Capillaria hepatica in wild Norway rats (Rattus norvegicus) from Vancouver, Canada.

Jamie L. Rothenburger,[1,7] **Chelsea G. Himsworth,**[2,3] **Victoria Chang,**[4] **Mani Lejeune,**[5] **and Frederick A. Leighton**[1,6]

[1] Department of Veterinary Pathology, Western College of Veterinary Medicine, University of Saskatchewan, 52 Campus Drive, Saskatoon, Saskatchewan S7N 5B4, Canada; [2] Animal Health Centre, British Columbia Ministry of Agriculture, 1767 Angus Campbell Road Abbotsford, British Columbia V3G 2M3, Canada; [3] School of Population and Public Health, University of British Columbia, 2206 East Mall, Vancouver, British Columbia V6T 1Z3, Canada; [4] Ontario Veterinary College, University of Guelph, 50 Stone Road, Guelph, Ontario N1G 2W1, Canada; [5] Canadian Cooperative Wildlife Health Centre, Alberta Branch, Faculty of Veterinary Medicine, University of Calgary, 3280 Hospital Drive NW, Calgary, Alberta T2N 4Z6, Canada; [6] Canadian Cooperative Wildlife Health Centre, National Headquarters, University of Saskatchewan, 52 Campus Dr., Saskatoon, Saskatchewan S7N 5B4, Canada; [7] Corresponding author (email: jamie.rothenburger@usask.ca)

ABSTRACT: *Capillaria hepatica* is a parasitic nematode that infects the liver of rats (*Rattus* spp.), and occasionally other mammalian species, including humans. Despite its broad geographic distribution and host range, the ecology of this parasite remains poorly understood. We characterized the ecology of *C. hepatica* in urban Norway rats (*Rattus norvegicus)* in Vancouver, Canada. The overall prevalence of *C. hepatica* among Norway rats was 36% (241/671); however, there was significant variation in prevalence among city blocks. Using a generalized linear mixed model to control for clustering by block, we found *C. hepatica* infection was negatively associated with season (spring [OR = 0.14, 95% CI = 0.05 – 0.39]; summer [OR = 0.14, 95% CI = 0.03 – 0.61]; winter [OR = 0.34, 95% CI = 0.13 – 0.84], compared to fall) and positively associated with sexual maturity (OR: 7.29, 95% CI: 3.98 – 13.36) and presence of cutaneous bite wounds (OR = 1.87, 95% CI = 1.11 – 3.16). Our understanding of the ecology of *C. hepatica* in rats is hindered by a paucity of data regarding the main mechanisms of transmission (e.g., environmental exposure vs. active cannibalism). However, associations between infection, season, maturity, and bite wounds could suggest that social interactions, possibly including cannibalism, may be important in transmission.

*Key words*: Black rat, *Calodium hepaticum, Capillaria hepatica*, ecology, Norway rat, *Rattus*, urban.

Capillaria hepatica (syn *Calodium hepaticum*) is a parasitic nematode with a broad host range that includes at least 80 species in the rodent family Muridae (Fuehrer et al. 2011). Infection has also been reported in at least 24 other mammalian families and in humans (Fuehrer et al. 2011). Animals become infected with *C. hepatica* by ingesting embryonated eggs, which hatch in the intestine, releasing larvae that migrate via the portal vein to the liver where they develop into adults, mate, and produce eggs (Farhang-Azad 1977b). Eggs are released into the environment by postmortem decomposition or in the feces of predators or scavengers of rats (Farhang-Azad 1977b). Rats ingest eggs from the environment or cannibalism (Farhang-Azad 1977b).

