Echinococcus multilocularis infection is more prevalent in young coyotes (*Canis latrans*) with varied effects of diet

by

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Abstract

Urban environments can influence parasite transmission and prevalence by altering the diets, distribution, abundance, and behaviour of wildlife. *Echinococcus multilocularis* is a zoonotic cestode that typically parasitizes coyotes (*Canis latrans*) and rodents (*Myodes* spp., *Microtus* spp.) as definitive and intermediate hosts, respectively. *E. multilocularis* has historically been widespread among Canadian wildlife, however, it is of emerging concern because a variant of a European strain is associated with 17 human infections, primarily in Alberta. This variant is now widespread among coyotes in the province and the pathogen appears to be especially prevalent among urban coyotes in Edmonton. I hypothesize that an altered diet in urban coyotes contributes to a higher prevalence of *E. multilocularis* either by (a) greater exposure from consumption of infected rodents, or (b) increased overall susceptibility to infection from consumption of anthropogenic food that may reduce body condition.

I tested these hypotheses by examining coyote carcasses donated from urban and rural sources in and near Edmonton. In close collaboration with other researchers, I compared the presence and intensity of *E. multilocularis* infection in the carcass intestine to physiological data (cementum age, sex, body condition) and measures of short and long-term diet (stomach contents, stable isotope values). *E. multilocularis* was detected in the intestine by molecular testing (qPCR) and quantified by worm counts of filtered intestinal contents. Long-term diet was tested for prey ($\delta^{15}N$) and anthropogenic food ($\delta^{13}C$) stable isotopic values. Stomach contents were separated into 10 diet components: prey (ungulates, rodents, meso-mammals, birds), vegetation (herbaceous, woody), native fruit, insects, anthropogenic food (digestible, indigestible). I examined the effects that consumption of rodents and anthropogenic food had on the presence of *E. multilocularis* with logistic regression models and the intensity of infection

with a negative binomial generalized linear model. I analyzed each location separately and accounted for the effect of age.

I detected *E. multilocularis* DNA in 70% of coyote intestines and worms were detected in 48%. Edmonton's urban coyotes exhibited infection prevalence much higher than other Canadian locations. We found few direct short- and long-term diet differences between infected and uninfected coyotes until we assessed urban and rural coyotes separately, which revealed that the volumes of rodents and anthropogenic food related to *E. multilocularis* infection differently in each location. Unexpectedly, uninfected urban coyotes consumed large volumes of rodents, primarily as older adults. Similarly, the presence and intensity of *E. multilocularis* infection in rural coyotes was most strongly driven by young age, but uninfected rural coyotes also consumed large volumes of digestible anthropogenic food. Young coyotes hosted the most intense infections and young, urban coyotes that consumed greater quantities of rodents and anthropogenic food were more likely to be infected. Taken together, our results suggest that young age is the most important contributor to the presence and intensity of *E. multilocularis* infection in coyotes and aspects of young coyote ecology, such as diet composition, may increase the likelihood of becoming infected.

Preface

This thesis is an original work by Deanna K. Steckler. Publication of this research is intended with coauthors[1] S. Sugden, D. Sanderson, B. Abercrombie, M. A. Seguin, K. Ford, and C. C. St. Clair. Colleen Cassady St. Clair was the supervisory author and was involved in manuscript review as was Scott Sugden. The research project, of which this thesis is a part received animal ethics approval from the University of Alberta Research Ethics Board, Project Name "Edmonton Urban Coyote Project", No. AUP00002336, 2018.

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[1] The thesis is formatted for submission to a peer-reviewed journal with multiple authors and therefore uses the pronoun "we" throughout. Contributions by other authors are as follows: S. Sugden collected data on coyote body condition, age, and stable isotopes, D. Sanderson and K. Ford quantified stomach contents, B. Abercrombie oversaw collection of most rural coyote carcasses, M. A. Seguin oversaw molecular analysis of parasites in intestinal samples, and C. C. St. Clair supervised the project. I led collection of morphological parasite data, participated in all other forms of data collection, conducted the statistical analyses, and wrote the manuscript.

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This research was conducted as part of a research collaboration, led by Dr. Colleen Cassady St. Clair at the University of Alberta and could not have been completed without the contributions of others. The coyote carcasses were donated by Bill Abercrombie and his team at Animal Damage Control Inc., as well as the City of Edmonton Animal Care and Control Centre and Edmonton Police Service. I cannot thank Scott Sugden enough for completing the majority of necropsies, ageing the coyotes, and conducting stable isotope analysis. Further, Scott provided unwavering support throughout the production of the manuscript. I would like to thank Alexis Seguin and her team at IDEXX Laboratories for doing molecular identification of *Echinococcus multilocularis* (for free!). I also thank Dana Sanderson and Kyra Ford for their work on stomach contents, Arya Horon for her work on the worm counts, and all of my undergraduate volunteers: Jonathan Farr, Jordyn Berezen, Eric Friesen, Lissette Gervais, Heather Gleeson, Jamie Lam, Caitlin Mak, Alyssa Maurier, Kelby Ogryzlo, Millicent Omenta, Marla Roth, Anna Wang, Calum Ward, Derrick Zhang.

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I want to take a final moment to express some self-love and thank myself. I want to thank me for believing in me and for not giving up even when I was convinced that I couldn't finish. Lastly, I want to thank Pogi for sitting next to me all of these years patiently waiting for me to finish working. I'm done now, bud. Let's go to the park.

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Introduction

Urbanization converts rural lands into human-dominated settlements and by 2050, 68% of the global human population is predicted to reside in cities (United United Nations 2018). However, urban landscapes are also expanding at twice the rate of their populations, which results in the decline of many species (Seto et al. 2012). Alternatively, generalists thrive in urban environments and often exist in greater abundance due to their ability to exploit anthropogenic resources (Sorace and Gustin 2009; Fischer et al. 2012), which necessarily increases human exposure to the zoonotic diseases and parasites of those species. For example, rats (*Rattus* spp.) pervade anthropogenic habitats and can host dozens of zoonotic pathogens (Strand and Lundkvist 2019); several of which have caused significant illness and death in humans (Himsworth et al. 2013). Additionally, raccoons (*Procyon lotor*) introduced to Europe have contaminated chimneys, attics, and garages with raccoon roundworm (*Baylisascaris procyonis*) that can be fatal to humans (Mackenstedt et al. 2015). The transmission of zoonotic parasites from generalist species can cause significant risk to public health and may be exacerbated by anthropogenic food in urban landscapes (Werner and Nunn 2020). Food subsidies could increase parasite transmission because they cause hosts to aggregate, thereby increasing contact rates and exposure (Becker et al. 2018a; Moyers et al. 2018) or by greater susceptibility to infection caused by poor nutrition (Becker et al. 2018b). Generalists are more likely to use these anthropogenic resources in areas frequented by people, thereby creating potential routes of disease exposure for humans (Saito and Sonoda 2017; Becker et al. 2018b).

Food subsidies and other aspects of wildlife diets in urban areas potentially contribute to the zoonotic risks associated with *Echinococcus multilocularis*, a zoonotic, trophically-

transmitted tapeworm of emerging concern in Canada because of an increase in human infections (Massolo et al. 2019; Houston et al. 2021). Typical transmission occurs when rodent intermediate hosts (e.g., voles, *Microtus* spp., *Myodes* spp.) are consumed by canid definitive hosts (e.g., foxes, *Vulpes* spp.; coyotes, *Canis latrans*), within which *E. multilocularis* matures in the intestine and sheds eggs into the environment via canid feces (Deplazes and Eckert 2001). Humans can become infected by accidental ingestion of parasitic eggs, which causes human alveolar echinococcosis (Craig et al. 2004). The resultant hydatid cysts in human tissues can remain asymptomatic for 5-15 years but are fatal without treatment (Eckert and Deplazes 2004). Multiple genetic strains of *E. multilocularis* infect wild canids throughout the northern hemisphere (Snabel et al. 2020), but the native North American strain rarely infects humans (Massolo et al. 2014). However, a variant of a European strain, first reported in North American wildlife in 2012, appears to be more virulent for people and is now widespread throughout Canada (Gesy et al. 2013a; 2014). The new variant is the causative agent of likely locally acquired human infections, the majority of which are reported from Alberta (Massolo et al. 2019; Houston et al. 2021). In that province, *E. multilocularis* is most commonly found in coyotes, which are abundant in urban areas. Among tested coyotes, the parasite appears to be moderately common in the City of Calgary (~25% prevalence), which was similar to the prevalence in rural areas (Catalano et al. 2012) and other Canadian provinces (Gesy et al. 2013a, 2014; Kotwa et al. 2019). However, the parasite appears to be much more common in the City of Edmonton, (~60% prevalence: Catalano et al. 2012; Luong et al. 2018; Sugden et al. 2020). Urban animals from Edmonton had a 50% higher prevalence of the parasite, compared to rural animals in the surrounding area. These urban coyotes also consumed more anthropogenic food and less protein (Sugden et al. 2020), suggesting that diet may contribute to their unusually high infection rates.

Two diet-related mechanisms potentially contribute to the high prevalence of *E. multilocularis* in Edmonton's coyotes; greater exposure through access to infected rodents and higher susceptibility through consumption of nutrient-poor anthropogenic food. Greater exposure may occur because trophic parasite transmission is generally and intrinsically associated with the consumption of intermediate hosts by definitive hosts, which is likely the reason that *E. multilocularis* prevalence typically parallels the availability of rodents (Guerra et al. 2014; Baudrot et al. 2016). Studies of urban foxes have shown that *E. multilocularis* infections generally decline in urban environments (Stieger et al. 2002; Fischer et al. 2005; Raoul et al. 2015), which is attributed to both a lack of suitable rodent habitat and less predatory reliance on susceptible intermediate hosts (Hegglin et al. 2007; Robardet et al. 2008). However, *E. multilocularis* may be especially prevalent in Edmonton's coyotes because the city contains an abundance of rodent habitat and coyotes appear to specialize on rodents (Murray et al. 2015a), including intermediate hosts. Rodent habitat in Edmonton stems from a large, contiguous river valley of over 7400 ha (City of Edmonton 2008), abundant natural and naturalized greenspaces (City of Edmonton 1994, 2008), unusually low human density with vast areas of undeveloped land, and a partial ban, since 2003 on the use of pesticides and herbicides (City of Edmonton 2004, 2019). These characteristics create abundant habitat for intermediate host species of *E. multilocularis* that include meadow voles (*Microtus pennsylvanicus*), southern red-backed voles (*Myodes gapperi*), and deer mice (*Peromyscus maniculatus*; Liccioli et al. 2013; Romig et al. 2017). Furthermore, whereas urban foxes tend to have a wide diet breadth with less reliance on rodents (Hegglin et al. 2007; Robardet et al. 2008), the diets of urban coyotes include a large proportion of rodents and specifically, highly-susceptible voles (Liccioli et al. 2015; Murray et

al. 2015a; Poessel et al. 2017). These factors combine to make it plausible that coyotes in Edmonton have unusually frequent exposure to *E. multilocularis* via infected rodents.

