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Endoparasites in dogs and cats diagnosed at the Veterinary Teaching Hospital (VTH) of the University of Prince Edward Island between 2000 and 2017. A large-scale retrospective study

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1	Endoparasites in dogs and cats diagnosed at the Veterinary Teaching Hospital (VTH) of the
2	University of Prince Edward Island between 2000 and 2017. A large-scale retrospective study
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20	
21	Abstract
22	
23	Although many studies on the frequency of endoparasites in dogs and cats in Canada have been
24	reported, seasonal and/or annual patterns are often not evaluated. The frequency and risk factors of
25	endoparasite infections from fecal samples of cats and dogs submitted to the Veterinary Teaching
26	Hospital of the Atlantic Veterinary College, University of Prince Edward Island-Canada were

determined, using univariable and multivariable logistic regression analyses. Investigated predictors 27 28 of endoparasitism available in the 2000 to 2017 database included sex, age, geographic origin and seasonality. A total of 15,016 dogs and 2,391 cats were evaluated for endoparasite status using 29 specific diagnostic tests: direct smear, Baermann, and/or 33% zinc sulfate solution in a standardized 30 centrifugal flotation method. Overall, twelve and eight parasite genera were detected in dogs and cats, 31 respectively. The overall proportional infection was 14.6%, and the cat population showed a higher 32 33 frequency of positivity to parasites compared to the dog population (P<0.001). The most frequent genera recovered in the whole population (dogs and cats), were Giardia duodenalis (5.2%), 34 Cystoisospora spp. (3.3%) and Toxocara spp. (3.2%). Endoparasitism levels were diagnosed more in 35 36 feces submitted from young, female intact dogs from PEI compared to the baselines of mature, sterilized male dogs from other provinces, respectively, and diagnoses occurred more often in autumn 37 months than in winter months. There was no significant diagnostic trend across the years for the 38 39 individual parasites models. The frequency of detected potentially zoonotic parasites in this study highlights the veterinary public health and One Health context of parasitic infections in pets. Although 40 the presented results are not from a random sample and therefore frequency results should be 41 interpreted with caution, the model relationship results may still be relevant. In addition, results are 42 43 of value to estimate parasite impact and to assist researchers, veterinarians and pet-owners with 44 suitable information to control parasites.

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47 Keywords: Veterinary Teaching Hospital, Endoparasites; Fecal examination; Epidemiology; Risk
48 factors; Pets; Canada.

49 1. Introduction

Parasitic infections are endemic worldwide and have been described as constituting the greatest single worldwide cause of illness and disease in animals and humans (Steketee, 2003). Parasites of companion animals are the cause of several disorders, including anorexia, anemia and diarrhea, mostly in young, old and immunocompromised subjects (Epe, 2009). A wide range of endoparasites are known to cause significant morbidity and mortality in pets, including several species of nematodes, cestodes, trematodes and protists

Many species of parasites infect pets, with significant interspecies transmission (Vélez-Hernandez 56 et al., 2014; Zanzani et al., 2014). Companion animals have been shown to transmit zoonotic parasites 57 including, Giardia duodenalis, Cryptosporidium parvum, Toxocara spp., Echinococcus granulosus, 58 Echinococcus multilocularis and hookworms of various species (Kapel et al., 2006; Antolová et al., 59 2009; Deplazes et al., 2011; Chen et al., 2012; Macpherson, 2013; Baneth et al., 2016; Kostopoulou 60 et al., 2017). Thus, public health control measures depend on knowing the frequency of these parasites 61 in the population. This zoonotic concern emphasizes the value of routine fecal evaluations for parasite 62 diagnosis in companion animals, even if the sensitivity of a single fecal exam in time can change, 63 depending on parasite biology, fecal examination method, and laboratory technician skill (Broussard, 64 2003). The close contact between pets and humans sharing the same environment increases the 65 transmission risk of different zoonotic diseases (Seah et al., 1975; Chomel and Sun, 2011; Togerson 66 and Macpherson, 2011; Esch and Petersen, 2013; Baneth et al., 2016). The number of dogs and cats 67 in Canada is estimated to be around 8.2 and 8.3 million, respectively. Approximately, 41% of 68 69 Canadian households have a dog, while 38% have a cat (CAHI, 2019). Therefore, veterinarians play a crucial role in zoonotic risk assessment and communication (Malloy and Embil, 1978; Villeneuve 70 71 et al., 2015), acting as a main public health operator.

Frequency and prevalence of endoparasite species may vary seasonally and from region to region within a country, depending on the biological variation and weather conditions (Oliveira-Sequeira et al., 2002). In a country as large and environmentally diverse as Canada, data from one region may not be applicable to another area of the country, and furthermore, few published papers have explored
seasonal patterns of endoparasitism in owned dogs and cats in Canada (Conboy, 2004; Blagburn et
al., 2008).

This large-scale retrospective study aims to investigate the frequency of endoparasites and the relative risk factors, in owned dogs and cats, from samples submitted to the Veterinary Teaching Hospital (VTH), Atlantic Veterinary College (AVC), University of Prince Edward Island (UPEI), Canada. Where possible, associations between endoparasite infections and age, sex, geographic origin, and seasonality were evaluated, based on availability of animal data.