Norway rats (*Rattus norvegicus*) and black rats (*R. rattus*) are among the most common hosts of *C.*

hepatica, and infections appears to be endemic in rat populations around the world (Fuehrer et al. 2011). Reported prevalence of infection has varied from 11%–88% (Easterbrook et al. 2007; Kataranovski et al. 2010). Although a number of factors associated with infection status have been identified, including host maturity, weight, habitat type, season, and population density (Childs et al. 1988; Easterbrook et al. 2007; Milazzo et al. 2010), the ecology of *C. hepatica* in rats remains poorly understood. Here, we characterize the ecology of *C. hepatica* in urban rats from an inner-city neighborhood of Vancouver, Canada.

We trapped 725 rats in 43 contiguous city blocks and an international shipping port in Vancouver, Canada (49°17'N, 123°6'W) over the course of one year (September 2011- August 2012), as previously described (Himsworth et al. 2013). Data collected in the field included species (determined by external morphology), sex, weight, number of skin wounds, sexual maturity (open vaginal orifice in females and scrotal testes in males) and the location where each rat was trapped. Rats underwent a standardized necropsy and tissue collection protocol at the Animal Health Centre, British Columbia Ministry of Agriculture, Abbotsford, British Columbia. The liver of each rat was examined for the presence of multifocal to coalescing white, tortuous foci consistent with *C. hepatica* infection (Luttermoser 1938). The extent of grossly evident lesions varied among rats, from a single focus to coalescing lesions affecting the majority of the liver (Figs. 1, 2).
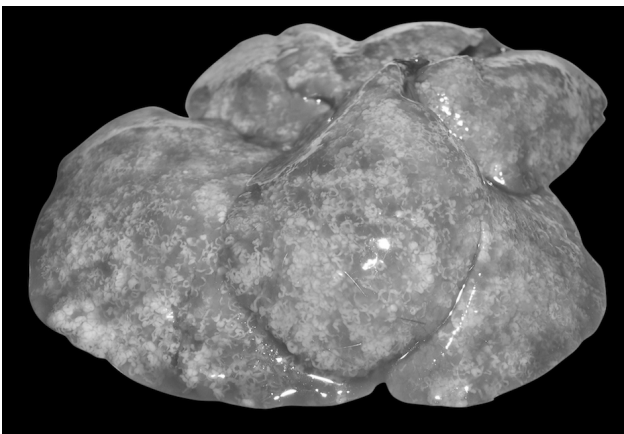
Figure 1. *Capillaria hepatica* infection in the liver (gross pathology) in an urban Norway rat (*Rattus norvegicus*) from Vancouver, Canada. Tortuous white foci affect all liver lobes.
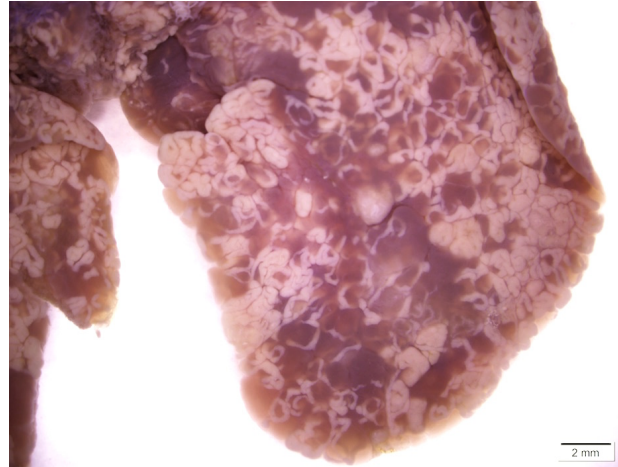

Figure 2. Direct stereoscopic image of fresh liver from an urban Norway rat *(Rattus norvegicus)* from Vancouver, Canada demonstrating infection with *Capillaria hepatica*. Tortuous, white foci affect multiple liver lobes.

Grossly affected samples of liver were examined using light microscopy in a subset of 108 rats. Microscopically, nematodes were characterized by thin cuticle and hypodermis with coelomyarian musculature and a small digestive tract lined by a single layer of low cuboidal epithelial cells. Eggs were nonembryonated and oval with thick, striated shells and bipolar plugs. Based on the morphology of the eggs, nematode cross sections, and location, the nematode was identified as *Capillaria hepatica* (Gardiner and Poynton 2006). Nematode infection was associated with varying amounts of hepatocyte necrosis, fibrosis, and inflammation (Fig. 3).