A second way that diet could contribute to the high prevalence of *E. multilocularis* in urban coyotes is via susceptibility caused by nutrient-poor food, which could occur through access to hyper-abundant, but low-quality anthropogenic waste. Consumption of anthropogenic food by wildlife has been associated with increased parasitic infections due to suppressed immune function (Cummings et al. 2020), poor nutrition (Ezenwa 2004), and aggregation of infected hosts (Murray et al. 2015b). Previous work in Edmonton showed that coyotes aggregate at large deposits of compost, which contain immune-suppressing mycotoxins that would exceed federal standards for animal feed if even small amounts were consumed daily (Murray et al. 2016). Additionally, protein-poor, anthropogenic diets appear to alter the microbiome and reduce the body condition of coyotes in the Edmonton area (Sugden et al. 2020), effects that might generally increase susceptibility to infection (Corbin et al. 2008). Further, coyote scats collected from large, unsecured compost piles were 10 times more likely to contain unspecified tapeworm (Taeniidae) eggs (Murray et al. 2016). However, it is unknown if the coyotes that deposited tapeworm eggs exhibited high infections because they consumed compost and experienced detrimental health effects, or because they were exploiting an abundance of rodents that were also attracted to compost (Hansen et al. 2020). The dietary resources that are most likely to attract infected coyotes to spaces of human use are currently unknown, which limits the ability to develop strategies to reduce the likelihood of human exposure, such as compost securement, reduction in rodent aggregation, and wildlife feeding policies.

The objective of this study was to determine whether consumption of one or both of rodents and anthropogenic food influenced the prevalence of *E multilocularis* in urban coyotes.

Because highly infected coyotes presumably deposit more eggs in their feces (Kapel et al. 2006), we also explored how these diet components influenced infection intensity. With a sample of animals that partially overlapped that of Sugden et al. (2020), we used coyote carcasses sourced from within and outside of the City of Edmonton to directly compare diet to the intestinal presence and intensity of *E. multilocularis*. We measured coyote diet in two ways; via stomach contents that reveal items consumed in the preceding few hours (Balestrieri et al. 2011) and via stable isotopes of carbon and nitrogen that represent longer-term consumption of anthropogenic food and prey, respectively (Newsome et al. 2010). Because others have shown that the prevalence and/or intensity of *E. multilocularis* infection in definitive hosts may vary with age (Yimam et al. 2002; Fischer et al. 2005; Liccioli et al. 2012; Kotwa et al. 2019) we accounted for this while testing our two main hypotheses.

Materials and Methods

Sample collection and necropsy

Coyote carcasses were sourced from areas within ("urban") and outside ("rural") the City of Edmonton between 2017 and 2020 (Appendix 1). Carcasses were donated by outside sources (urban: City of Edmonton Animal Care and Control Centre and Edmonton Police Service; rural: Animal Damage Control Inc.) following one of population management, roadkill, or conflict with humans. Prior to necropsy, the carcasses were stored at -80°C for at least five days (>120 hours) to neutralize the zoonotic risk of *E. multilocularis* (Veit et al. 1995). The majority of carcasses were shared with Sugden et al. (2020) and necropsy procedures were consistent across studies. We measured body size (i.e., mass, length, girth) and calculated the kidney fat index

(KFI) as a metric for body condition (Huot et al. 1995). We then removed the anterior lower jaw for ageing, the small intestine for parasite analysis, the outer hind claws for stable isotope analysis, and the stomach for diet analysis.

The age of each coyote at death was determined by counting the cementum annuli of the lower teeth, which was completed by Sugden et al. (2020). Teeth were fixed, decalcified, sectioned, and stained following published methods (Stewart et al. 1996), and the annuli were counted at 2.5x magnification (Linhart and Knowlton 1967). We calculated age to the nearest month by assuming each coyote had a birth date of May 1. See Appendix 2 for the distribution of coyote ages for each location.

E. multilocularis detection and quantification

To test our hypotheses about the relationship between diet and *E. multilocularis* infection, we used molecular methods to detect parasite presence and morphological worm counts to determine infection intensity. Adapting the Gesy et al. (2013b) method, we divided the small intestine into four equal lengths and removed two ~0.5g mucosal scrapings from the posterior end of the second quarter (Karamon et al. 2020) for molecular testing. To allow for a spatially paired molecular and morphological detection, we rinsed and filtered each quarter of the intestine separately and did not combine them as in the original protocol (Gesy et al. 2013b). Once filtered, the contents of each intestinal quarter were stored individually in 70% ethanol to create four 60mL samples. The second quarter sample was further divided into 25% aliquots (15mL) for morphological parasite detection and quantification.

In collaboration with IDEXX Laboratories, Inc., a commercially available real-time polymerase chain reaction (qPCR) assay (IDEXX Laboratories, *Echinococcus* RealPCR™

Panel) was used to detect the presence of *E. multilocularis* in the small intestine. Intestinal samples were stored in sterile microtubes at -20°C until transported to the commercial laboratory (IDEXX Laboratories, Inc., West Sacramento, CA). Real-time PCR was performed using the LightCycler 480 system (Roche) with proprietary forward and reverse primers and hydrolysis probes. The *E. multilocularis* qPCR assay targets a ribosomal RNA sequence between the Cox 1 and Cox 2 genes. Real-time PCR was performed with seven quality controls, including PCR-positive controls, PCR-negative controls, negative extraction controls, DNA preanalytic quality control targeting the host 18S rRNA gene complex, RNA preanalytic quality control targeting the host 18S rRNA gene complex, an internal positive control spiked into the lysis solution, and an environmental contamination monitoring control.

We conducted morphological worm counts to assess the intensity of *E. multilocularis* infection by observing one 25% aliquot of intestinal contents for each carcass under a dissection microscope. All parasitic scoleces were morphologically identified and counted (Stock 2017; Thompson 2017). For three samples that contained >1,000 worms in the first 1mL, we estimated the number of worms in the remaining sample by multiplying the count by the full volume (i.e., 15mL). All scoleces present were morphologically identified as *E. multilocularis*, but due to the potential for coinfection with the related zoonotic *Echinococcus canadensis* (Santa et al. 2018*)*, we only included counts for carcasses that had molecular confirmation of the presence of *E. multilocularis*.

Due to the high sensitivity of the qPCR test, some samples tested positive for *E. multilocularis* DNA but contained no visible worms. We attributed this to either the presence of an exceptionally light infection (e.g., single worm) or remnant DNA from a new or recently concluded infection. To distinguish animals with active infections, we retained our designation

of coyotes as infected or uninfected based on qPCR results, but also added an additional response variable of "biologically active" (hereafter bioactive) infections, which we defined as including both of confirmed molecular detection of *E. multilocularis* and visual detection of *Echinococcus* spp.

Coyote diet analysis

We used measurements of short- and long-term diet to test our hypotheses that relate diet to *E. multilocularis* infection. Short-term diet was assessed using stomach contents to determine the last meal eaten prior to death. To overcome biases associated with the method (e.g., varied digestibility of contents, overemphasizing recently eaten prey), we also evaluated longer-term diet (i.e., 2-5 months; Bearhop et al. 2003) with stable isotope values of nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) determined from claw samples (full method: Sugden et al. 2020). In general, greater $\delta^{15}N$ values are representative of protein (i.e., prey) consumption and $\delta^{13}C$ is associated with corn-based (i.e., anthropogenic) food (Newsome et al. 2010). The claw samples were rinsed in 2:1 chloroform:methanol solution three times, dried for 5 days at 37 °C, and 5mm of the claw tip was manually ground (Sugden et al. 2020). A 1.5mg subsample of the homogenized claw was combusted with a Vario Pyrocube and then analyzed with an Isoprime Vision Mass Spectrometer at the Biogeochemical Analytical Service Laboratory (Dept. of Biological Sciences, Univ. of Alberta).

To explore short-term diet, we analyzed the stomach contents of each animal by first measuring the total stomach content volume (mL) via water displacement (Wolfert and Miller 1978). Contents were then rinsed, sorted into 10 mutually exclusive diet components, identified to the lowest possible taxonomic level, and each component was quantified by volumetric

displacement. The diet components included prey items (ungulates, rodents, meso-mammals, birds), vegetation (herbaceous, woody), insects, native fruit, and anthropogenic food, which we defined as human-sourced items that originated from human food production or waste processes. We further divided anthropogenic food into "digestible anthropogenic food" that we defined as items of at least minimal nutritive value (e.g., apples, dog kibble, chicken bones, birdseed) and "indigestible anthropogenic food" that included non-nutritive trash items such as food wrappers, plastic, and scraps of leather. Trace components with volumes less than our minimum measurable sensitivity (0.5mL) were assigned a value of 0.1mL.

Statistical analysis

All statistical analyses were conducted using R version 4.0.4 (R Core Team 2021). *E. multilocularis* detection by molecular and bioactive metrics was analyzed as present/absent binary variables. To test the effect of diet on the intensity of infection, we removed samples with a negative qPCR result and analyzed only those that were confirmed infected by molecular detection. This allowed us to increase the sensitivity of the infection intensity analysis and avoid redundancy because the absence of infection was previously tested with the binary variables. To address outlying values of worm counts, we truncated this variable at 20,000, added a pseudo-count of 1 to zero values, and log-transformed the counts. A small number of cementum age values were missing, so a linear regression model was built with the remaining physiological measurements (i.e., mass, length, girth, KFI) and used to interpolate the missing values. To compare differences in diet and infection by functional age classes of juvenile and adult, we added a binary "age group" variable based on the 50^{th} percentile (1.78 years), which corresponded with the approximate age of reproductive maturity in female coyotes (Green et al.

2002); individuals were included in either the "young age group" (<1.78 years) or "old age group" (>1.78 years) and no individuals were precisely 1.78 years old. We also calculated Shannon's diversity index (H) for each stomach's contents to provide a single measure of diet diversity (Amundsen and Sanchez-Hernandez 2019).