83 Outcomes originated from this study should be an aid for clinical veterinarians to better educate
84 their clients about parasite occurrence and to use good preventive programs.

85

86 2. Materials & Methods

87 2.1 Study population and fecal examination

88 Between January 1, 2000 and November 17, 2017, fecal samples from privately owned pets from Prince Edward Island or other Canadian Provinces (primarily Atlantic Canadian provinces of Nova 89 Scotia, New Brunswick, and Newfoundland and Labrador) were submitted to the Parasitology 90 Laboratory of Diagnostic Services at the VTH, Atlantic Veterinary College (AVC), University of 91 Prince Edward Island (UPEI) for coprological analyses. The samples represent a mix of routine 92 diagnostic surveillance on clinically normal animals and diagnostic investigations on pets exhibiting 93 clinical signs suggestive of parasitism. Inclusion of a detailed history on sample submission by 94 95 referring veterinarians was inconsistent such that the true proportion of samples from normal animals and those with clinical signs was unknown. Stored data were organized in a database of variables 96 97 including; animal species, age, sex, geographic origin (based on home address), date of sample submission, diagnostic technique employed, and tests results. There were substantial missing data for 98 certain variables (e.g. age) because not all relevant data were submitted with the fecal sample. 99

For each sample, parasite eggs, cysts, oocysts and larvae were concentrated from fecal specimens 100 101 by using a 33% zinc sulfate solution (specific gravity = 1.18) in a standardized centrifugal flotation 102 method (Zajac and Conboy, 2012). Additional methods were employed, including: the Baermann technique for suspected lungworm cases, based on reported clinical signs of coughing and/or dyspnea, 103 to detect the presence of first-stage larvae (L1); and direct smears primarily for trophozoites and cysts 104 of Giardia duodenalis, based on reported clinical signs of diarrhea (Zajac and Conboy, 2012). For 105 106 much of the study period, capillarid eggs were not specifically identified at the Parasitology Laboratory of the VTH. Detection of capillarid eggs in dog fecal samples included Eucoleus 107 aerophilus and Eucoleus boehmi and those in cats would have been Aonchotheca putorii and E. 108 109 aerophilus.

110

111 2.2 Data management and statistical analysis

The initial dataset counted 36,838 rows, representing all the animal species fecal samples which passed through the Parasitology Laboratory at the VTH-Diagnostic Services during the study period. In order to deal with just dogs and cats, all other species were removed from the dataset, leaving 24,220 observations. All possible duplicate observations were removed (with the first occurrence of the animal in the dataset retained to enable age risk factor assessment), leaving 17,407 observations, 15,016 dogs and 2,391 cats.

Two datasets were constructed, one for dogs and one for cats. The first step was to give to each 118 single observation an unequivocal identification number because the numbering system used by the 119 120 Parasitology Laboratory ran sequentially but reverted back to 1 each year. It was possible to extrapolate the age of each subject by subtracting the date of birth from the date of the visit. In order 121 to make predictor results meaningful and statistically relevant, certain variables were recategorized. 122 The date of testing was used to create season of testing, according to 3-month categories following 123 124 the astronomical calendar, thus winter was December 21 to March 20; Spring was March 21 to June 125 20; Summer was June 21 to September 20; and Autumn was September 21 to December 20. Age was divided into two categories, ≤ one year old and > one year old. The main part of our sample originated
primarily from Prince Edward Island and the rest came from other Canadian provinces, so the variable
"geographic origin" was dichotomized as PEI and other. Unfortunately, some of the predictor
variables presented with missing values which led to a reduction in the number of samples available
for those analyses.

The frequency of isolated parasites was estimated as the number of positive animals/number of 131 132 examined animals, in total, and by animal species. Age, sex, animal species, geographic origin (region), year and seasonality were examined as putative risk factors for the commonly isolated 133 parasites using Pearson's χ^2 test. In order to assess the chance of there being significant differences 134 135 between the means of the annual numbers of samples from dogs and cats submitted to the VTH during the study period, a Student's t-test was conducted. Results were considered significant when $P \le 0.05$. 136 Five multivariable logistic regression analyses were also performed, keeping as our model 137 outcome the combined test results (parasite presence/absence) for dogs and for cats, and one model 138 for each of the 3 most common dog parasites. Backward stepwise regression was employed for the 139 model building, and model fitness was assessed by means of the Hosmer-Lemeshow goodness-of-fit 140 test. Odds ratios (OR) and their 95% confidence intervals (CI) were reported. Testing and analytic 141 142 control for confounding of model variables was done throughout the model-building process (Dohoo 143 et al., 2009). Interactions between significant model main effects were built and assessed for significant associations with the outcomes of interest. Stata Statistical Software (Release 15, College 144 Station, TX: StataCorp LLC) was utilized for the descriptive statistical analysis and the model 145 146 building, analyses and evaluations.

