



Original Investigation

Prevalence of renal calculi in wolverine (*Gulo gulo*) from northwestern CanadaMichelle P. Oakley^{a,1}, Thomas S. Jung^{a,*}, Piia M. Kukka^a, Jean-François Robitaille^b^a Yukon Department of Environment, P.O. Box 2703, Whitehorse, Yukon Y1A 2C6, Canada^b Department of Biology, Laurentian University, 935 Ramsey Lake Road, Sudbury, Ontario P3E 3C6, Canada

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ABSTRACT

Renal calculi (kidney stones) are often reported in domestic animals and occasionally wildlife; however, prevalence is rarely reported for free-ranging wildlife. Our aim was to determine the prevalence of renal calculi in a large sample of free-ranging wolverine (*Gulo gulo*) from a harvested population in Yukon, Canada. We tested for an effect of sex, age, and body condition, on the presence of renal calculi. Macroscopic examination revealed renal calculi in 48 of 537 (8.9%) wolverine. Bilateralism was low, with only 6 of 48 (12.5%) affected wolverine having calculi in both kidneys. Calculi were found in similar prevalence between the sexes. A significantly higher percentage of adults (≥ 2 years old) had renal calculi than sub-adults (< 2 years old). When considering adults alone, prevalence was 12.7% for males and 17.8% for females. The mean age of affected females was not statistically different than the sample population, but the mean age of affected adult males was 2 years older than unaffected adult males. Mean body condition scores for wolverine with and without calculi were not statistically different for females or males. Mineral composition was determined for calculi from 29 wolverine. Calculi from most wolverine (90%) were composed of 95–100% ammonium acid urate, with magnesium ammonium phosphate (struvite) and calcium phosphate (apatite) as minor ($\leq 5\%$) constituents. Our study is one of the first to document the prevalence of renal calculi in a free-ranging population of wildlife. Prevalence of renal calculi in adult wolverine from northwestern Canada was substantial; however, the pathogenesis and clinical significance of nephrolithiasis in wolverine is unknown.

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Introduction

Renal calculi (kidney stones) are solid mineral concretions that form in the kidneys and may be present throughout the urinary tract. They may develop as a result of a variety of factors, including a sharp decrease in urine volume, an increase in stone-forming minerals such as calcium, urate, or cysteine, in the urine, or as a result of a purine-rich diet, decreased water intake, a urinary tract infection, or genetic predisposition (Houston et al. 2011). Renal calculi vary in size, shape and color (Houston and Moore 2009; Osborne et al. 2009), and can produce clinical symptoms, including pain and predisposition to infection, when they are large enough to block the urinary tract and cannot be expelled in urine. In some instances they can predispose affected individuals to infections of

the urinary system or hamper the urine flow. Affected individuals may remain sub-clinical during their life, or show clinical signs leading in some severe cases to renal failure (e.g., Calle 1988; Hope et al. 1989; Venn-Watson et al. 2010).

Nephrolithiasis is occasionally observed in domestic and captive mammals, where it may be largely related to diet and obesity (e.g., Mussart and Coppo 1998; Houston and Moore 2009; Osborne et al. 2008, 2009; Robinson et al. 2008). However, the condition is rarely reported from free-ranging wildlife (Simpson et al. 2011; Niemuth et al. 2014). The occurrence of renal calculi has been reported in the wild for several species of marine mammals (e.g., Dennison et al. 2007; Keller et al. 2008; Venn-Watson et al. 2010), ungulates (e.g., Reynolds 1982; Hope et al. 1989; Larsen et al. 2000) and carnivores (e.g., Simpson et al. 2011; Tordiffe et al. 2012; Niemuth et al. 2014). However, most cases in the literature involving renal calculi in free-ranging wildlife are based on small samples and prevalence rates could not be established. Exceptions include a study of renal calculi in 492 European otters (*Lutra lutra*) killed on roads in England (Simpson et al. 2011), and a study of 229 North American river otters (*Lontra canadensis*) harvested for fur in the southeastern

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Table 1
Annual variation in the number, percentage, and sample size (*n*) of free-ranging wolverine (*Gulo gulo*) affected with renal calculi in Yukon, Canada.

Year (winter)	Age-class	Males			Females		
		Affected	<i>n</i>	%	Affected	<i>n</i>	%
2005/2006	Adults	1	19	5.3	1	11	9.1
	Sub-adults	1	28	3.6	0	9	0
2006/2007	Adults	7	20	35.0	0	8	0
	Sub-adults	0	30	0	2	19	10.5
2007/2008	Adults	3	21	14.3	4	10	40.0
	Sub-adults	2	30	6.7	0	6	0
2008/2009	Adults	2	26	7.7	2	13	15.4
	Sub-adults	1	27	3.7	0	7	0
2009/2010	Adults	1	15	6.7	4	14	28.6
	Sub-adults	2	45	4.4	0	22	0
2010/2011	Adults	3	19	15.8	0	9	0
	Sub-adults	2	30	6.7	1	15	6.7
2011/2012	Adults	0	18	0	2	8	25.0
	Sub-adults	2	25	8	3	24	12.5

United States (Niemuth et al. 2014). These studies reported prevalence rates of 10.1% and 16.1% in European and North American otters, respectively, demonstrating that a substantial proportion of free-ranging wildlife populations may be affected by renal calculi.