Prior to testing our hypotheses with multivariable models, we used univariate tests to compare the effects of diet components (volume of stomach content components) and diet composition ($\delta^{15}N$ and $\delta^{13}C$ stable isotopes, Shannon's H) on molecular and bioactive infection status, infection intensity, location (urban/rural), and age group (young/old: 50^{th} percentiles of 1.78 years). To analyze diet metrics by molecular and bioactive infection status, univariate logistic regression models were constructed, and significance was determined with likelihood ratio tests. Diet metrics were related to infection intensity by Spearman's rank correlation. To determine if the mean diet metrics varied by location and age group, Kruskall-Wallis tests were performed. Lastly, diet metrics were related to the cementum age (years) with Spearman's rank correlation. For these descriptive analyses, we considered variables with $P<0.1$ significant.

In order to determine how the consumption of rodents and/or anthropogenic food corresponded with molecular and bioactive *E. multilocularis* infection status and infection intensity, we built regression models for each response variable. Predictor variables were selected for inclusion in the final models with a two-step, purposeful selection method modified from Hosmer et al. (2013). Each predictor was scaled to mean 0 and unit variance and tested in a univariate regression model, which was then assessed for significance with a likelihood ratio test. Predictors that were found to be liberally significant ($p < 0.25$) in the univariate tests were then combined into subsequent multivariable models to determine the overall best predictors of infection.

We determined the focal diet components (volume of rodents, digestible anthropogenic food, indigestible anthropogenic food), cementum age (years), carcass location (urban/rural), and coyote sex that predicted *E. multilocularis* infection with univariate logistic regression (for molecular and bioactive infection) and negative binomial regression (for infection intensity). We assessed the significance of each explanatory variable in univariate models with likelihood ratio tests. Body size and condition were excluded from further consideration due to a correlation with cementum age (Spearman's $r > 0.6$). Early observation (Table 1, 2) revealed that both location and cementum age were significant predictors of molecular and bioactive infection status, so we also tested the volumes of rodents and anthropogenic food in interactions with these two variables. To determine the best predictors of *E. multilocularis* prevalence and intensity, all explanatory variables and interaction terms that had liberal significance ($p < 0.25$) in univariate tests were then combined into multivariable regression models. The fit of the resultant global model was assessed with Nagelkerke's pseudo R^2 and area under the receiver operating characteristic curve (reviewed in Hosmer et al. 2013). The global models were then assessed using an all-subsets approach; those with a delta Akaike Information Criterion (ΔAIC) < 2 were averaged after standardizing predictor coefficients by their partial standard deviation and adjusting them based on model weight (Cade 2015). The relative importance of predictors was assigned by summing the weights of the pre-averaged top models in which the predictors appeared.

Results

E. multilocularis prevalence and infection intensity

We obtained and studied 112 coyote carcasses generated by rural population management (n = 66), roadkill in the City of Edmonton (n = 35), and conflict with humans (urban: n = 6; rural = 5). We detected *E. multilocularis* in 70% (78/112) of carcass intestines via molecular methods and 48% (54/112) via worm detections signifying biologically active infections. The mean parasite count was 1312 ± 4115 SD (range: 0 - 55,000), but three individuals had parasite counts >10,000. These represented only 5% of bioactive infections (3/54), but 72% of all parasites we counted. In univariate tests, infection prevalence differed significantly by both location and age group, but not sex (Table 1) and infection intensity negatively correlated with cementum age (Table 2). Infection prevalence was 27% greater in urban coyotes by both detection methods (molecular: 80% vs. 63%; bioactive: 56% vs. 44%). Although infection intensity was 4 times greater in urban than rural coyotes (Table 1), high variance prevented statistical significance. Compared to coyotes in the older age group (upper 50^{th} percentile, >1.78 years), coyotes in the younger age group (<1.78 years) had 35% greater molecular prevalence (80% vs. 59%), 69% greater bioactive prevalence (61% vs. 36%), and 6.2 times greater mean infection intensity (Table 1). By molecular prevalence, uninfected coyotes were approximately 50% older (3.25 ± 2.37 years vs. 2.14 ± 2.23 years) and 66% older by bioactive prevalence (3.06 ± 2.52 years vs. 1.84 ± 1.90 years; Table 2). There were negligible differences in infection prevalence by sex, but male coyotes had a mean infection intensity 26% greater than females (Table 1).

Overview of coyote diet

Measured with Shannon's H, the diversity of stomach contents was similar for coyotes compared by infection status for molecular and bioactive detections and did not correlate with

infection intensity (Table 2). Food was present in 91% (102/112) of coyote stomachs and the volume in each ranged from 0 to 2560.1 mL (mean ± SD = 179.9 ± 331.7 mL). Summed across all coyote stomachs, the proportion of food types by volume included 53% ungulates, 14% rodents, 13% meso-mammals, 13% digestible anthropogenic food, 3% indigestible anthropogenic food, 2% birds, 1% vegetation, 0.4% native fruit, and 0.05% insects with little variation between samples divided by infection status. When infection status was measured molecularly, univariate tests revealed that insects exhibited greater volumes in infected coyote stomachs (Table 2a; Figure 1a). When infection status was measured as bioactive worm counts, infected coyote stomachs contained lower volumes of vegetation (Table 2b; Figure 1b). While not statistically significant, birds exhibited 7.3 times and 3.5 times greater volumes in infected coyote stomachs by molecular and bioactive detection methods, respectively. Additionally, the volume of birds positively correlated with worm counts (Table 2c). Stable isotopic values of $\delta^{15}N$ and $\delta^{13}C$ neither differed by infection status nor correlated with infection intensity (Table 2).

Separate from infection status, diet composition differed significantly by location and age group and several diet components correlated with cementum age (Table 3, Figure 1c,d). Rural coyote stomach contents consisted of 88% prey (77% ungulates, 14% meso-mammals, 6% rodents, 3% birds) and 10% anthropogenic food (64% digestible, 36% indigestible), whereas urban coyote stomachs contained 60% prey (74% rodents, 23% meso-mammals, 1% birds, 0.7% ungulates) and 38% anthropogenic food (99% digestible, 0.6% indigestible). Statistically, urban coyote stomachs contained lower volumes of ungulates, but more insects. In rural coyotes, the volume of birds positively correlated with indigestible anthropogenic food. Urban coyotes also had lower values of $\delta^{15}N$ and higher values of $\delta^{13}C$ (Table 3a). Significant diet differences were

also detected when compared to age (Table 3b,c; Figure 1d). Shannon's H correlated with cementum age (Table 3c) and coyotes in the young age group (<1.78 years) had significantly more diverse diets (Table 3b). The stomach contents of both age groups contained approximately 80% prey. However, coyote stomachs of the older age group (>1.78 years) relied most on ungulates (80% ungulates, 11% meso-mammals, 9% rodents, <0.1% birds), whereas coyote stomachs of the younger age group (<1.78 years) contained greater proportions of other prey types (40% ungulates, 24% meso-mammals, 29% rodents, 6% birds) and a greater proportion of anthropogenic food (22% vs. 13%). Furthermore, cementum age positively correlated with ungulates and negatively correlated with rodents, birds, vegetation, native fruit, and insects, which further supports increased diet diversity among young coyotes (Table 3c). Lastly, cementum age positively correlated with $\delta^{15}N$ and $\delta^{13}C$ stable isotopic values.

E. multilocularis infection, rodents, and anthropogenic food

We hypothesized that the presence or intensity of *E. multilocularis* infection in coyotes would result from greater exposure to infected prey or greater susceptibility to infection, which we predicted would correspond with the volume of rodents and anthropogenic food, respectively. The volume of rodents did not differ by infection status for either molecular (Table 2a, Figure 1a) or bioactive metrics (Table 2b, Figure 1b) and marginally, negatively correlated with infection intensity (Table 2c). By molecular detection, digestible anthropogenic food exhibited 2.3 times greater volume in uninfected coyote stomachs and 53% greater volume in coyotes without bioactive infections. Conversely, the volume of indigestible anthropogenic food was greater in infected coyote stomachs; 30% greater by molecular detection and 2.7 times greater by bioactive detection. Neither type of anthropogenic food correlated with infection intensity. These

focal diet components also varied by location (Table 3a; Figure 1c) and urban coyote stomachs exhibited a 3.8 times greater mean volume of rodents and 2.6 times greater mean volume of digestible anthropogenic food, whereas rural coyotes were the primary consumers of indigestible anthropogenic food. Furthermore, coyotes in the younger age group (<1.78 years) exhibited 86% greater mean volume of rodents and 36% greater mean volume of indigestible anthropogenic food than coyotes in the older age group (>1.78 years; Table 3b; Figure 1d) and both diet components negatively correlated with cementum age (Table 3c).

Univariate variable selection. To further explore the effects that intermediate hosts (rodents) and anthropogenic food had on infection, we examined them as interactions with both location and cementum age because of the importance of these variables for predicting both molecular and bioactive infections (Table 1, 2). This exploration revealed that the presence and intensity of infection were best predicted by multiple three-way interactions with diet, location, and cementum age (Appendix 3). These relationships, in addition to the differences in diet composition by location (Table 3a; Figure 1c), suggested that the relationships between infection, diet, and age behaved differently in the urban and rural locations. Therefore, we assessed the effects of diet and age on infection for each location separately (Table 4, Figure 2).

Among urban coyotes, cementum age predicted infection only when interacting with diet variables. Lower cementum age and greater volumes of rodents predicted bioactive infections (Figure 2d, Table 4b) and intensity (Figure 2g, Table 4c). Similarly, lower cementum age and greater volumes of digestible anthropogenic food in the stomachs predicted the presence of molecular (Figure 2b, Table 4a) and bioactive (Figure 2e, Table 4b) infections. In urban coyotes, increasing cementum age and volumes of these diet components predicted lower likelihood of

infection and lower infection intensity. Descriptively, infected coyotes in the older age group (>1.78 years) had lower volumes of rodents in their stomachs (Figure 3a). Volumes of digestible anthropogenic food were lower in the stomachs of infected coyotes in the older age group and uninfected coyotes in the younger age group (<1.78 years).

In the rural sample, lower cementum age independently predicted the presence of molecular and bioactive infection as well as the intensity of infection, though diet interactions were also evident (Table 4). Lower cementum age combined with greater volumes of rodents predicted the presence of molecular infections (Figure 2a). Greater volumes of digestible anthropogenic food were present in uninfected rural coyote stomachs (Figure 3b) and predicted a lower likelihood of detecting *E. multilocularis* infection with either detection method (Figure 2b,e). Greater volumes of digestible anthropogenic food also predicted less intense infections, especially as cementum age increased (Figure 2h). Lastly, greater volumes of indigestible anthropogenic food predicted the presence of bioactive infections (Figure 2f) but corresponded with lower infection intensity in coyotes with lower ages (Figure 2i).