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148 3. Results
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¹⁵⁰ *3.1 Descriptive statistics*

Between January 1, 2000 and November 17, 2017, a total of 17,407 fecal samples from privately owned companion animals (15,016 dogs and 2,391 cats) were processed in order to detect endoparasites. On average, the frequency of samples submitted was 967.1 per year (SD=347.94), with a range from 580 to 1654. Dogs represented a large majority of the overall samples, with a mean of 834.2 submissions per year (SD=290.68; range: 508-1385), whereas cats represented a minority of the overall samples (mean samples per year = 132.8; SD=61.12; range: 64-269). A comparison of the two means resulted in a statistically significant difference on a t-test (t=3.75, P<0.001).

Other information collected at the time of the sample submission is summarized in Table 1. Of the 158 animals with age identified in the database, the proportion of cats less than 12 months of age was 159 160 twice the proportion for dogs (P<0.001). Of the female dogs identified in the database, nearly 2/3rds were spayed, whereas nearly 2/3rds of female cats were not spayed (P<0.001). Approximately 60% 161 of male dogs and cats identified in the database were neutered. Regarding the season of the submitted 162 163 animals to the VTH, 1/3rd of submitted samples for the dogs occurred in spring, and nearly 60% were during spring and summer, whereas 1/3rd of tested cats were in summer, and nearly 60% in summer 164 and autumn (P<0.001). Of dogs and cats where the origin was known, approximately 78% were living 165 in PEI (P<0.001). 166

The proportion of animals within the entire population found to be infected (eggs, cysts, oocysts, L1, and trophozoites) with at least one parasite genus was 14.6% (95%CI: 0.146±0.0052), with 17.2% (95%CI: 0.172±0.014) and 14.2% (95%CI: 0.142±0.002) from cats and dogs, respectively. This species difference was statistically significant on the Pearson χ^2 -test (P<0.001).

Table 2 shows the frequency for each parasite genus found separately in the dog and cat populations. Frequent parasites detected, both in dogs and cats, were *Giardia duodenalis* at 5.4% in dogs and 4.18% in cats, followed by *Cystoisospora* spp. at 2.9% and 5.81% in dogs and cats, respectively. Finally, *Toxocara* spp. was detected in 362 dogs and in 196 cats. The remaining genera of parasites were found less frequently.

Multiple infections were far less common than those with a single parasite type in both dogs and 176 177 cats (Table 3). Multiple infections were slightly more common in cats (13.3%) than in dogs (12.5%), although not statistically significant. Out of the 237 dogs with double infections, 103 (43.5%) 178 included G. duodenalis and Cystoisospora spp. parasites followed by G. duodenalis and Toxocara 179 canis (13.1%). Of the 27 dogs with triple infections, 9 (33.3%) included G. duodenalis, Cystoisospora 180 spp.and T. canis parasites, while only one dog had 4 parasite genera diagnosed: Crenosoma vulpis, 181 182 T. canis, Trichuris vulpis and Uncinaria. Of the 41 cats harbouring two different parasites, 11 (26%) included Cystoisospora spp. and Toxocara cati, whereas 9 (22%) had capillarids and Toxocara cati, 183 and another 9 had G. duodenalis and Cystoisospora spp.combined infections. Of the 9 cats positive 184 185 to 3 parasite genera, 4 (44.4%) included capillarids, Cystoisospora spp.and T. cati. Three cats were positive for 4 parasite genera, with two cats having Taenia spp. along with capillarids, Cystoisospora 186 spp.and T. cati, and the other one harboured G. duodenalis instead of capillarids. Only one cat hosted 187 188 five different parasites, and they were represented by capillarids, G. duodenalis, Cystoisospora spp., Taenia spp. and T. cati. 189

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191 *3.2 Univariable analytical statistics*

192 Tables 4 and 5 show the differences in proportions of samples diagnosed positive, by factors of 193 interest, for parasitism in general, and for the main parasite genera detected in dogs and cats, respectively. Determination of the Pearson γ^2 -test showed seasonal patterns for G. duodenalis. in dogs 194 (P=0.03), while there were no significant seasonal differences in cats (P=0.12). Conversely, cats were 195 196 more likely to be *Cystoisospora* spp. positive during summer than other seasons (P<0.001), whereas the probability of *Cystoisospora* spp. being positive was higher during autumn than other seasons in 197 dogs (P<0.001). For *Toxocara* spp., dogs were more likely to be positive in the autumn than other 198 times of the year (P<0.001), but there were no significantly different proportions among seasons 199 within the feline population (P=0.10). 200

Unfortunately, the dataset had numerous missing values; age, sex and province of origin were included in only 3,247 dogs and 412 cats. For this reduced dataset, younger dogs were parasitized more with *G. duodenalis* than older dogs (P<0.001), however this association was not found in cats (P=0.311). *Cystoisospora* spp. was significantly more often diagnosed in puppies than adult dogs (P<0.001), but age of kittens was not associated with diagnosis of *Cystoisospora* spp. (P=0.49). *Toxocara* spp. showed a significantly higher frequency in the young pets of both species (dogs: P<0.001 and cats: P=0.004).

The proportions of animals diagnosed positive for at least one parasite genus, by geographic region (Table 4 and 5), were found to differ significantly in dogs (P<0.001) but not in cats (P=0.29).

