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Ecotoxicoparasitology: Understanding mercury concentrations in gut contents, intestinal helminths and host tissues of Alaskan gray wolves (*Canis lupus*)

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HIGHLIGHTS

- [THg] and stable isotopes together provide insight on host-parasite-Hg interactions.
- A significant positive association exists between host liver and taeniid [THg].
- [THg] varies within the GI tract and may be influenced by the presence of parasites.
- [THg] is higher in ascarids & taeniids than in the lumen contents in the GI tract.

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ABSTRACT

Some gastrointestinal helminths acquire nutrients from the lumen contents in which they live; thus, they may be exposed to non-essential elements, such as mercury (Hg), during feeding. The objectives of this study were: 1) determine the total mercury concentrations ([THg]) in Gray wolves (*Canis lupus*) and their parasites, and 2) use stable isotopes to evaluate the trophic relationships within the host. [THg] and stable isotopes (C and N) were determined for helminths, host tissues, and lumen contents from 88 wolves. Sixty-three wolves contained grossly visible helminths (71.5%). The prevalence of taeniids and ascarids was 63.6% (56/88) and 20.5% (18/88), respectively. Nine of these 63 wolves contained both taeniids and ascarids (14.3%). All ascarids were determined to be *Toxascaris leonina*. *Taenia* species present included *T. krabbei* and *T. hydatigena*. Within the GI tract, [THg] in the lumen contents of the proximal small intestine were significantly lower than in the distal small intestine. There was a significant positive association between hepatic and taeniid [THg]. Bioaccumulation factors (BAF) ranged from <1 to 22.9 in taeniids, and 1.1 to 12.3 in *T. leonina*. Taeniid and ascarid BAF were significantly higher than 1, suggesting that both groups are capable of THg accumulation in their wolf host. $\delta^{13}C$ in taeniids was significantly lower than in host liver and skeletal muscle. [THg] in helminths and host tissues, in conjunction with stable isotope (C and N) values, provides insight into food-web dynamics of the host GI tract, and aids in elucidating ecotoxicoparasitologic relationships. Variation of [THg] throughout the GI tract, and between parasitic groups, underscores the need to further evaluate the effect(s) of feeding niche, and the nutritional needs of parasites, as they relate to toxicant exposure and distribution within the host.

1. Introduction

Mercury (Hg) is a non-essential element that exists in numerous chemical forms, occurring both naturally in the environment and as the result of human activities. Major anthropogenic sources include the burning of fossil fuels (especially coal), municipal waste incineration (Virtanen et al., 2007), and other point sources such as amalgam waste from dental clinics (Shraim et al., 2011). Emissions from geological sources (e.g. cinnabar), as well as emissions from forest fires and volcanoes, also contribute Hg to the environment. Hg is not only ubiquitous, but also environmentally persistent (Balshaw et al., 2007), and can have detrimental effects on the health of organisms. Methylmercury (MeHg) is the highly toxic form (Kruzikova et al., 2008; Virtanen et al., 2007), which includes monomethylmercury ($MeHg^+$) and dimethylmercury.

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The $MeHg^{+}$ form has been shown to bioaccumulate and/or biomagnify in food webs, and often reaches high concentrations in specific tissues of long-lived fish-eating animals (Clarkson and Magos, 2006; Balshaw et al., 2007; Bridges and Zalups, 2010; Virtanen et al., 2007).

Historically, studies on food webs have rarely included parasites, partly due to the complex nature of host-parasite interactions (Gómez-Díaz and González-Solís, 2010). The term *ecotoxicology* has been applied to the field of study focused on the effects of toxic chemicals on biological organisms, at the population, community and ecosystem level (Moriarty, 1988); *ecotoxicoparasitology*, however, relates to the interface of three disciplines, and is concerned with complex systems including parasites, their hosts, and the toxicants to which they are exposed.

Several gastrointestinal helminths feed on the intestinal contents of the hosts in which they live, thereby, putting them in direct competition with their host for nutrients (Coop and Kyriazakis, 1999). These parasites are exposed to the same dietary and elimination pathways (e.g. bile) of the host; thus, uptake of toxicants by the parasites likely occurs as a direct result of their feeding activities within the host's gastrointestinal (GI) tract. One previous study showed that GI helminths are capable of bioaccumulation and/or biomagnification of non-essential elements at concentrations that are orders of magnitude higher than those in host tissues (Sures et al., 1999). However, the process by which parasites acquire, concentrate, and/or biotransform non-essential elements, such as Hg, and their potential impact on host-toxicant dynamics, is not well understood.

One tool that may be used to explore aspects of host-parasite relationships and Hg exposure is stable isotope analysis. In many systems, carbon (C) and nitrogen (N) stable isotopes have been used in feeding ecology studies to determine food web length and structure (Gómez-Díaz and González-Solís, 2010), often facilitating assessment of consumer feeding habits (Daugherty and Briggs, 2007). Because parasites utilize various feeding strategies, it may be challenging to interpret trophic relationships using isolated approaches (Gómez-Díaz and González-Solís, 2010). Previously stable isotope and total mercury (THg) analysis have been used to provide insight into sources of Hg exposure in gray wolves (McGrew et al., 2014). N signatures have also been shown to have a positive association with total mercury concentrations [THg] in arctic food webs (Atwell et al., 1998). The aim of this study was, therefore, to use stable isotope signatures (C and N) and [THg] together, to better understand the influence of the host-parasite relationship on [THg], and provide insight into the trophic relationships that exist within gray wolves from Alaska.