The inflammatory reaction varied from predominantly granulocytic (suggestive of an acute infection) to predominantly lymphoplasmacytic and histiocytic (suggestive of a chronic infection). Given that no pathologic process, other than nematode infection, was found to cause the gross liver lesions, any rat with those gross lesions was considered to be infected with *C. hepatica*. It is notable that liver *C. hepatica* infection is grossly evident as early as six days postinfection (Luttermoser 1938).

For statistical analysis, the primary outcome variable was *C. hepatica* infection (positive vs. negative). Explanatory variables considered included season (September–November = fall; December–February = winter; March–May = spring; June–August = summer), sex, sexual maturity (immature vs. mature), body condition as assessed by volume of internal fat stores (score of 0–2), weight, and presence or absence and number of cutaneous bite wounds (Table 1). A generalized linear mixed model
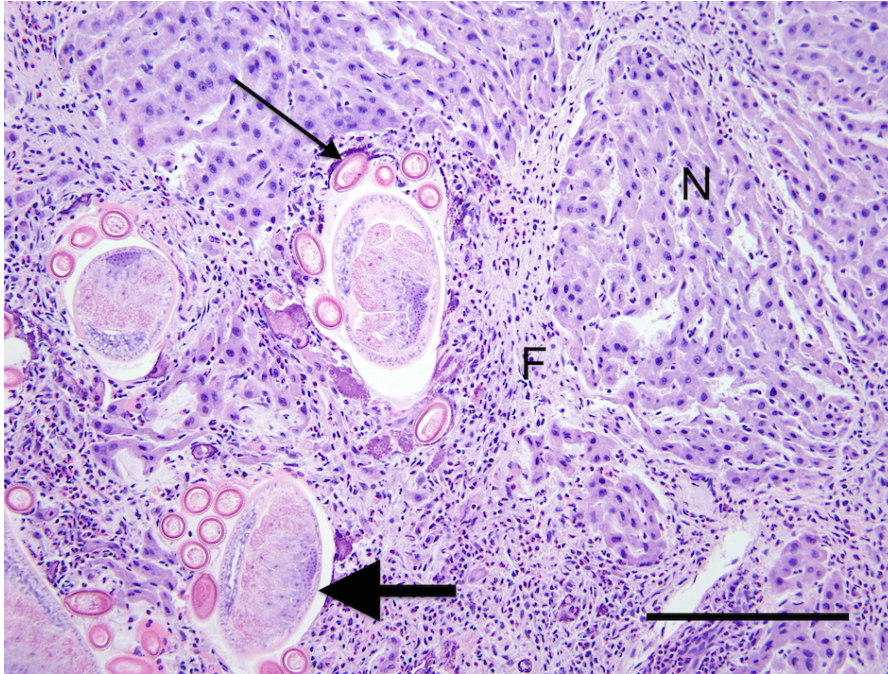
Figure 3. Histologic features of the liver in a wild, urban Norway rat (*Rattus norvegicus*) from Vancouver, Canada with *Capillaria hepatica* infection. Cross-sections of *C. hepatica* adults (wide arrow) and eggs with polar plugs (narrow arrow) within the liver parenchyma. Granulocytes infiltrate the affected area and there is loss of hepatocytes. Dissecting fibrosis (F) separates the affected area from normal hepatocytes (N). H&E stain. 20X; Bar=200 μm.

(GLM), controlling for clustering by block of origin, was used to identify the most parsimonious set of variables that explained the outcome. For the GLM models, individuals for which data were missing for one or more variables under study were excluded. All statistical analyses were conducted using R (R Development Core Team, Vienna, Austria). The University of British Columbia's Animal Care Committee approved this study (A11-0087).