No statistical significances were found in the proportions positive for all genera combined, by sexual status, in both cats and dogs. When looking specifically at intact versus sterilized animals, which could change the possibility of exposure to parasites, there was no evidence for significant differences in cats for each of the three common parasites of interest (*G. duodenalis* P=0.107, *Cystoisospora* spp. P=0.724 and *T. cati* P=0.09). Conversely, intact dogs were more likely to be diagnosed infected for *G. duodenalis* (P<0.001), *Cystoisospora* spp. (P<0.001) and *T. canis* (P<0.001) than those that were sterilized.

These univariable analytical statistics should be interpreted with caution. The multivariable analytical statistics section provides more definitive results because there is statistical control for confounding of all variables in the final models.

220

221 *3.3 Multivariable analytical statistics*

Based on our 3,242 dog observations with data for the final multivariable logistic regression model (controlling for confounding - Table 6), being older than one year old was a protective factor against a diagnosis of parasitic infections by a factor of 4.3 times (4.3 is the inverse of the OR= 0.232) compared to puppies less than 1 year old. Sterilization, both male and female, was associated with decreased odds of having a parasite diagnosis by a factor of 1.60 compared to intact dogs (while male or female dogs had very similar odds). The province of origin also appeared to have an influence on parasite diagnosis; dogs from Prince Edward Island were 92% more likely to be infected than dogs not from PEI (1.92 is the inverse of the OR=0.521). Without controlling for confounding, 9.2% of PEI dogs were infected versus 4.0% of other dogs. Seasonality indicated that dogs were 65% more likely to be infected in the autumn compared to the winter season baseline (OR=1.65). The significant (P=0.012) trend across the years showed a decreasing odds of diagnosis by a factor of 1.04 or 4% for each additional year within the database (1.04 is the inverse of the OR=0.961).

A similar multivariable logistic regression model for cat parasite diagnoses was built through stepwise backward elimination, but age was the only predictor remaining in the model. The odds of being diagnosed positive for at least one parasite genus decreased after the first year of life by a factor of 2.44 (2.44 is the inverse of the OR=0.42; 95%CI: 0.24-0.72), as previously determined in the preliminary univariable logistic regressions for putative risk factors.

239 In order to better understand the epidemiology and risk factors of the 3 common parasites of dogs (G. duodenalis, Cystoisospora spp. and T. canis), a model was built using the frequency of diagnosis 240 for each parasite. No final logistic regression model was possible for Giardia duodenalis because no 241 predictor variables remained significant in the final model. Conversely, age was significant (P<0.001) 242 as a predictor for the diagnosis of Cystoisospora spp. (Table 7), where a dog being more than 1 year-243 244 old was 5.21 time less likely to be diagnosed positive than young dogs. Being sterilized, both for 245 females (OR=3.67) and males (OR=2.42), appeared to be a protective factor compared to the intact female baseline, while intact male dogs had similar odds to intact female dogs. Dog samples coming 246 247 from outside of PEI were 4.61 times less likely to be diagnosed positive for *Cystoisospora* spp. than dogs from PEI. Additionaly, autumn appeared as a risk factor for Cystoisospora spp. with an OR=2.76 248 compared to the winter baseline (Table 7). Spring and summer only had trends toward higher risk for 249 Cystoisospora spp. infection. 250

The *T. canis* final logistic regression model (Table 8) showed that dogs older than 1-year were close to 6 times less likely of being diagnosed positive for *T. canis* compared to younger dogs. Samples submitted from PEI resulted in 1.7 times higher odds of being *T. canis* positive than other provinces (listed in Table 1), although the odds ratio was not significant (P=0.132), but the variable was retained in the model because it was a confounder for other variables in the final model. Regarding the sexual status, only the castrated male category showed a significant protective association of 2.72 compared to the intact female baseline as with the *Cystoisospora* spp. model, dogs with *T. canis* were 2.67 times more likely to be positive in autumn than the winter baseline (Table 8). There was no significant diagnostic trend across the years for the individual parasites models.

260

261 **4. Discussion**

This study of fecal samples of cats and dogs submitted to the Veterinary Teaching Hospital of the University of Prince Edward Island has provided useful annual and seasonal information on their proportional endoparasitic infection levels, as well as risk factors (i.e. year, season, age, sex and region) for endoparasite infections diagnosed within the study population in Canada.

The cat population has always been estimated to be larger than the dog population in Canada, however the two populations are nearly equal now, with 8.3 million cats and 8.2 million dogs (CAHI, 2019). Conversely, the proportion of dog samples submitted was 7 times higher than cat samples, which was consistent with other studies of similar methodology (Lue et al., 2008; Sánchez-Vizcaíno et al., 2017). In our population, dogs were more often neutered (63.8%) than cats (51.2%), with other studies showing similar owners' attitudes toward pet neutering (Lund et al., 1999; Sánchez-Vizcaíno et al., 2017) in the USA and UK, respectively.

The results presented in this paper show a relatively low frequency of fecal endoparasites in pets; 17.2% of the examined cats and 14.2 % of the dogs were shown to be infected with at least one parasite genus. A conference abstract of a nationwide study on dogs with constant or regular access to the outdoors, carried out in Italy by Brianti et al. (2018), reports twice the percentage of our survey. However, the samples submitted to the VTH in our study were from a population of dogs that would

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have been a mix of animals from urban, suburban and rural areas and included both clinically normalanimals and those exhibiting clinical signs of disease.