Multivariable regression models. To identify the combination of predictors for each location that best predicted each of the response variables (molecular, bioactive, and intensity), we constructed global models with the liberally-significant variables (P<0.25) from our univariate tests (Table 4). For urban coyotes (Figure 4a), molecular infections had no significant predictors of (Table 5a). For urban bioactive infections (Table 5b) the best predictor was an interaction between lower quantities of rodents and lower cementum age, which supported Figure 3a that showed that infected coyotes were in the younger age group (<1.78 years) and uninfected coyotes in the older age group (1.78 years) ate more rodents. Additionally, there was a marginal

effect (within 90% C.I.) of lower volumes of rodents in urban coyotes independent of cementum age. Similarly, urban infection intensity (Table 5c), was predicted by lower volumes of rodents as well as lower rodent volume combined with lower cementum age. Further, there was an additional marginal effect of decreasing cementum age independent of interactions (Figure 4a). Evaluation of the final global models with Nagelkerke's pseudo R^2 (Table 6) revealed that the molecular and bioactive models demonstrated good fit (R^2>0.2), and the intensity of infection model demonstrated excellent fit (R^2>0.4; McFadden 1979).

For the rural sample (Figure 4b), the best predictor of the presence of molecular infection (Table 5a) was lower cementum age. For rural bioactive infections (Table 5b), lower cementum age best predicted infection with a marginal effect of lower volume of digestible anthropogenic food. For rural infection intensity (Table 5c), both of lower cementum age and lower volumes of digestible anthropogenic food best predicted infection intensity (Figure 4b). Rural coyotes with bioactive infections consumed very low quantities of digestible anthropogenic food and infected coyotes in the older age group (>1.78 years) consumed almost none (Figure 3b). Evaluation of the final global models with Nagelkerke's pseudo R^2 (Table 6) revealed that the molecular model demonstrated good fit (R^2>0.2), and the bioactive and intensity of infection model demonstrated excellent fit (R^2>0.4; McFadden 1979).

Discussion

Understanding the dietary drivers of *E. multilocularis* infection in urban coyotes is important for mitigating the spread of this increasingly common zoonosis and protecting human health. We hypothesized that coyote diets would relate to increased *E. multilocularis* infection via two potential mechanisms; greater consumption of rodent intermediate hosts that increased

exposure to the parasite, and/or consumption of poor-quality anthropogenic food that increased overall susceptibility to infection. Overall, we found few direct, short-term diet effects, measured with stomach contents, or long-term ones, measured with stable isotopes, between infected and uninfected coyotes. When we divided coyotes by urban and rural locations, we found effects of both rodents and anthropogenic food, but with further variation between coyote age and the three measures of infection. In urban coyotes, the presence of bioactive *E. multilocularis* infection and infection intensity increased for younger coyotes that ate rodents, but it simultaneously declined for older coyotes that ate rodents. A similar pattern occurred for molecular and bioactive infection for digestible anthropogenic food wherein the likelihood of being infected with *E. multilocularis* increased for younger coyotes that ate rodents and decreased for older coyotes that ate rodents. In rural coyotes, the presence and intensity of *E. multilocularis* infection was most strongly predicted by younger age, with a lesser effect of decreased consumption of digestible anthropogenic food. Taken together, our results suggest that young age is the most important contributor to the presence and intensity of *E. multilocularis* infection in coyotes. Although diet composition appeared to influence the likelihood or intensity of infection, it did so in complex ways that differed by location and may relate to several variables that we could not measure, such as aggregation by coyotes or their prey.

Our estimates of the prevalence of *E. multilocularis* in Edmonton support estimates from other studies of 53% (Sugden et al. 2020) to 65.2% (Luong et al. 2018) and extend, with a partially shared sample, the observation by Sugden et al. (2020) that prevalence is higher in Edmonton than the surrounding rural area. Our use of qPCR confirmed *E. multilocularis* presence in significantly more coyote intestines than Luong et al. (2018) and Sugden et al. (2020; 80% vs. 53-65%) presumably because PCR-based detection has a sensitivity >94% (Eckert

2003) and we targeted for analysis material extracted from the location in the intestine where *E. multilocularis* was most expected to occur (Thompson 2017; Karamon et al. 2020). Our more conservative bioactive detection was within the previously reported prevalence range. However, we may have not detected very light infections because, although morphological identification is considered the "gold standard" method (Eckert 2003), maximum sensitivity depends on analysis of the full intestinal contents, whereas we only viewed a subsample. Comparison by location revealed an infection prevalence was 27% greater in the urban sample, a smaller difference than the 50% greater urban prevalence reported by Sugden et al. (2020). Although some samples were shared across studies, this discrepancy is likely due to our 37% larger urban sample size and use of a more sensitive molecular test (qPCR vs. conventional PCR) conducted on material extracted from a lower section of the intestine (jejunum vs. duodenum; Karamon et al. 2020). Several of the animals that Sugden et al. (2020) classified as negative were positive in our more sensitive test. However, regardless of detection method, the prevalence of *E. multilocularis* in Edmonton's coyotes is considerably higher than other Canadian locations (Table 7), except Saskatchewan and a national park in Manitoba and much higher than the only two other cities tested (Calgary, AB: 21-29%; Winnipeg, MB: 7%).

Coyotes are generalists and their diets reflected the wide breadth of forage available in heterogeneous landscapes, including mammals, birds, anthropogenic food, vegetation, fruit, and insects. Similar to other studies, the stomach contents of coyotes were diverse and mammalian prey was the most important component (McVey et al. 2013; Latine and Giuliano 2017; Metzger et al. 2017), but Guislain et al. (2008) detected a greater dependency on meso-mammals inconsistent with our findings. The composition of diets varied by location and, in agreement with Murray et al. (2015a) and Poessel et al. (2017), urban coyotes demonstrated heavier reliance

on anthropogenic food and rodents, with less dependence on ungulates. Although birds constituted only 2% of the volume of food consumed across all the coyotes we measured, 73% of the volume in this category was consumed by infected, young, rural coyotes. The volume of birds also correlated with infection intensity. Birds are not competent intermediate hosts for *E. multilocularis* and likely do not play a role in transmission (Moreira et al. 1978), but it may be indicative of scavenging (Hinton et al. 2017; Latine and Giuliano 2017). Positive effects of birds and indigestible anthropogenic food on infection status or intensity may have reflected correlations with young or undernourished animals.

We predicted that greater volumes of rodents in coyote stomachs would contribute to a greater likelihood of infection with *E. multilocularis* due to the requirement of rodent consumption for parasite transmission (Rausch 1995). We found a complex pattern in urban coyotes, for which rodent consumption increased bioactive infection status and intensity for young animals but decreased it for older coyotes. This finding opposes a previously reported positive correlation between rodents and infection intensity in foxes (Robardet et al. 2008), but might be explained by acquired immunity (Torgerson 2006) stemming from prior reliance on rodent prey in urban environments (Poessel et al. 2017). It is unlikely that canids become entirely immune to infection by *E. multilocularis* (Torgerson 2006), but studies in domestic dogs (*Canis lupus familiaris*) experimentally reinfected four times with *E. multilocularis* showed a tremendous reduction in infection intensity with ~90% fewer adult worms than never-exposed individuals (Kouguchi et al. 2016; 2020). In our study, this type of immunological defence may be plausible because though young coyotes consumed great quantities of rodents, they also had high *E. multilocularis* infection prevalence and high infection intensities. By contrast, older urban coyotes that consumed rodents were generally uninfected even though rodents have been

documented as the most important prey type in urban environments (Liccioli et al. 2015; Murray et al. 2015a; Poessel et al. 2017).

Our second hypothesis of diet's contribution to infection status is that a poor quality, protein-poor diet might worsen coyote condition (Sugden et al. 2020) and nutrient-poor diets might increase coyote susceptibility to parasites generally (Murray et al. 2016). We expected to find a positive correlation between consumption of digestible anthropogenic food and infection, but that trend was only detected in young, urban coyotes. As for rodents, older urban coyotes that consumed digestible anthropogenic food were less likely to be infected. A plausible interpretation for these results is that urban coyotes that forage at compost sites (as described by Murray et al. 2016) consume high quantities of both rodents and anthropogenic food but acquire immunity to biologically active infections over time (Torgerson 2006). Alternatively, rural coyotes that consumed digestible anthropogenic food were overall less likely to be infected, which may suggest that habitual reliance on anthropogenic food combined with less reliance on rodent prey in rural environments decreases the likelihood of infection by trophic parasites because there are fewer opportunities for parasitic exposure. This trend appears to have occurred for *E. multilocularis* in urban foxes (Hofer et al. 2000; Robardet et al. 2008) and for different trophic parasites in urban ring-billed gulls (*Larus delawarensis*; Aponte et al. 2014).

We found mixed support for our hypotheses, but our findings underscore the variability with which coyotes exploited anthropogenic resources, which likely has equally variable impacts on their health and likelihood of parasitism (Werner and Nunn 2020). For example, mechanical damage to intestines could explain why we found weak evidence that consumption of indigestible anthropogenic food by rural coyotes corresponded to greater *E. multilocularis* prevalence. Research on the effects of foreign body ingestion by land mammals is lacking, but in

avian and marine systems, plastic intake has been associated with poorer nutrition and body condition, exposure to immuno-compromising toxins, and intestinal inflammation caused by physical trauma. All of these factors might contribute to increased susceptibility to disease (Puskic et al. 2020).

Young age was the strongest predictor of the presence and intensity of *E. multilocularis* infection overall; it was especially important to prevalence in rural coyotes. Our data agree with previous reports of higher parasite prevalence among young coyotes (Liccioli et al. 2012; Kotwa et al. 2019) and greater infection intensities in young foxes (Yimam et al. 2002; Fischer et al. 2005). However, some studies found no effect of age on infection status (Catalano et al. 2012; Luong et al. 2018). The higher prevalence of infection we found in young coyotes further supports a hypothesis (above) that canids become less susceptible to *E. multilocularis* with repeated exposures (Torgerson 2006). That hypothesis is amplified by our findings that 73% of coyotes with infection intensities >1000 were less than 1 year old and the fact that the three individuals with infection intensities >10,000 were less than 6 months old.

Our study had several limitations for exploring how rodents or anthropogenic food in the recent diet might contribute to parasite presence or intensity and characteristics that may have inflated our estimate of parasite prevalence. First, our coyote carcasses were collected opportunistically and may not represent the population of coyotes and their recent diets in either Edmonton or the surrounding area. Second, our sample may have contained a younger age distribution than is representative of the population. Greater susceptibility of young animals to traps, roadkill, and conflict likely inflated our population-wide measures of parasite prevalence and intensity (Van Deelen and Gosselink 2006; Kreling et al. 2019), although a previous sample of road-killed coyotes in Edmonton was not biased toward younger animals (Murray and St.