Of 725 rats trapped, 15 were excluded due to incomplete records or autolysis. Of the rats assessed for *C. hepatica* infection, 95% (672/710) were Norway rats and 38 were black rats. *Capillaria hepatica* infection was found in 241/672 Norway rats (36%) and 32% (9/38) of black rats. Prevalence of *C. hepatica* varied by block, ranging from 0% to 81% (Fig. 4), and block of origin was significantly associated with the odds of *C. hepatica* infection on bivariate logistic regression (data not shown). Given the low number of black rats and the differing biology of Norway and black rats (Feng and Himsworth 2013), black rats were excluded from further analysis. The following statistics pertain to Norway rats only (*n*=672).

Characteristics of the trapped population and associations among these characteristics and *C. hepatica* infection are summarized in Tables 1 and 2. In the final GLM model, only season, maturity, and presence of bite wounds were retained. Specifically, the odds of being infected with *C. hepatica* were less in the spring (OR = 0.14, 95% CI = 0.05–0.39), summer (OR = 0.14, 95% CI = 0.03–0.61) and winter (OR = 0.34, 95% CI = 0.13–0.84) compared to fall. The odds of being infected with *C. hepatica* were greater in mature rats (OR = 7.29, 95% CI = 3.98–13.36) than in immature rats and those with bite wounds (OR = 1.87, 95% CI = 1.11–3.16) than those without.

The ecology of *C. hepatica* is evidently complex, with infection being associated with geographic location, maturity, season, and bite wounds. Accurate interpretation of these findings, however, is impeded by a lack of understanding regarding how *C. hepatica* is transmitted among rats (i.e., through incidental exposure in the environment [with eggs released through decomposition or in the feces of nonrat predators or scavengers] or conspecific scavenging on dead rats). Given that, within urban centers, 1) rat populations can reach high densities; 2) rats seldom live more than 1 year; 3) there is a dearth of predators and non-*Rattus* spp. scavengers capable of effective carcass removal, particularly within burrow systems (Feng and Himsworth 2013), the eventual fate of rat carcasses is perplexing. Rats are averse to feeding on freshly-dead conspecifics; however, rats that are hungry or have opportunity to consume partially decomposed rat carcasses overcome this aversion (Calhoun 1963; Carr et al. 1979). Additionally, cannibalistic behavior can be acquired through observational learning (Carr et al. 1979). Although rats that die as a result of wounds sustained during aggressive interactions are often cannibalized (Calhoun 1963), it is not clear whether aggressor rats or other conspecifics are the main scavengers. Insects are not thought to be major disseminators of *C. hepatica* eggs (Farhang-Azad 1977b) and the role of non-*Rattus* spp. predators (e.g., cats) in egg dissemination has not been thoroughly investigated.

This study demonstrates marked variation in prevalence by block. This is consistent with the ecology of other pathogens in this study population, and likely reflects the fact that urban rats live in discrete colonies with small home ranges (often limited to a city block) and limited movement of rats among colonies (Himsworth et al. 2013). Geographic variation in *C. hepatica* prevalence may also be related to environmental differences, with some environments being more conducive to egg survival and transmission than others (Farhang-Azad 1977b). Additionally, in some blocks, increased resource competition or learned behaviors could lead to increased cannibalism (Calhoun 1963).

Some investigators found no association between *C. hepatica* prevalence and season (although their analyses were not cluster controlled; Easterbrook et al. 2007; Kataranovski et al. 2010), while others have noted seasonal variation, attributed to increased prevalence among noninfected, immature rats in spring vs. fall (Childs et al. 1988). We found the association between season and *C. hepatica* to be independent of sexual maturity. Therefore intra-annual variation in *C. hepatica* infection is likely a result of other factors, such as seasonal differences in egg exposure, resource availability and the propensity for cannibalism.