Cats were significantly more parasitized than dogs in our study population, likely due to the higher 280 likelihood of cats being free to roam outdoors compared to dogs, thereby increasing the probability 281 282 of contact with infectious parasitic stages from environmental sources. This is opposite to the results of Barutzki and Schaper (2011), in a similar large-scale study carried out on fecal samples from 283 284 German dogs and cats submitted to a Veterinary Laboratory for parasitological examination. On the contrary a survey carried out in Italy by Riggio et al. (2013) showed the same discrepancy in parasite 285 prevalence of 35% and 31% in cats and dogs, respectively. Additionally, Tamponi et al. (2017) 286 287 pointed out a higher infection risk in cats compared to dogs in companion animals living in Sardinia 288 region, an Italian Mediterranean Island; unfortunately, no hypotheses were provided. Compared with other Canadian studies, we found a lower frequency of infection, which again may be due to our study 289 290 population being from VTH submissions rather than a random sample. Most other Canadian study results are from western Canada (Stull et al., 2007; Bridger and Whitney, 2009; Joffe et al., 2011) or 291 Ontario and Quebec (Shulka et al., 2006; Blagburn et al., 2008) or from shelter dogs and cats 292 (Villeneuve et al., 2015). However, in a country as large as Canada, data and risk factors from one 293 region could be different from another region. 294

The final model result of dogs coming from provinces other than Prince Edward Island having significantly lower odds of being positive for parasites is also likely due to differences in the many host, agent and environmental factors that affect the probability of a dog harboring parasites in a country as large and heterogeneous as Canada.

Proportionately, all Canadian studies have shown that *G. duodenalis* and *Toxocara* spp. are the most common parasites infecting cats and dogs (Seah et al., 1975; Malloy and Embil, 1978; Shulka et al., 2006; Villeneuve et al., 2015). The infection risk for *G. duodenalis* in Europe is reported to be approximately 25% and 20% in dogs and cats, respectively (Epe et al., 2010) by using antigen snaptests. Carlin et al. (2006) reported a lower infection rate than Epe et al. (2010) but still higher than the

present study; 15.6% among dogs tested, and 10.8% among cats. utilizing the same diagnostic test 304 used by Epe et al. (2010). These two European studies with the snap-test likely have higher estimates 305 306 because antigen can be detected even after the organism has been eliminated. Our lab does perform G. duodenalis diagnosis by zinc sulfate solution, having a Se=86%, Sp=98%, which is similar to the 307 308 snap-test of Se=91%, Sp=96%. Additionally, Uehlinger et al. (2017) have demonstrated the reliability of ZnSO4 floatation technique compared to snap antigen-test. Both G. duodenalis and Toxocara spp. 309 310 parasite species are responsible for human infections. It is estimated that about 200 million people in Asia, Africa and Latin America have shown giardiasis clinical manifestations (Feng and Xiao, 2011). 311 Regarding human toxocariasis, Lee et al., (2014) reported 10% seroprevalence in North America from 312 313 a meta-analysis.

According to some studies, monoparasitism appears to be much more common than polyparasitism in pets presented to a teaching hospital (Kirkpatrick, 1988; Sudan et al., 2015), monoparasitism (38.1%) was less frequent than polyparasitism (46.7%). Additionally, the low socio-economic conditions can affect the host-parasite relationship, leading to more pet scavenging for food and therefore more parasite exposure (Schurer et al., 2014).

Regarding seasonal variation in endoparasite frequency, submitted samples from dogs more 319 320 frequently harboured parasites during autumn in our study for the main endoparasites found, whereas 321 cats were much more frequently and significantly diagnosed in summer just for Cystoisospora spp. 322 Dogs may have increased likelihood of exposure during the summer months when there are longer days with more light, and the fact that people and their dogs spend more time outside, leading to more 323 324 diagnosed infections in autumn. Favourable climatic conditions occurring during the summer may also allow for better parasite development within the environment and hosts. Seasonal effects on 325 parasitism may reflect parasitic responses to changes in photoperiod, leading to lower shedding in 326 winter (Polley and Thompson, 2009). 327

In Italy, the seasonal pattern was studied only for *G. duodenalis* and only for dogs, where a higher prevalence was found in winter (Bianciardi et al., 2004). A similar result was found in Argentina in the late winter (Fontanarrossa et al., 2006). Similar seasonal results were obtained by Kirkpatrick
(1988) in dogs of Pennsylvania in the 1980s, whereas no seasonality was detected by Nolan and Smith
(1995) in the following decade in the same area. Due to the historic nature of these surveys in
Pennsylvania, and the effects of climate change on parasite and host physiology, their results should
be interpreted with caution (Polley and Thompson, 2009).

Regarding dog-level risk factors associated with being positive for parasites, the logistic model built for dogs indicated that neutering was associated with 60% lower parasitic infections compared to intact animals. Possible reasons for this association (not causal) could be that sterilization may reduce the natural instinct of dogs to roam in search of a mate, thereby reducing exposure to parasitic infections. Additionally, owners who sterilized their pets could be more likely to prevent roaming and to use preventative anti-parasitic products or treat dogs for parasites than those who did not.