Clair 2015). Our molecular measure of parasite prevalence may also have been further inflated due to the high sensitivity of qPCR, which may have detected fragments of *E. multilocularis* DNA that would not convey bioactive infection or capacity to transmit viable eggs. Conversely, morphological assessment by us and others (Robardet et al. 2008; Gesy et al. 2013b) necessarily examines a subsample of the intestinal contents and may have missed very light infections. Without the molecular verification we used, worm counts may also fail to distinguish between *Echinococcus* spp. in coyote intestines (Santa et al. 2018). Most important for the testing of our hypotheses about diet contributions to infection status is that stomach contents may not reflect longer-term diet composition. Because *E. multilocularis* requires up to 60 days to mature in coyote intestines (Liccioli et al. 2015), stomach contents might have been quite different at the time of parasitic exposure. Unfortunately, our longer-term measures of diet via stable isotope analyses were too coarse to reflect specific components, such as the distinction between rodents and other prey or many components of anthropogenic food.

Despite these limitations, our results have some implications for the management of urban coyotes in relation to human safety. Foremost among these is that higher rates of infection with *E. multilocularis* in young coyotes means that their use of resources and habitat identifies the locations where eggs are more likely to be shed in the environment and, consequently, cause potential exposure for people. Egg deposition in feces is substantial because each adult worm can shed 27-114 eggs per oviposition event for up to 90 days (Kapel et al. 2006). This means the most heavily infected animals might produce millions of eggs over the infection term, which can persist in the environment for over a year (Veit et al. 1995; Thevenet et al. 2005). Interestingly, the three 'super spreaders' (i.e., > 10,000 parasites counted) in our study were urban, road-killed, and young with stomachs that were either empty or contained signs of scavenging (e.g., maggots,

nylon strap). Dispersing young coyotes are highly mobile (Kolbe and Squires 2004; Sasmal et al. 2019), but motility may also vary with condition. In Edmonton, GPS-collared coyotes with mange made greater use of residential areas than healthy coyotes (Murray et al. 2015b) and a similar tendency may attend starving young coyotes. The extended asymptomatic period of alveolar echinococcosis creates challenges in confirming sources of human exposure (Massolo et al. 2019; Houston et al. 2021). Therefore, the most logical and available form of mitigation for the risk of transmission of the tapeworm to people is public education that alerts citizens to the presence of infected wildlife, the need to wash things that potentially come in contact with eggs (e.g., hands, toys, tools, garden produce), and the value of deworming dogs, especially if they are prone to consuming rodents (Deplazes et al. 2011). Citizens should identify and secure sources of food and shelter that could attract coyotes to residential areas. Bylaws that prohibit intentional feeding of wildlife, including coyotes, may also be needed to limit use by coyotes of residential areas. Other options to control coyotes or their parasites are likely less effective. Although some citizens call for the removal of hosts (Hegglin et al. 2015), culling of coyotes does not appear to be an effective means of reducing population sizes (Mitchell et al. 2004; Mosnier et al. 2008; Morin and Kelly 2017). Attempts to reduce *E. multilocularis* prevalence with fox culling did not reduce the fox population size and resulted in a 37% increase in parasite prevalence because a high proportion of infected young individuals dispersed into the unoccupied territory (Comte et al. 2017). Similarly, anticoagulant poisons used to limit rodent populations degrade the immune system of predators and could increase overall susceptibility to disease (Serieys et al. 2018), potentially including this parasite. In European foxes, *E. multilocularis* has been partially controlled by treating foxes with medicated praziquantel baits (Tackmann et al. 2001), but this treatment is difficult to achieve in wild animals (König et al. 2008; Comte et al. 2013). Evidence

that the more virulent variant of a European strain of *E. multilocularis* is now widespread in Canada (Gesy et al. 2014) and that it has infected over a dozen people in Alberta (Massolo et al. 2019; Houston et al. 2021) speaks to the need for more research on how best to limit both rates of infection in wildlife and the sites or activities that increase transmission to humans.

Tables and Figures

Table 1. *Echinococcus multilocularis* a) molecular and b) bioactive infection status and c) infection intensity in coyote (*Canis latrans*) intestines compared across location (urban/rural), age group (young/old based on 50th percentiles, 1.78 years), and sex. Test statistics (χ^2) and P-values were calculated from the likelihood ratio of univariate generalized linear models (logistic regression: molecular, bioactive infections; negative binomial: intensity). Bold values denote P-values<0.25, signalling the variables that were retained for use in subsequent analyses.

		a) molecular infection				b) bioactive infection				c) infection intensity (n = 78)		
variable	n	% (n) infected	% (n) uninfected	χ^2 (df = 1)	p-value	% (n) infected	% (n) uninfected	χ^2 (df = 1)	p-value	mean worms (SD)	χ^2 (df = 1)	p-value
all samples	112	70 (78)	30 (34)	-	-	48 (54)	52 (58)	-	-	1312 (4115)	-	-
location												
urban	41	80 (33)	20 (8)	3.75	**0.053**	56 (23)	44 (18)	1.61	**0.204**	2307 (5940)	0.10	0.747
rural	71	63 (45)	37 (26)			44 (31)	56 (40)			582 (1638)		
age group[2]												
young	56	80 (45)	20 (11)	6.18	0.013	61 (34)	39 (22)	7.08	0.008	1574 (4676)	2.14	0.143
old	56	59 (33)	41 (23)			36 (20)	64 (36)			253 (1303)		
sex												
male	60	68 (41)	32 (19)	0.10	0.746	47 (28)	53 (32)	0.12	0.725	1456 (4512)	0.26	0.608
female	52	71 (37)	29 (15)			50 (26)	50 (26)			1153 (3681)		

[2] binary age group included for descriptive purposes only and not included as a covariate in subsequent statistical analyses.

Table 2. Mean and standard deviation of coyote (*Canis latrans*) cementum age (years) and diet variables (volume [mL] of stomach contents, Shannon's H, stable isotopes) compared across *Echinococcus multilocularis* a) molecular and b) bioactive infection status and c) infection intensity in coyote intestines. Test statistics and P-values were calculated from the likelihood ratio of univariate logistic regression models (χ^2, for molecular and bioactive infections), and Spearman's rank correlation (R_S, for intensity). Bold values denote $P<0.10$ signalling significance.

	a) molecular infection				b) bioactive infection				c) intensity	
	mean (SD) infected	mean (SD) uninfected	χ^2 (df = 1)	p-value	mean (SD) infected	mean (SD) uninfected	χ^2 (df = 1)	p-value	R_S	p-value
cementum age	2.14 (2.23)	3.25 (2.37)	9.98	**0.002**	1.84 (1.90)	3.06 (2.52)	10.65	**0.001**	-0.23	**0.044**
ungulate	84.2 (322.7)	128.8 (330.2)	0.05	0.821	86.0 (360.9)	108.7 (288.6)	0.01	0.931	-0.09	0.432
rodent	25.6 (61.4)	23.5 (61.6)	0.05	0.824	23.7 (64.1)	26.1 (59.0)	0.03	0.853	-0.14	0.206
meso-mammal	26.0 (93.5)	17.7 (62.0)	0.44	0.507	26.2 (106.3)	21.0 (59.5)	0.17	0.680	-0.02	0.863
bird	5.1 (32.6)	0.7 (3.5)	1.11	0.292	6.0 (38.1)	1.7 (9.5)	0.37	0.375	0.23	**0.041**
digestible anthropogenic	17.2 (55.3)	40.3 (80.3)	2.51	0.113	19.0 (64.3)	29.0 (67.0)	0.61	0.433	-0.06	0.607
indigestible anthropogenic	6.0 (22.5)	4.6 (17.1)	0.10	0.751	8.3 (26.7)	3.0 (13.2)	1.99	0.158	0.07	0.522
vegetation	2.1 (4.1)	3.9 (8.9)	1.77	0.184	1.6 (2.8)	3.6 (7.8)	3.73	**0.053**	-0.09	0.458
native fruit	0.4 (2.0)	1.9 (10.3)	1.38	0.240	0.5 (2.4)	1.1 (7.9)	0.28	0.594	0.13	0.268
insects	0.1 (0.8)	<0.1 (<0.1)	2.90	**0.089**	0.1 (0.8)	0.1 (0.5)	0.23	0.629	0.03	0.781
total food	163.7 (332.8)	217.0 (331.2)	0.61	0.434	164.7 (374.0)	194.0 (289.5)	0.14	0.712	-0.08	0.470
Shannon	0.38 (0.40)	0.44 (0.41)	0.47	0.578	0.41 (0.41)	0.43 (0.41)	0.06	0.857	0.06	0.577
$\delta^{15}N$	8.64 (1.05)	8.74 (0.85)	0.29	0.591	8.71 (0.90)	8.70 (0.93)	<0.01	0.952	-0.08	0.470
$\delta^{13}C$	-22.53 (1.1)	-22.29 (0.8)	0.99	0.297	-22.39 (0.97)	-22.33 (1.32)	0.07	0.787	-0.06	0.577

Table 3. Mean and standard deviation of coyote (*Canis latrans*) cementum age (years) and diet variables (volume [mL] of stomach contents, Shannon's H, stable isotopes) compared across carcass a) location (urban/rural) and b) age group (young/old: 50^{th} percentiles of 1.78 years) groups and c) cementum age (years). Test statistics and P-values were calculated from univariate Kruskal-Wallis tests (χ^2; location, age group) and Spearman's rank correlation (R_S: cementum age). Bold values denote $P<0.10$ signalling significance.