The association between maturity and *C. hepatica* infection may simply suggest that the longer a rat lives the more likely it is to be infected. This inference has been made in previous studies that found a positive association between infection and weight (Farhang-Azad 1977a; Childs et al. 1988; Easterbrook et al. 2007). However, in our study, sexual maturity was a stronger predictor than weight (the effect of maturity was not specifically examined in the aforementioned studies), suggesting that physiologic, social, or behavioral differences that occur with sexual maturity may be responsible for the observed association. Associations between bite wounds and infection may support a social basis for transmission. Since bite wounds reflect dominance and social interaction within a rat colony (Calhoun 1963; Feng and Himsworth 2013), infected rats may be more vulnerable to socially dominant rats.

Despite the apparent ubiquity of *C. hepatica*, its true impact on individual and population health remains unknown. Some have suggested that population impacts are negligible (Davis 1951). Given the degree of pathology found in some infected livers, however, it is plausible that infections are costly to the host and possibly result in subclinical effects on factors such as reproduction. Ultimately, future studies of *C. hepatica* in rats will be required to fully elucidate the ecology of this parasite, particularly its transmission and impacts on population health.

LITERATURE CITED

Calhoun JB. 1963. *The ecology and sociology of the Norway rat*. US Department of Health, Education, and Welfare, Public Health Service Publication No. 1008, Bethesda, Maryland, USA, 288 pp.

Carr WJ, Hirsch JT, Campellone BE, Marasco E. 1979. Some determinants of a natural food aversion in Norway rats. *J Comp Physiol Psychol* **93**:899–906.

Childs JE, Glass GE, Korch JR GW. 1988. The comparative epizootiology of *Capillaria hepatica* (Nematoda) in urban rodents from different habitats of Baltimore, Maryland. *Can J of Zool* **66**:2769–2775.

Davis DE. 1951. The relation between the level of population and the prevalence of *Leptospira*, *Salmonella*, and *Capillaria* in Norway rats. *Ecology* **32**:465–468.

Easterbrook JD, Kaplan JB, Vanasco NB, Reeves WK, Purcell RH, Kosoy MY, Glass GE, Watson J, Klein SL. 2007. A survey of zoonotic pathogens carried by Norway rats in Baltimore, Maryland, USA. *Epidemiol Infect* **135**:1192–1199.

Farhang-Azad A. 1977a. Ecology of *Capillaria hepatica* (Bancroft 1893) (Nematoda). 1; Dynamics of infection among Norway rat populations of the Baltimore Zoo, Baltimore, Maryland. *J Parasitol* **63**:117–122.

Farhang-Azad A. 1977b. Ecology of *Capillaria hepatica* (Bancroft 1893)(Nematoda). II. Egg-releasing mechanisms and transmission. *J Parasitol* **63**:701–706.

Feng AY T, Himsworth, CG. 2013. The secret life of the city rat: A review of the ecology of urban Norway and black rats (*Rattus norvegicus* and *Rattus rattus*). *Urban Ecosyst*. DOI 10.1007/s11252-013-0305-4.

Fuehrer HP, Igel P, Auer, H. 2011. *Capillaria hepatica* in man—An overview of hepatic capillariosis and spurious infections. *J Parasitol Res* **109**:969–979.

Gardiner, CH, Poynton, SL. 2006. *An Atlas of Metazoan Parasites in Animal Tissues*, 2nd Ed. Armed Forces Institute of Pathology, Washington, DC. USA, 64 pp.

Himsworth, CG, Bidulka J, Parsons KL, Feng AYT, Tang P, Jardine CM, Kerr T, Mak S, Robinson J, Patrick DM. 2013. Ecology of *Leptospira interrogans* in Norway rats (*Rattus norvegicus*) in an inner-city neighborhood of Vancouver, Canada. *PLoS Negl Trop Dis* **7**:e2270.

Kataranovski M, Zolotarevski L, Belij S, Mirkov I, Stosic J, Popov A, Kataranovski D. 2010. First record of *Calodium hepaticum* and *Taenia taeniaeformis* liver infection in wild Norway rats (*Rattus norvegicus*) in Serbia. *Arch Biol Sci* **62**:431–440.