The observed trend across the 18 years of this large-scale retrospective study found a significant decrease in the number of parasite-positive dogs (Table 6). This trend likely reflects an increased awareness among dog owners about parasitic diseases, increased use of deworming and preventative protocols, and the improved efficacy of new products on the market (Palmer et al., 2010). The odds of being positive for parasites decreased by 4% each year, which is in agreement with other studies (Barutzki and Schaper, 2013; Sudan et al., 2015; Tamponi et al., 2017).

347 The final logistic model for all parasites combined in cats showed only that age was a putative risk factor. The odds of a cat being positive for at least one parasite genus was 2.44 times less in adults 348 compared to kittens, likely due to parasite-specific immunity that is usually acquired with age, and 349 350 the eventual single or repeated exposures to parasites during the mature life of a cat (Ramirez-Barrios et al., 2004). In Canada, cats come into heat any time between January and October, although they 351 are focused around the longest day of the year in June (Bronwyn Crane personal communication). 352 Dogs are not seasonal, with the exception of the Basenji breed. All other breeds usually have a 6-353 354 month inter-estrus interval and heats are randomly spread throughout the year.

The two genus-specific parasite models in dogs, for *Cystoisospora* spp. and *T. canis* (Tables 7-8), found similar results compared to the model built for predictors associated with the presence/absence of at least one parasite (Tables 6). With these genera making up a large portion of the parasites found in the dogs in the dataset, it is not surprising to find these similarities in results. Due to the smaller number of dogs specifically with *T. canis* infections, sterilized females and geographic origin were no longer significantly associated with infection, but the odds ratios were similar to the model for combined parasite presence.

As previously indicated, the main limitation to the interpretation of the study results is that 362 endoparasite data from diagnostic laboratories often carry a selection biases, in that they are based on 363 364 incomplete data submitted, and they reflect the situation in well-cared for animals that are being 365 checked for endoparasite. Our results from the fecal analyses provide an overview about the frequency of which endoparasites are diagnosed in dogs and cats. Despite the selection biases, the 366 367 results of the risk factor analyses may still reflect relationships found in a randomly selected population. In particular, the longitudinal nature of this dataset provided results regarding annual and 368 seasonal patterns. An additional limitation is the imperfect sensitivities of the tests utilized by the 369 laboratory which would likely lead to under-estimates of the frequency of infection. 370

371 Based on the public health concern of our results, control measures need to be considered for both 372 Toxocara spp. and G. duodenalis. Prevention of initial contamination of the environment can be 373 achieved by eliminating patent infections in dogs and cats, and/or preventing defecation by pets in public areas unless owners clean up after their pets. Furthermore, education of the public on proper 374 375 hygiene prior to ingestion of food is important (Overgaauw and van Knapen, 2013). As G. duodenalis is only minimally zoonotic, it may be necessary to treat and isolate positive dogs (or isolate their fecal 376 377 matter) in order to protect other dogs and cats from infection and to prevent environmental contamination with potentially zoonotic cysts (Bowman and Lucio-Forster, 2010). 378

Future research that compares longitudinal data from other parts of Canada or North Americawould help to corroborate the results found from the VTH database in PEI. Furthermore, evaluating

if owners' attitudes toward parasitic infections affect the probability of parasite infections in pets inPEI or elsewhere would also be helpful.

383

384 5. Conclusion

This secondary-data study provides an impression of the parasitic infection status in submitted 385 fecal samples from dogs and cats in Prince Edward Island and other Canadian provinces, during an 386 eighteen-year period. Endoparasitism was diagnosed less commonly in mature, sterilized male dogs, 387 from other provinces compared to the baseline of young, female intact dogs from PEI, and diagnoses 388 occurred more often in autumn months than in winter months. G. duodenalis, Cystoisospora spp. and 389 390 Toxocara spp. were the three most common genera of parasites found in dogs, and the same significant risk factors were also found in the models for the last two parasites. Only age was a 391 392 significant risk factor to fecal parasitism in cats in the dataset, with young cats having higher endoparasitism levels compared to mature cats. 393

Although these data represent only the group of concerned pet owners who used the services of a 394 395 referral veterinary teaching hospital, it is still relevant to obtain information about the presence of parasites, especially in relation to the zoonotic potential of pets sharing the same environment with 396 human beings. These concerned pet owners are likely more intimately sharing their environment with 397 their pets than owners who are less concerned about their pets. Knowing the trend of parasitic 398 infections across the geography, years, seasons, species, sexual status and age provides a perspective 399 to clinicians and researchers that can guide them when estimating the risk and impact of parasites and 400 401 when communicating control measures using a One Communication concept (Cipolla et al., 2015).