	a) location				b) age group (binary)				c) cementum age (continuous)	
	mean (SD) urban	mean (SD) rural	χ^2 (df = 1)	p-value	mean (SD) young	mean (SD) old	χ^2 (df = 1)	p-value	R_S	p-value
cementum age	2.63 (2.39)	2.20 (2.20)	1.26	0.262	-	-	-	-	-	-
ungulate	153.9 (397.3)	0.04 (2.0)	22.07	**<0.001**	44.6 (171.0)	150.8 (420.8)	2.53	0.112	0.17	**0.070**
rodent	12.4 (33.2)	46.7 (87.8)	6.08	**0.014**	32.8 (71.5)	17.6 (48.4)	2.94	**0.087**	-0.22	**0.020**
meso-mammal	28.6 (98.8)	14.6 (53.3)	0.27	0.600	26.8 (101.3)	20.2 (65.4)	0.01	0.905	-0.06	0.513
bird	5.4 (34.1)	0.9 (3.33)	0.30	0.586	6.3 (37.4)	1.3 (9.3)	11.49	**0.001**	-0.37	**<0.001**
digestible anthropogenic	15.3 (46.7)	39.6 (88.0)	2.83	**0.092**	25.2 (69.4)	23.2 (62.2)	0.768	0.381	-0.12	0.210
indigestible anthropogenic	8.6 (25.8)	0.2 (0.6)	2.45	0.117	6.4 (17.7)	4.7 (23.8)	5.14	**0.024**	-0.20	**0.032**
vegetation	3.4 (7.2)	1.4 (2.7)	2.36	0.124	2.1 (3.9)	3.2 (7.5)	2.25	0.134	-0.17	**0.080**
native fruit	1.0 (7.2)	0.5 (2.6)	0.40	0.528	1.6 (8.3)	<0.1 (<0.1)	8.35	**0.004**	-0.24	**0.010**
insects	<0.1 (<0.1)	0.3 (1.1)	5.67	**0.017**	0.2 (0.9)	<0.1 (<0.1)	4.91	**0.027**	-0.30	**0.001**
total food	228.7 (399.7)	104.7 (124.7)	1.93	0.165	145.7 (201.1)	220.9 (422.6)	0.08	0.780	-0.07	0.434
Shannon	0.45 (0.42)	0.38 (0.39)	0.92	0.338	0.51 (0.44)	0.33 (0.35)	5.10	**0.024**	-0.27	**0.005**
$\delta^{15}N$	8.80 (1.00)	8.54 (0.071)	4.84	**0.028**	8.60 (0.96)	8.82 (0.85)	1.86	0.172	0.16	**0.088**
$\delta^{13}C$	-22.94 (0.66)	-21.36 (1.14)	43.35	**<0.001**	-22.46 (1.27)	-22.27 (1.01)	2.18	0.140	0.16	**0.090**

Table 4. Cementum age (years), focal diet variables (stomach content volume: rodents, digestible anthropogenic food, indigestible anthropogenic food), and age/diet interactions that predict *Echinococcus multilocularis* a) molecular and b) bioactive infection status and c) intensity in coyote (*Canis latrans*) intestines for each location (urban/rural). Test statistics (χ^2) and P-values were calculated from the likelihood ratio of univariate generalized linear models (logistic regression: molecular, bioactive infections; negative binomial: intensity). Bold values denote P-values<0.25, signalling the variables that were retained for use in subsequent analyses.

		a) molecular infection				b) bioactive infection				c) infection intensity			
		linear		interact age		linear		interact age		linear		interact age	
location	predictor variable	χ^2 (df = 1)	p-value	χ^2 (df = 1)	p-value	χ^2 (df = 1)	p-value	χ^2 (df = 1)	p-value	χ^2 (df = 1)	p-value	χ^2 (df = 1)	p-value
urban	cementum age	0.07	0.794	-	-	0.02	0.874	-	-	0.13	0.722	-	-
	rodents	0.05	0.830	0.07	0.784	0.13	0.722	9.66	**0.002**	0.01	0.935	10.89	**0.001**
	digestible	0.32	0.574	5.65	**0.017**	0.17	0.677	2.79	**0.095**	0.10	0.747	0.07	0.786
	indigestible	0.54	0.463	0.23	0.630	0.02	0.890	0.26	0.608	1.37	**0.241**	0.05	0.821
rural	cementum age	11.95	**<0.001**	-	-	17.75	**<0.001**	-	-	4.54	**0.033**	-	-
	rodents	0.02	0.891	1.94	**0.164**	0.10	0.750	0.13	0.717	0.01	0.937	2.43	**0.119**
	digestible	5.33	**0.021**	0.70	0.404	7.99	**0.005**	2.81	**0.094**	8.46	**0.004**	3.09	**0.078**
	indigestible	0.47	0.492	0.29	0.593	2.87	**0.090**	0.19	0.663	<0.01	0.967	5.05	**0.025**

Table 5. Results from regression analyses to determine the combination of cementum age (years), focal diet variables (stomach content volume: rodents, digestible anthropogenic food, indigestible anthropogenic food), and age/diet interactions that best predict *Echinococcus multilocularis* a) molecular and b) bioactive infection status and c) infection intensity in coyote (*Canis latrans*) intestines for each location (urban/rural). Beta coefficients (β) were calculated from generalized linear models (logistic regression: molecular, bioactive infections; negative binomial: intensity) averaged with partial standard deviation. Bolded values denote significant predictors (95% C.I.). Asterisks denote marginal values (significant within 90% C.I.).

location	predictor	a) molecular infection		b) bioactive infection		c) infection intensity	
		β	95% CI	β	95% CI	β	95% CI
urban	cementum age	-0.08	-0.70, 0.54	-0.18	-0.99, 0.64	-0.29	-0.63, 0.04*
	rodents	-	-	-0.87	-1.79, 0.04*	**-0.56**	**-0.99, -0.12**
	age x rodents	-	-	**-1.11**	**-2.17, -0.05**	**-0.53**	**-0.88, -0.17**
	digestible anthropogenic	-0.21	-1.31, 0.89	0.10	-0.42, 0.62	-	-
	age x digestible	-0.61	-2.81, 1.58	-	-	-	-
	indigestible anthropogenic	-	-	-	-	0.11	-0.20, 0.43
	total food	-0.25	-1.14, 0.65	0.13	-0.76, 1.03	-0.04	-0.41, 0.33
rural	cementum age	**-0.95**	**-1.56, -0.34**	**-1.27**	**-1.97, -0.57**	**-0.47**	**-0.80, -0.14**
	digestible anthropogenic	-0.61	-1.62, 0.41	-3.38	-6.87, 0.12*	**-0.93**	**-1.64, -0.22**
	indigestible anthropogenic	-	-	0.54	-0.45, 1.54	0.05	-0.17, 0.27
	total food	0.01	-0.52, 0.55	0.11	-0.44, -0.66	0.08	-0.20, 0.36

* = significant within 90% confidence interval

Table 6. Evaluation of model fit for the final multivariable generalized linear models that combined cementum age (years) and focal diet components (stomach content volume: rodents, digestible anthropogenic food, indigestible anthropogenic food) to predict *Echinococcus multilocularis* molecular and bioactive infection status (logistic regression) and infection intensity (negative binomial) in coyote (*Canis latrans*) intestines for each location (urban/rural). Values were calculated from Nagelkereke's pseudo R^2, and area under the receiver operating characteristic curve (AUC).

location	response variable	R^2	AUC
urban	molecular	0.249	0.651
	bioactive	0.321	0.766
	intensity	0.449	-
rural	molecular	0.324	0.782
	bioactive	0.481	0.857
	intensity	0.402	-

Table 7. Summary of studies that reported *Echinococcus multilocularis* infection prevalence in wild coyotes (*Canis latrans*) from urban and rural locations within Canada between 2012 and 2021. Included is the morphological and/or genetic method by which *E. multilocularis* infection was detected and the material sampled (intestinal contents, feces).

reference	location type	location	% positive	no. positive	no. test	detection method	material
current study	urban	Edmonton, AB	56%	23	41	morphology	intestine
Luong et al. (2018)	urban	Edmonton, AB	65%	10	15	morphology	intestine
Catalano et al. (2012)	urban	Edmonton, AB	62.5%	5	8	morphology	intestine
current study	urban	Edmonton, AB	80%	33	41	qPCR	intestine
Sugden et al. (2020)	urban	Edmonton, AB	53%	16	30	PCR	intestine / feces
Catalano et al. (2012)	urban	Calgary, AB	20.5%	17	83	morphology	intestine
Liccioli et al. (2012, 2014)	urban	Calgary, AB	29.5%	18	61	morphology/ PCR	intestine
			21.4%	82	385	PCR	feces
current study	rural	Alberta	44%	31	71	morphology	intestine
current study	rural	Alberta	63%	45	71	qPCR	intestine
Sugden et al. (2020)	rural	Alberta	35%	23	65	PCR	intestine / feces
Gesy et al. (2013b, 2014)	rural	Quesnel, BC	37%	10	27	PCR	intestine
Gesy et al. (2014)	rural	Sathu, NT	8%	6	73	PCR	intestine
Kolapo et al. (2021)	rural	Saskatchewan	72%	150	208	coproPCR	intestine
Gesy et al. (2014)	rural	Saskatchewan	24%	4	17	PCR	intestine
Gesy et al. (2014)	rural	Riding Mt, MB	67%	2	3	PCR	intestine
Tse et al. (2019)	urban	Winnipeg, MB	7.3%	9	122	PCR	feces
Kotwa et al. (2019, 2020)	rural	Ontario	24%	100	416	qPCR	feces
Schurer et al. (2018)	rural	Quebec	0%	0	77	PCR	intestine