Luttermoser GW. 1938. An experimental study of *Capillaria hepatica* in the rat and the mouse. *Am J Epidemiol* **27**:321–340.

Milazzo C, Ribas A, Casanova JC, Cagnin M, Geraci F, Bella C. 2010. Helminths of the brown rat (*Rattus norvegicus*) (Berkenhout, 1769) in the city of Palermo, Italy. *Helminthologia* **47**:238–240.
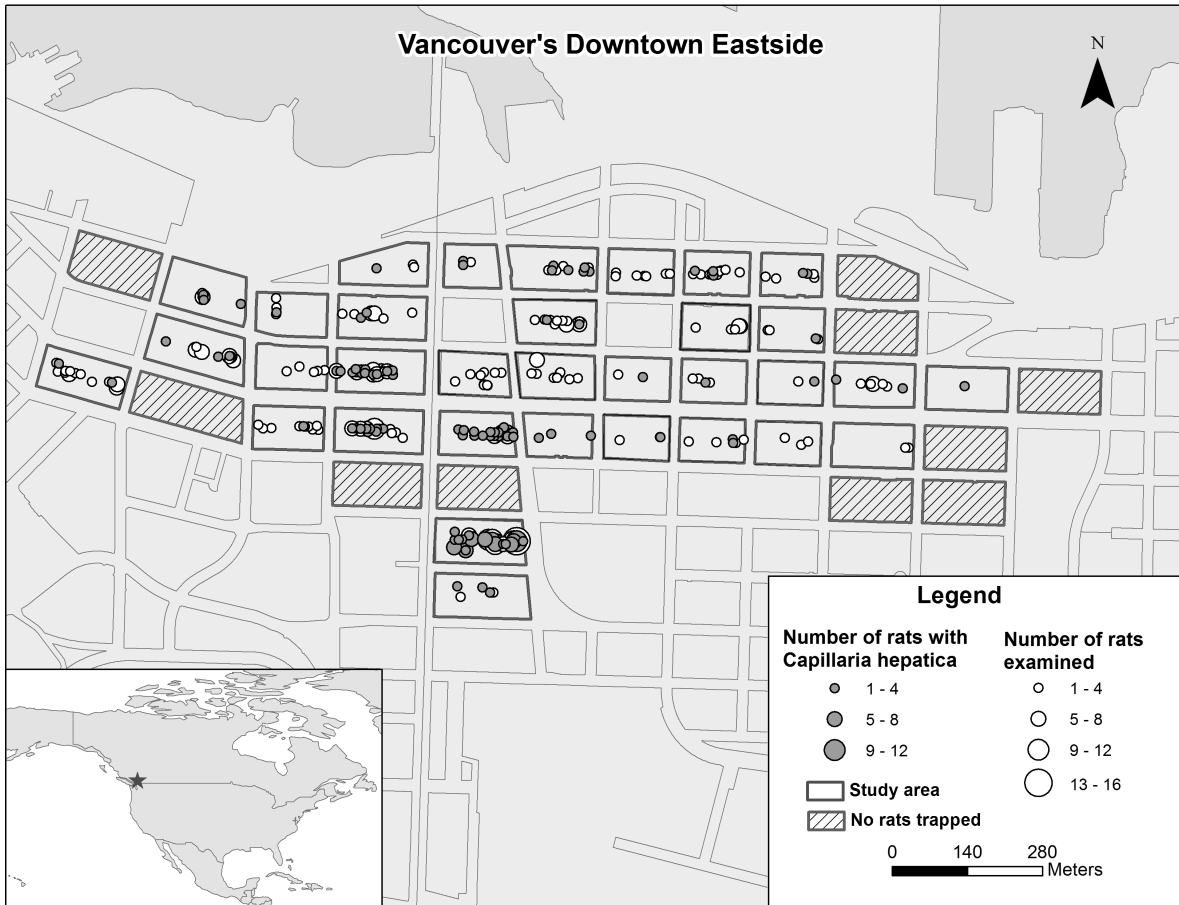
Figure 4. Distribution of *Capillaria hepatica* -positive Norway rats (*Rattus norvegicus)* relative to the number of rats examined in each city block in the Downtown Eastside, Vancouver, Canada.