402

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552 Highlights:

553	•	Parasites occur even in owned companion animals under veterinary care
554	•	Cats are significantly more frequently diagnosed positive for parasites than dogs
555	•	Two of the three most frequently diagnosed parasite genera in dogs are zoonotic or
556		potentially zoonotic: Toxocara and Giardia, respectively
557	•	Parasites were significantly more likely to be diagnosed in young dogs in PEI in autumn in
558		the first years of the laboratory results, compared to mature sterilized dogs outside of PEI
559		during other times of the year

Predictors	Categories	Dogs (%)	Cats (%)	P-Value
	$\leq 12 \text{ months}$	709 (21.8)	164 (39.8)	-0.001
Age*	>12 months	2,538 (78.2)	248 (60.2)	<0,001
	Total	3,247	412	
	Male	641 (19.7)	86 (21.4)	
Sexual status*	Female	534 (16.5)	110 (27.4)	
Sexual status*	Castrated male	1,047 (32.2)	127 (31.6)	< 0.001
	Sterilized female	1,025 (31.6)	79 (19.6)	
	Total	3,247	402	
	PEI	2,479 (76.3)	362 (87.7)	
Geographic	Others**	769 (23.7)	51 (12.3)	< 0.001
origin*	Total	3,248	413	
	Winter	2,905 (19.3)	497 (20.8)	
C 114-	Spring	4,564 (30.4)	503 (21)	
Seasonality	Summer	4,162 (27.7)	686 (28.7)	< 0.001
	Autumn	3,385 (22.6)	705 (29.5)	
	Total	15,016	2,391	

Table 1: Descriptive statistics of predictors, by category, for 15,016 dogs and 2,391 cats diagnosed at the Atlantic Veterinary College from 2000-2017, along with comparisons between dogs and cats.

*Predictors with missing values. PEI= Prince Edward Island. **Other Provinces include (Alberta,

British Columbia, New Brunswick, Nova Scotia, Newfoundland and Labrador, Ontario, Quebec, and Saskatchewan). P-value based on the Pearson χ^2 -test.

	Descritter	Dogs (n	=15,016)	Cats (r	n=2,391)	T (1 0 (
	Parasites	n	%	n	%	Total %
Nematoda	Ancylostoma	110	0.73	1	0.04	0.63
	Angiostrongylus vasorum	145	0.97	-	-	
	Capillarids*	17	0.11	24	1.00	2.35
	Crenosoma vulpis	280	1.86	-	-	
	Toxascaris leonina	17	0.11	2	0.08	0.11
	Toxocara canis/cati	362	2.41	196	8.2	3.21
	Trichuris vulpis	64	0.43	-	-	
	Uncinaria	114	0.76	1	0.04	0.66
Trematoda	Alaria	27	0.18	-	-	
Cestoda	Taenia	17	0.11	21	0.88	0.22
Protozoa	Giardia duodenalis	811	5.4	100	4.18	5.23
	Cystoisospora	437	2.91	139	5.81	3.31

Table 2: Frequency of the endoparasites found in 15,016 dogs and 2,391 cats diagnosed at the Atlantic Veterinary College during 2000-2017.

*Includes Aonchotheca putrorii and Eucoleus aerophilus (cats) and Eucoleus aerophilus and Eucoleus boehmi (dogs).

# of different	Positive dogs (n=2,128)		Positive of	Te4al 0/	
parasitism	n	%	n	%	Total %
1	1,863	87.55	358	86.89	87.4
2	237	11.14	41	10.19	9.95
3-4	28	1.31	12	2.91	1.58
5	-	-	1	0.25	0.04

Table 3: Proportions of single and multiple parasitism infections in 2,128 positive dogs and 412 positive cats diagnosed at the Atlantic Veterinary College from 2000-2017

Table 4: Frequency of the three main parasite genera in dogs, by age group, sex, geographical origin and seasonality of diagnosis, diagnosed at the Atlantic Veterinary College from 2000-2017.

Indonendant	No. of		No. of po	sitive dogs (%)	
Independent variables/category	tested dogs	Toxocara canis	Giardia duodenalis	<i>Cystoisospora</i> spp.	All genera
Age group*					
≤ 12 months	709	47 (6.6)	57 (8)	43 (6.1)	146 (20.6)
>12 months	2,538	21 (0.8)	22 (0.9)	21 (0.8)	112 (4.4)
Total	3,247	132 (4.1)	79 (2.4)	64 (2)	258 (7.9)
Sexual status*					
Male	641	25 (3.9)	30 (4.7)	19 (3.0)	82 (12.8)
Female	534	23 (4.3)	21 (3.9)	27 (5.1)	72 (13.5)
Sterilized female	1,025	12 (1.2)	18 (1.8)	7 (0.7)	51 (5)
Castrated male	1,047	8 (0.7)	9 (0.9)	11 (1.1)	52 (5)
Total	3,247	68 (2.1)	79 (2.4)	64 (2.0)	257 (7.9)
Geographical origin*					
Prince Edward Island	2,479	60 (2.4)	67 (2.7)	61 (2.5)	227 (9.2)
Other	769	8 (1.0)	12 (1.6)	3 (0.4)	31 (4)
Total	3,248	68 (2.1)	79 (2.4)	64 (2.0)	258 (7.9)
Seasonality					
Winter	2,905	83 (2.9)	171 (5.9)	55 (1.8)	474 (16.3)
Spring	4,564	76 (1.7)	212 (4.7)	106 (2.3)	558 (12.2)
Summer	4,162	74 (1.8)	225 (5.4)	151 (3.6)	568 (13.6)
Autumn	3,385	129 (3.8)	203 (6.0)	125 (3.7)	528 (15.6)
Total	15,016	362 (2.4)	811 (5.4)	437 (2.9)	2,128 (14.2)

*Variables containing missing values

Table 5: Frequency of the three main parasites genera in cats, by age group, sexual status, geographical origin and season of diagnosis, diagnosed at the Atlantic Veterinary College from 2000-2017.