In this study we provide the first reported observations of renal calculi in free-ranging wolverines (*Gulo gulo*) and estimate prevalence based on gross examination of renal calculi in a large sample of free-ranging wolverines (*n* = 537). In addition, we compared prevalence among sex and age-classes, and in relation to body condition. Finally, we undertook chemical analyses of calculi to determine their mineral composition.

Material and methods

In Yukon, Canada, wolverines are legally harvested during winter (November to March) by licensed fur trappers (Slough 2007). We solicited fur trappers to voluntarily submit wolverine carcasses during the 2005/2006 to 2011/2012 winter fur trapping seasons (i.e., seven winters). Skinned carcasses were kept frozen at -20°C for 6–10 months prior to necropsy.

During necropsy, we took morphometric measurements (e.g., mass, total body length), determined sex, collected a premolar for aging, and excised and weighed various fat depots (see Robitaille et al. 2012; Kukka and Jung 2015). Age was determined via cementum analysis (Poole et al. 1994) at a commercial laboratory (Matson's Laboratory LLC, Milltown, Montana, USA). Wolverine < 2 years old were classified as sub-adults and those ≥ 2 years old as adults. In addition, we calculated a body condition index (BCI) for each individual, using the mass of sternal fat and applying the equations provided by Robitaille et al. (2012) for each sex.

Kidneys were removed, sliced open with longitudinal incisions, and macroscopically examined visually and by feeling for grit to determine the presence of calculi. Our search was focused within the renal pelvis and surrounding portions of the medulla. We did not specifically examine the lower urinary tract for calculi, although in some cases the bladder was checked for comparative purposes. Our methods allowed us to observe very small (<1 mm) concretions, however we acknowledge that some smaller calculi may have been missed during our examinations because we did not use diagnostic imaging techniques to detect smaller calculi (Niemuth et al. 2014). To assess bilateralism we noted in which kidney calculi were found. The mineral composition of a subsample of renal calculi was determined via optical crystallography using polarized light microscopy (Moore 2007; Appel et al. 2010) at a commercial laboratory (Canadian Veterinary Urolith Centre, University of Guelph, Guelph, Ontario, Canada).

We tested for statistical differences in the mean age and body condition of affected and unaffected adult wolverine using

two-sample *t*-tests. Data were visually examined for normality using box plots, prior to using parametric statistical tests. A *P*-value of ≤ 0.05 denoted statistical significance. SYSTAT (ver. 13) was used for statistical tests.

Results

Renal calculi were found in 48 of 537 (8.9%) free-ranging wolverine over the seven consecutive trapping seasons. We observed calculi in wolverine in all years; but, annual prevalence of renal calculi was variable (Table 1). Prevalence was similar for male (29 of 362; 8.0%) and female (19 of 175; 10.9%) wolverine.

Typically, calculi were observed in only one kidney (53% right kidney and 47% left kidney). The rate of bilateralism was low: only 6 of 48 (12.5%) wolverine with calculi had them in both kidneys. Small calculi were observed throughout the medulla, and as expected, large calculi were in the renal pelvis. Virtually no calculi were observed near the poles, as seen in otter (Simpson et al. 2011).

Calculi varied in size, shape and color (Table 2). In some animals a single large calculus was observed, sometimes approaching one-third the size of the kidney. In other instances (41%), wolverine harbored several small (≤ 1 mm) calculi scattered throughout the medulla (Table 2). Calculi from most animals (85%) were irregular in shape, with a rough surface (Table 2). The color varied, including white, tan/grey, green, and brown, with most (65%) being tan/grey (Table 2). We did not observe macroscopic change in kidneys of wolverine with renal calculi.

Mineral composition was determined for calculi from 29 wolverine (Table 2). For most of these (90%), calculi were composed of 95–100% ammonium acid urate, with calcium phosphate (apatite) being a minor constituent (5%) for one animal, and magnesium ammonium phosphate hexahydrate (struvite) for four others (Table 2). Calculi from two wolverine (6%) were composed of 65% and 50% ammonium acid urate and the remainder was apatite. For one other wolverine (4%), the calculus was composed of 60% struvite and 40% apatite.

Age data was available for 524 of 537 (98%) of the wolverines examined, with most (60.5%) being classified as sub-adults. Renal calculi were more prevalent in adult than sub-adult wolverines of both sexes. The prevalence of renal calculi was 5.0% and 17.8% for sub-adult and adult females, respectively (Fig. 1). Prevalence of renal calculi was 4.7% and 12.7% for sub-adult and adult males, respectively. The mean age of adult females with and without renal calculi did not differ statistically ($t_{71} = 0.859$; $P = 0.393$; Fig. 2). However, adult males with renal calculi were on average 2 years older than unaffected adult males ($t_{132} = 3.030$; $P = 0.003$; Fig. 2).

Body condition index values were estimated for 514 of 537 (96%) of the wolverines examined for renal calculi. BCI scores varied

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