Table 1. Characteristics and associations with *Capillaria hepatica* infection among a group of Norway rats (*Rattus norvegicus*) trapped in Vancouver, Canada from September 2011 to August 2012.

| Category | Subcategory[a] | Number of rats (%)[a] (*n*=672) | C. hepatica infection | | *P*-value[c] |
			Present (%)[b] (*n*=241)	Absent (%)[b] (*n*=431)	
Season	Fall	237 (35.3)	161 (66.8)	76 (17.6)	<0.001
	Winter	134 (19.9)	44 (18.3)	90 (20.9)	
	Spring	217 (32.3)	29 (12.0)	188 (43.6)	
	Summer	84 (12.5)	7 (2.9)	77 (17.9)	
Sex	Male	374 (55.7)	146 (60.6)	228 (52.9)	0.096
	Female	290 (43.2)	95 (39.4)	195 (45.2)	
Sexual maturity	Mature	389 (57.9)	208 (86.3)	181 (42.0)	<0.001
	Immature	219 (32.6)	25 (10.4)	194 (45.0)	
Weight (per 10 g)	Median (IQR)	145 (66 – 257)	238 (167 – 301)	77 (57 – 199)	<0.001
Fat score (categorical)	Poor (0)	277 (41.2)	41 (17.0)	236 (54.8)	<0.001
	Moderate (1)	193 (28.7)	90 (37.3)	103 (23.9)	
	Good (2)	196 (29.2)	107 (44.4)	89 (20.6)	
Fat score (continuous)	Median (IQR)	1 (0 – 2)	1 (1 – 2)	0 (0 – 1)	<0.001
Wound Presence	Yes	167 (24.9)	82 (34.0)	85 (19.7)	<0.001
	No	504 (75.0)	158 (65.6)	346 (80.3)	
Wound Number	Median (IQR)	0 (0 – 0)	0 (0 – 1)	0 (0 – 0)	0.004

[a] IQR = interquartile range
[b] Frequencies and percentages may not add to 100% because of exclusion of rats with missing data for the variable in question.
[c] Determined using logistical regression models

Table 2. Odds ratios (OR) with 95% confidence intervals (CI) for association between the presence of *Capillaria hepatica* with other variables among Norway rats (*Rattus norvegicus*) trapped in Vancouver, Canada from September 2011 to August 2012.

| Category | Subcategory | Unadjusted | | Adjusted (GLMM[b]) | |
		OR	95% CI	OR	95% CI
Season	Fall	Ref[a]		–	–
	Spring	0.07	0.04 – 0.12	0.14	0.05 – 0.39
	Summer	0.04	0.02 – 0.09	0.14	0.03 – 0.61
	Winter	0.23	0.15 – 0.36	0.34	0.13 – 0.84
Sex	Female	Ref		–	–
	Male	1.31	0.95 – 1.82	–	–
Maturity	Immature	Ref		–	–
	Mature	8.92	5.71 – 14.4	7.29	3.98 – 13.36
Weight (10g)		1.09	1.08 – 1.11	–	–
Fat score (categorical)	Poor (0)	Ref			
	Moderate (1)	5.03	3.27 – 7.84	–	–
	Good (2)	6.92	4.51 – 10.78	–	–
Fat score (continuous)		2.56	2.09 – 3.17	–	–
Wound Presence	No	Ref		–	–
	Yes	2.11	1.48 – 3.02	1.87	1.11 – 3.16
Wound Number		1.19	1.05 – 1.34	–	–

[a] Reference value for analysis.
[b] GLMM = Generalized linear mixed model (controlled for clustering by block)