Indonondont	No. of		No. of pos	sitive cats (%)	
Independent variables/category	tested cats	Toxocara cati	Giardia duodenalis	<i>Cystoisospora</i> spp.	All genera
Age group*					
≤ 12 months	164	22 (13.4)	6 (3.7)	9 (5.5)	36 (21.9)
>12 months	248	13 (5.2)	5 (2.0)	10 (4.0)	26 (10.5)
Total	412	35 (8.5)	11 (2.7)	19 (4.6)	62 (15.0)
Sexual status*					
Male	86	5 (5.8)	4 (4.6)	2 (2.3)	12 (14.0)
Female	110	13 (11.8)	4 (3.6)	7 (6.4)	23 (20.9)
Sterilized female	79	2 (2.5)	-	2 (2.5)	4 (5.1)
Castrated male	127	8 (6.3)	3 (2.4)	6 (4.7)	15 (11.8)
Total	402	28 (7.0)	11 (2.7)	17 (4.2)	54 (13.4)
Geographical origin*					
PEI	362	33 (9.1)	11 (3.0)	15 (4.1)	56 (15.5)
Other	51	2 (3.9)	-	4 (7.8)	6 (11.8)
Total	413	35 (8.5)	11 (2.7)	19 (4.6)	62 (15.0)
Seasonality					
Winter	492	44 (8.9)	23 (4.7)	14 (2.8)	73 (14.8)
Spring	505	30 (5.9)	14 (2.8)	14 (2.8)	55 (10.9)
Summer	701	53 (7.6)	25 (3.6)	62 (8.8)	142 (20.2)
Autumn	693	69 (9.9)	38 (5.4)	49 (7.1)	142 (20.5)
Total	2,391	196 (8.2)	100 (4.2)	139 (5.8)	412 (17.2)

*Variables containing missing values. PEI= Prince Edward Island

Table 6: Predictors of the final logistic regression model of factors associated with parasites presence in 3,242 dogs diagnosed at the Atlantic Veterinary College from 2000-2017.

Predictors	Odds Ratio	95%CI	P-value	Overall P-value
Year	0.961	0.931-0.991	0.012	
Age*				
≤ 12 months	baseline	baseline	-	
>12 months	0.232	0.174-0.311	< 0.001	
Sexual status*				
Female	baseline	baseline	-	
Male	0.978	0.687-1.392	0.904	0.026
Sterilized female	0.627	0.419-0.938	0.023	0.026
Castrated male	0.624	0.419-0.931	0.021	
Geographic origin*				
PEI	baseline	baseline	-	
Others	0.521	0.351-0.774	0.001	
Seasonality				
Winter	baseline	baseline	-	
Spring	1.201	0.781-1.845	0.404	0.011
Summer	1.461	0.974-2.191	0.067	
Autumn	1.647	1.076-2.523	0.022	

* Variables containing missing values; PEI= Prince Edward Island. P-value based on the Wald test

Predictors	Odds Ratio	95% CI	P-value	Overall P-value
Age*				
≤ 12 months	baseline	baseline	-	
>12 months	0.192	0.108-0.341	< 0.001	
Sexual status*				
Female	baseline	baseline	-	
Male	0.577	0.314-1.062	0.077	0.005
Sterilized female	0.272	0.113-0.652	0.004	0.005
Castrated male	0.413	0.195-0.876	0.021	
Geographic				
origin*				
PEI	baseline	baseline	-	
Others	0.217	0.065-0.676	0.009	
Seasonality				
Winter	baseline	baseline	-	
Spring	2.181	0.844-5.634	0.107	0.042
Summer	2.399	0.960-5.993	0.061	0.042
Autumn	2.759	1.074-7.085	0.035	

Table 7: Predictors of the final logistic regression model of factors associated with *Cystoisospora* spp. presence in 3,242 dogs diagnosed at the Atlantic Veterinary College from 2000-2017.

*Variables containing missing values; PEI=Prince Edward Island. P-value based on the Wald test

Table 8 Predictors of the final logistic regression model of factors associated with Toxocara
<i>canis</i> presence in 3,242 dogs diagnosed at the Atlantic Veterinary College from 2000-2017.

Predictors	Odds Ratio	95% CI	P-value	Overall P-value
Age*				
≤ 12 months	baseline	baseline	-	
>12 months	0.168	0.094-0.297	< 0.001	
Sexual status*				
Female	baseline	baseline	-	
Male	0.948	0.524-1.713	0.860	0.012
Sterilized female	0.589	0.277-1.250	0.168	0.012
Castrated male	0.368	0.157-0.862	0.021	
Geographic origin*				
PEI	baseline	baseline	-	
Others	0.558	0.262-1.191	0.132	
Seasonality				
Winter	baseline	baseline	-	
Spring	0.756	0.309-1.850	0.541	-0.001
Summer	1.232	0.566-2.681	0.598	< 0.001
Autumn	2.674	1.270-5.628	0.010	

*Variables containing missing values; PEI=Prince Edward Island. P-value based on the Wald test.