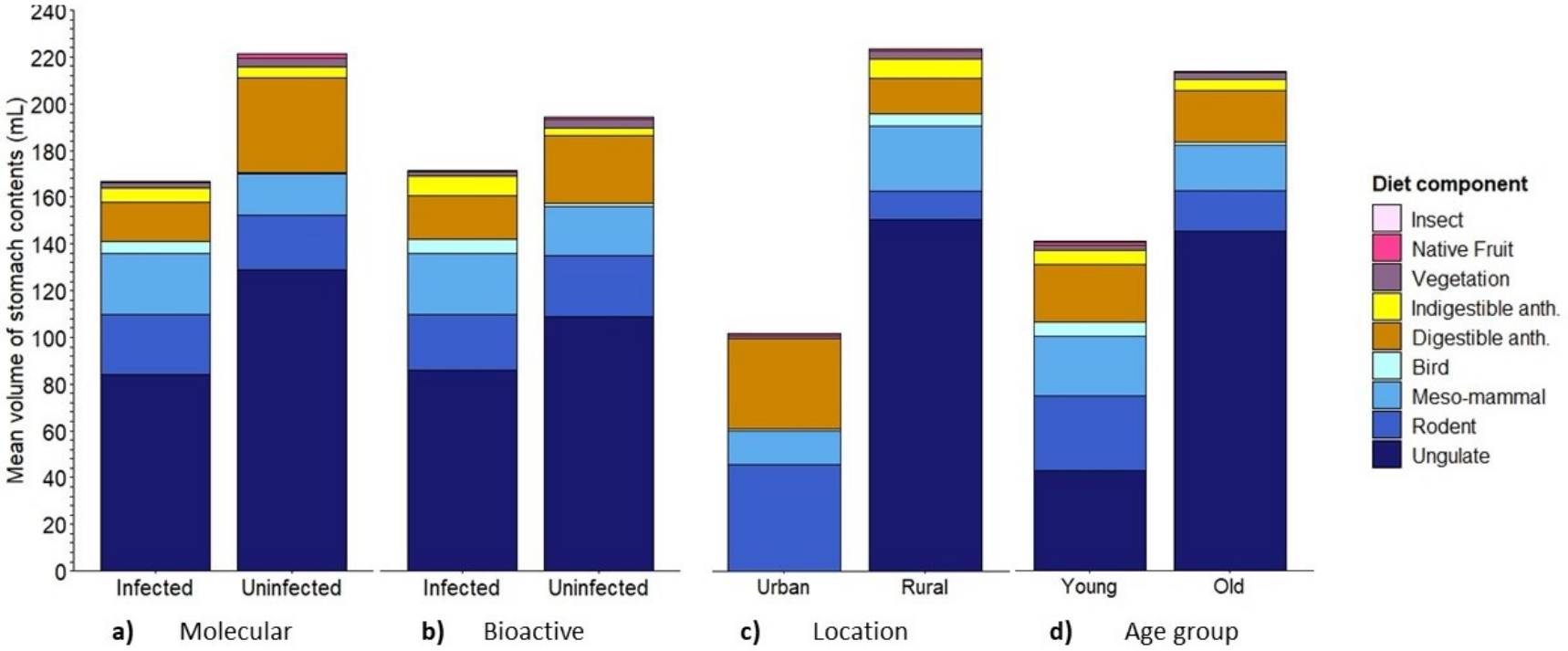

Figure 1. Mean volume of diet components recovered from the stomach contents of coyotes (*Canis latrans*) compared across *Echinococcus multilocularis* a) molecular and b) bioactive infection status, location (urban/rural) and age group (young/old: 50th percentiles of 1.78 years). Colour schemes represent types of prey (blue), anthropogenic food (yellow), and other food (pink).

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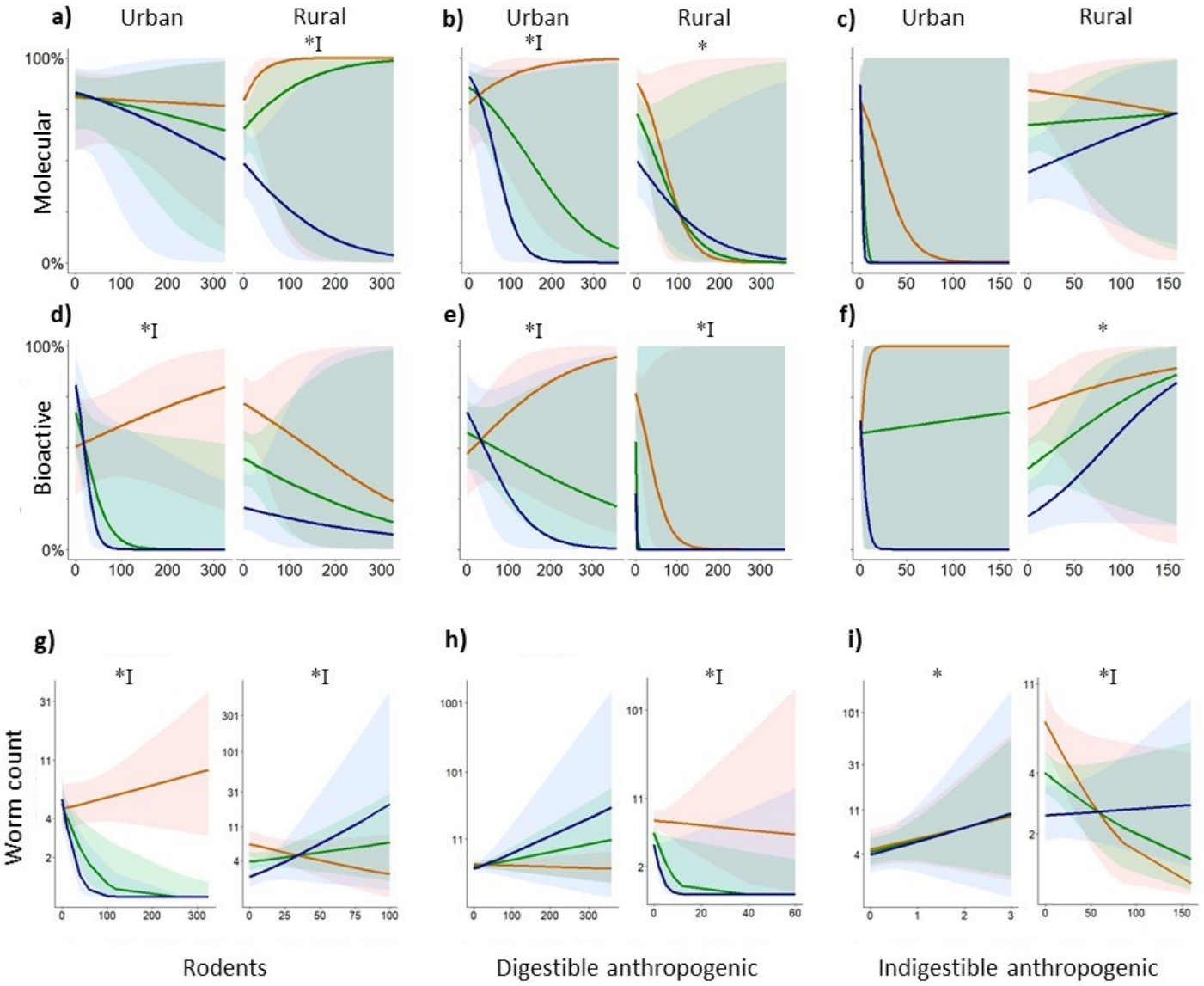

* → significant diet variable

*I → significant interaction with age

Figure 2. *Echinococcus multilocularis* infection measured as molecular (a, b, c) bioactive (d, e, f) and intensity (g, h, i) from stomach content volumes (mL) of rodents (a, d, g), digestible anthropogenic food (b, e, h), and indigestible anthropogenic food (c, f, i) compared by age (years) for urban and rural coyotes (*Canis latrans*). Confidence intervals represent 95% C.I.

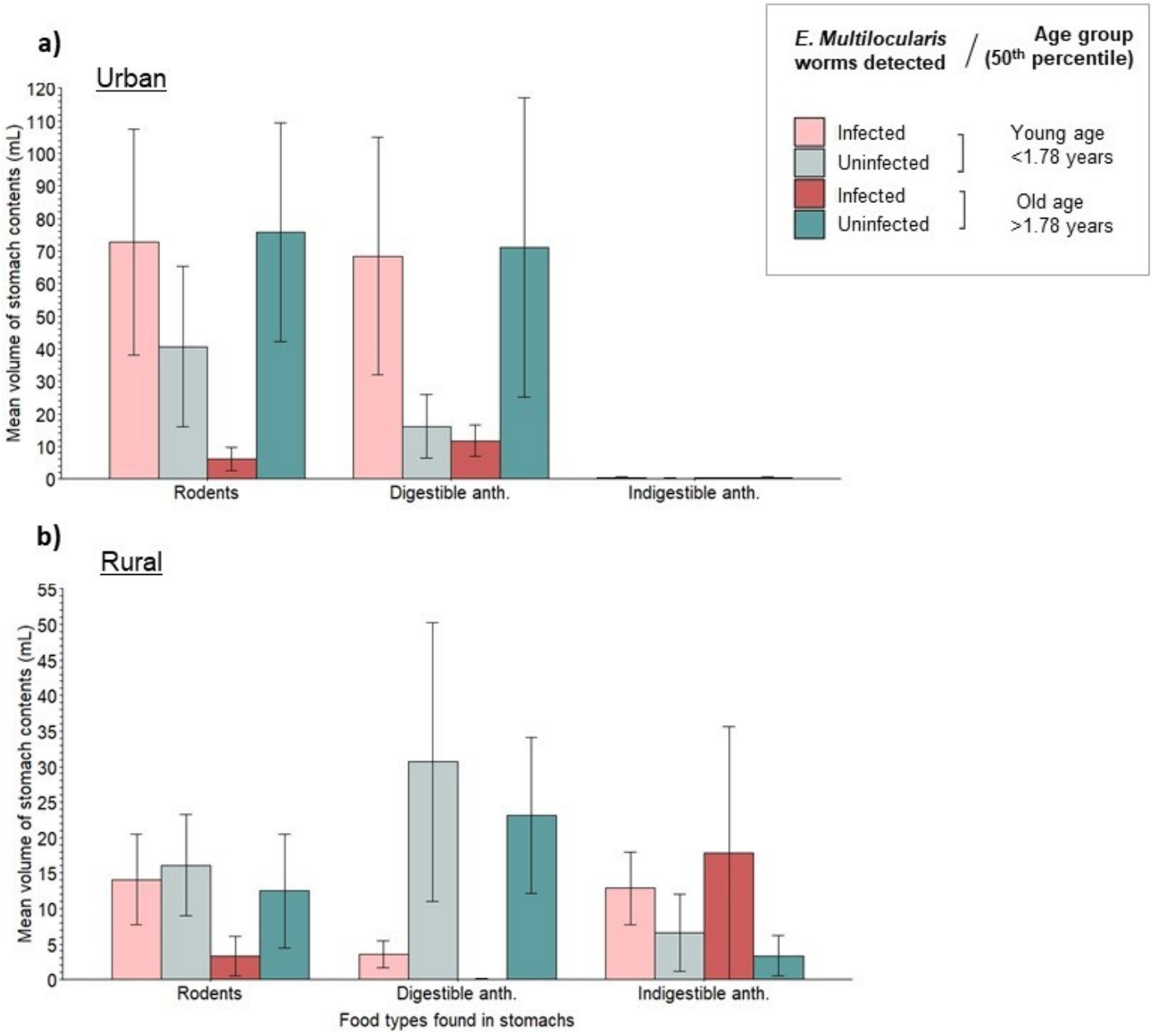

Figure 3. Focal diet variables (stomach content volume: rodents, digestible anthropogenic food, indigestible anthropogenic food) compared by binary age group (young/old: 50th percentiles of 1.78 years) and the presence of *Echinococcus multilocularis* bioactive infection in a) urban and b) rural coyote (*Canis latrans*) intestines. Error bars represent 1 standard error.