2. Materials and methods

2.1. Sample collection

Samples from gray wolves from across the state of Alaska were collected by the Alaska Department of Fish & Game, between 2006 and 2009, as part of ongoing studies. Previously, McGrew et al. (2014) measured C and N stable isotope signatures and [THg] from the liver, kidney, and skeletal muscle of this wolf population. In this work, the GI tracts, GI helminths, and host tissues were evaluated from a subset of these individuals, as well as other wolves from Alaska ($n = 88$). At necropsy, sex and age class were determined, and tissues, including liver, skeletal muscle, and GI tracts (with lumen contents and parasites) were collected. Nine of 88 GI tracts were processed immediately, whereas the remaining GI tracts were transferred to the University of Alaska Fairbanks (UAF) and stored at −80°C until further processing could take place. Wolves were divided into two age classes, <12 months and ≥12 months, based on previous studies (Gese et al., 1997; McGrew et al., 2014; Zarnke et al., 2004).

During processing, the large and small intestines were opened longitudinally using clean stainless steel instruments, and all grossly visible parasites were removed, rinsed with ultrapure water, and weighed. Due to freezing, only the ascarids were able to be enumerated. A limited number of representative ascarids were fixed in 10% formalin for morphologic identification; likewise, single terminal proglottids from representative taeniids were frozen for molecular identification (Obwaller et al., 2004). All remaining ascarids and taeniids were frozen for subsequent THg analysis, and C and N stable isotope analysis. Lumen contents and full-thickness sections of GI wall were collected from proximal small intestine (pSI), distal small intestine (dSI), and colon, and were weighed prior to freezing at −80 C. Determination of [THg] and stable isotopes was carried out for host tissues, lumen contents, and parasites.

2.2. Total mercury (THg) analysis

Host liver and skeletal muscle are commonly sampled tissues in THg and SI studies due to their intrinsic properties and rate of cellular turnover. In this study, these tissues were thawed at room temperature, and sub-sampled (70–150 mg) using clean stainless steel forceps and scissors. Instruments were washed with ultrapure water and dried between each sample. Samples were analyzed with a direct mercury analyzer (DMA) using a Milestone DMA-80 instrument as described previously (Butala et al., 2006; McGrew et al., 2014; Field measurement technology for mercury in soil et al., DC. May 2004). Groups of ascarids or taeniids from each host were freeze-dried, homogenized using a mortar and pestle, and analyzed for THg on a dry weight basis. Ascarids and taeniids were homogenized and analyzed separately in instances of mixed infection. The homogeneity ensured [THg] were representative of the parasite group(s) collected from each host. [THg] were not determined for taeniids from 6/56 wolves due to low biomass. While host tissue [THg] were determined on a wet weight (ww) basis, both ww and dry weight (dw) values are presented for comparative purposes. Dry weight [THg] was calculated as ww [THg]/(1 − fraction moisture content). Percent moisture was also used to calculate [THg] in parasites on a ww basis.

2.3. Stable isotope analysis

Stable isotope values ($\delta^{13}C$ and $\delta^{15}N$) were determined as previously described (McGrew et al., 2014). Briefly, all tissues to be analyzed were freeze-dried and homogenized prior to analysis, which was carried out by continuous-flow isotope-ratio mass spectrometry using an elemental analyzer (Carlo Erba NC1500 or Thermo Flash 2000) interfaced to a mass spectrometer (Micromass Optima or Thermo-Finnigan Delta Plus XP). Isotope values are reported in delta (δ) notation:

$$\delta X = \left(R_{sample} / R_{standard}\right) - 1$$

where X represents ^{13}C or ^{15}N in parts per thousand (‰) deviation relative to a standard (monitoring) gas and R_{sample} and $R_{standard}$ represent the ratio of $^{13}C/^{12}C$, or $^{15}N/^{14}N$, for sample and standard, respectively. Analytical error was assessed by replicate measures of primary standards (<0.2‰ for both isotopes across all analytical sequences) and quality control was assessed using several secondary standards, analyzed several times within individual analytical sequences (<0.3‰); accuracy was assessed using primary standards as unknowns, and was within 0.2‰ for both isotopes. Reproducibility of values was generally better than 0.2‰.

2.4. Helminth identification

Prevalence was defined as the proportion of wolves infected with a given parasite, and intensity was defined as the number of individuals of a particular parasite species, in a given host (Bush et al., 1997). Ninety-five percent confidence intervals were determined using the Clopper-Pearson method (Newcombe, 1998).

Ascarids were identified based on morphological criteria (Anderson et al., 1974). Three of 15 wolves harbored only a single ascarid; thus, species identification could not be performed without adversely affecting [THg] and stable isotope determination. Taeniids were identified using