basis of the few animals examined, whether a relationship between *F. magna* infection and cadmium levels existed independently of age. The possibility of some degree of immunotoxicity related to cadmium levels (possibly tempered by selenium concentrations) warrants further investigations in larger populations of animals.

The caribou we examined were representative of those typically harvested by Innu hunters. Caribou are an important source of country food for Innu people in the area; in addition to concerns about the potential impact of environmental contaminants on animal health, questions have been raised about potential human health effects from eating country foods. A detailed discussion of potential risks of eating caribou is beyond the scope of this study; overall, however, the levels of environmental contaminants found were low except for cadmium in the kidneys of some adult

caribou. From a public health perspective, the issue of cadmium in George River herd caribou has been thoughtfully addressed elsewhere (Archibald and Kosatsky, 1991; Robillard and Bélanger, 1997). Specific recommendations for Innu people in Labrador in this regard are best made at the local level in conjunction with the communities and local public health representatives, with careful consideration of the many benefits of hunting and eating country foods.

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