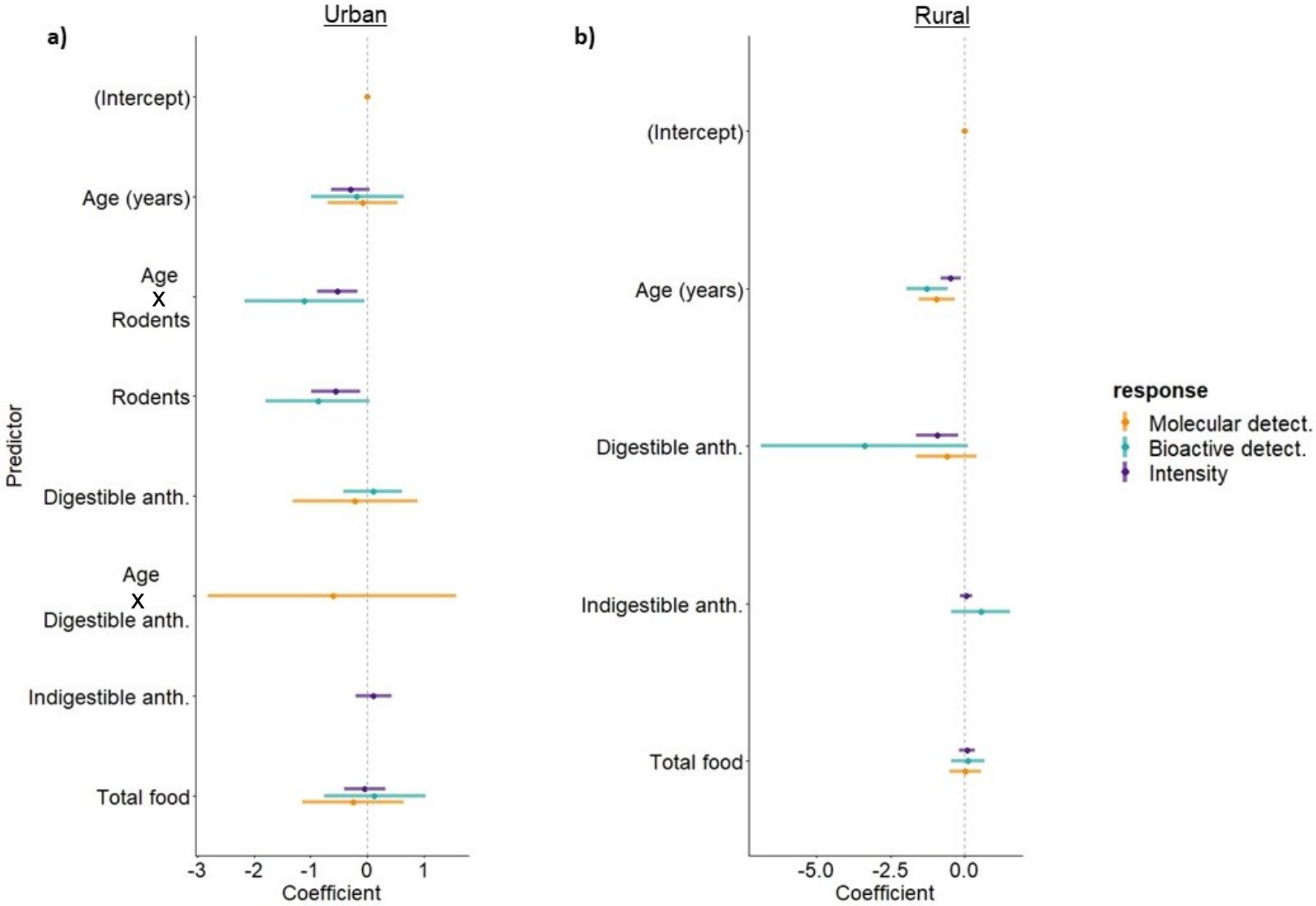
a)
Urban
(Intercept)
Age (years)
Age
X
Rodents
Rodents
Digestible anth.
Age
X
Digestible anth.
Indigestible anth.
Total food
Predictor
-3
-2
-1
0
1
Coefficient
b)
Rural
(Intercept)
Age (years)
Digestible anth.
Indigestible anth.
Total food
-5.0
-2.5
0.0
Coefficient
response
Molecular detect.
Bioactive detect.
Intensity

Figure 4. Results from regression models of cementum age (years), focal diet variables (stomach content volume: rodents, digestible anthropogenic food, indigestible anthropogenic food), and age/diet interactions that predict *Echinococcus multilocularis* molecular and bioactive infection status and infection intensity in a) urban and b) rural coyote (*Canis latrans*) intestines. Predictors are ranked by the frequency of inclusion in top models prior to model-averaging. Error bars denote 95% C.I

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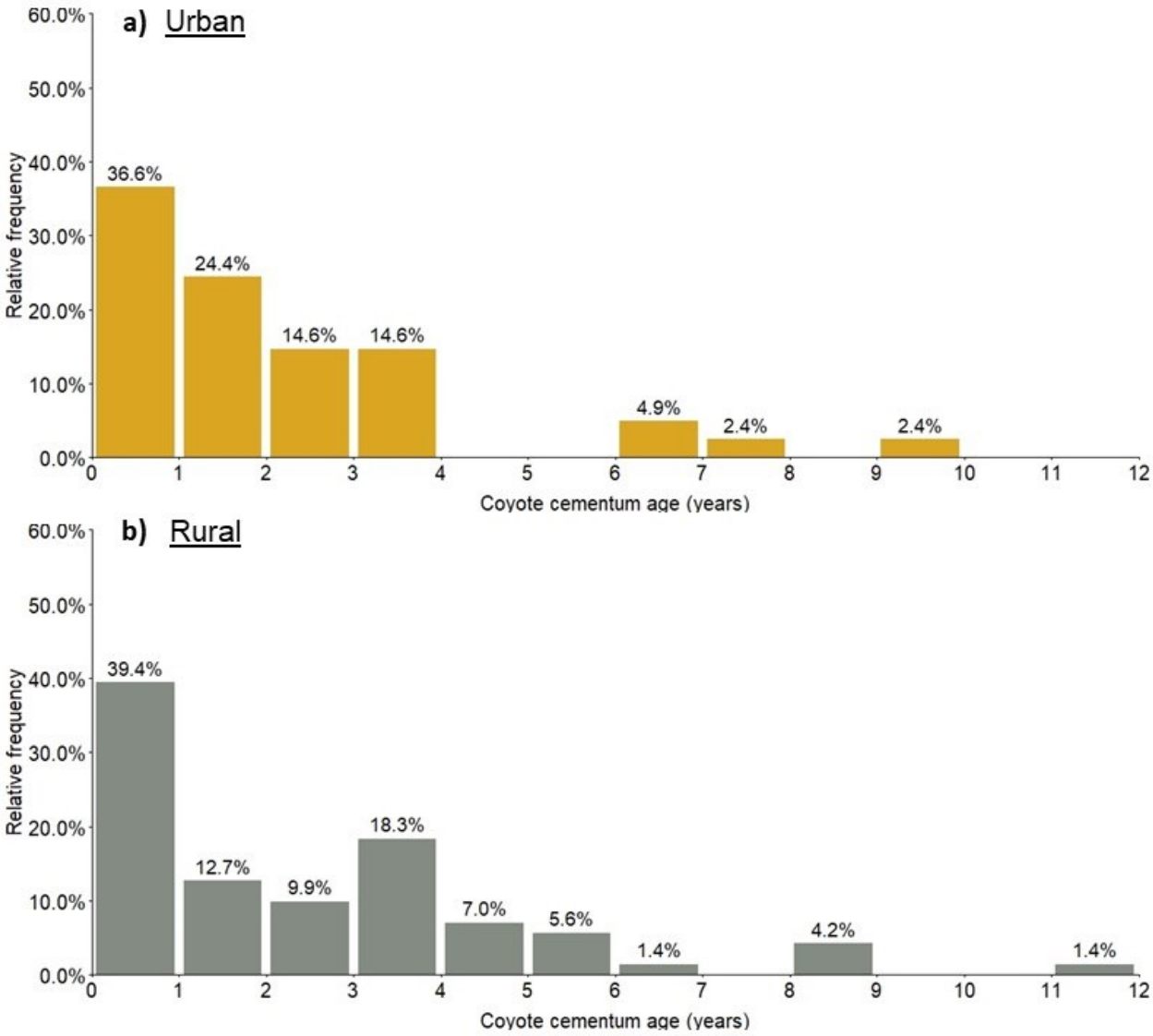
Appendix

Appendix 1. Number of coyote (*Canis latrans*) carcasses collected for study organized by location (urban/rural), year, and season of coyote death. Months are assigned to seasons based on the climatic trends in Edmonton, AB, Canada.

Location	Year	Spring Mar - May	Summer Jun - Aug	Fall Sep - Nov	Winter Dec - Feb*	**Total**
urban	2017	0	1	3	1	**5**
	2018	5	0	5	13	**23**
	2019	0	5	2	3	**10**
	2020	2	1	0	0	**3**
	Total	**7**	**7**	**10**	**17**	**41**
rural	2017	0	0	10	48	**58**
	2018	7	0	0	1	**8**
	2019	0	0	1	4	**5**
	Total	**7**	**0**	**11**	**53**	**71**

* January and February included in the previous year's winter



Appendix 2. Relative frequency distribution of cementum age (years) of a) urban (n = 41) and b) rural (n = 71) coyotes (*Canis latrans*).

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Appendix 3. Cementum age (years), focal diet variables (stomach content volume: rodents, digestible anthropogenic food, indigestible anthropogenic food), and 2- and 3-way interactions with location (urban/rural) and age that predict *Echinococcus multilocularis* a) molecular and b) bioactive infection status and c) intensity in coyote (*Canis latrans*). Test statistics (χ^2) and P-values were calculated from the likelihood ratio of univariate generalized linear models (logistic regression: molecular, bioactive infections; negative binomial: intensity). Bold values denote P-values<0.25, signalling the variables that were retained for use in subsequent analyses.

		a) molecular infection				b) bioactive infection				c) infection intensity			
sample	variable	linear	interact location	interact age	interact location x age	linear	interact location	interact age	interact location x age	linear	interact location	interact age	interact location x age
all coyotes	location	**0.053**	-	**0.093**	-	**0.204**	-	**0.006**	-	0.747	-	**0.247**	-
	cementum age	**0.002**	**0.093**	-	-	**0.001**	**0.006**	-	-	**0.063**	**0.247**	-	-
	rodents	0.824	0.996	0.634	**0.243**	0.853	0.940	**0.085**	**0.049**	0.866	0.986	**0.048**	**0.004**
	digestible	**0.113**	**0.164**	**0.157**	**0.046**	0.433	**0.009**	**0.190**	**0.111**	0.894	**0.004**	0.898	**0.089**
	indigestible	0.751	0.505	0.721	0.512	**0.158**	0.900	0.945	0.565	0.913	**0.227**	**0.084**	0.869