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Short Communication

Prevalence and distribution of *Dirofilaria immitis* infection in wild canids in southern Ontario

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ABSTRACT

Wild canids represent a potential reservoir host for *Dirofilaria immitis* infection in dogs in Ontario. Since wild canids are not protected by chemoprophylaxis, understanding the epidemiology of *D. immmitis* in these populations may help elucidate the background risk of infection for dogs. The aim of this study was to determine the prevalence and distribution of *D. immitis* infection in wild canids across southern Ontario. From February 2016 to March 2017, 290 wild canid carcasses (273 coyotes and 17 foxes) were collected from across the region and assessed for the presence of *D. immitis* at the time of necropsy. Overall, *D. immitis* infection was identified in 4.8% (95% CI 2.8–8.0%) of these wild canid carcasses. Among coyotes, 5.1% (95% CI 3.0–8.5%) were positive; no evidence of *D. immitis* was found in the 17 foxes. *Dirofilaria immitis* infections in wild canids were detected in two regions of southern Ontario: 12 of the 14 *D. immitis* infections were detected in the south-western region and two were detected in the eastern region. Our findings provide preliminary insights into the prevalence and geographical distribution of *D. immitis* in coyotes and foxes in southern Ontario.

1. Introduction

Dirofilaria immitis (heartworm) has been recognized as endemic in Canada since 1977, with the majority of cases occurring in Ontario (Slocombe, 1978). As such, Ontario has been a major focus of *D. immitis* chemoprophylaxis and surveillance programs for dogs in Canada (Klotins et al., 2000). What is known about *D. immitis* infection in dogs in the province is largely based on two sources. The first consists of survey data from 1977 to 2010 that describes the number of *D. immitis* cases, the number of dogs tested, and the number of dogs on chemoprophylaxis, as voluntarily reported by veterinary clinics across the province (Slocombe, 1978; McGill et al., 2019). The second consists of *D. immitis* antigen test results from in-clinic testing and submissions to diagnostic laboratories from 2007 to 2016 (Herrin et al., 2017; Evason et al., 2019); preventive medication and travel history is not available for the dogs included in this data set. Based on these data, the prevalence of *D. immitis* infection in dogs in the province is low, with the most recent reported infection prevalence of 0.12% in dogs from 2008 to 2015 (Evason et al., 2019). However, the above data sources are from dogs that visit veterinary clinics; dogs that do not visit veterinary clinics are naturally underrepresented. This latter subset of dogs is unlikely to be on heartworm chemoprophylaxis as all heartworm preventive products in Canada are prescription-only medications. Since such dogs are at greater risk for *D. immitis* infection, it is likely that the true transmission rates and background risk of infection for dogs in Ontario are being underestimated.

With respect to spatial distribution, the risk of *D. immitis* infection in dogs in Canada varies geographically and is considered highest in southern Ontario. Historically, an endemic focus for *D. immitis* infection in dogs in southern Ontario has been the south-western region. A second endemic focus in the eastern region was recognized in the early 1990's (Slocombe and Villeneuve, 1993). These foci continue to represent higher risk areas for dogs in Ontario; McGill et al. (2019) found that the risk of *D. immitis* infection for dogs was 7 times and 2.6 times greater in census divisions from the south-western and eastern regions, respectively, of southern Ontario compared to dogs from the rest of Ontario.

Untreated dogs and wild canids, including coyotes (*Canis latrans*), which thrive in rural and urban regions, are potential sources of *D. immitis* infection for dogs in North America (Brown et al., 2012). The

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presence of wildlife reservoirs increases the risk of *D. immitis* infection in dogs, even if most dogs in the area are on chemoprophylaxis (Bowman and Atkins, 2009). Since these wild populations are not protected by chemoprophylaxis, infection prevalence data are important for understanding the true transmission dynamics in a region; such information could help assess the background risk of infection for dogs (Brown et al., 2012; Wang et al., 2014).

In contrast to the data available for *D. immitis* infection in dogs, there is a paucity of knowledge regarding the infection in wildlife in Ontario. *Dirofilaria immitis* infections have been reported in foxes (*Vulpes vulpes*) and coyotes in southern Ontario since 1979 (Slocombe and McMillan, 1980; Slocombe, 2002, 2011); however, to the authors' knowledge, no large-scale survey appears to have been conducted. Thus, the role of wild canids in the epidemiology of *D. immitis* in the region is unknown. The objectives of the present work were to describe the prevalence and spatial distribution of *D. immitis* infection in coyotes and foxes across southern Ontario.

2. Methods

2.1. Study population

Wild canid carcasses were obtained as part of a study investigating *Echinococcus multilocularis* in wild canids in southern Ontario. Details pertaining to sample collection are described by Kotwa et al. (2019). Briefly, from February 2016 to March 2017, wild canid carcasses were collected from across southern Ontario and were obtained through collaboration with licensed hunters and trappers and the Ontario Ministry of Natural Resources and Forestry. The geographic location of origin (i.e., latitude and longitude) was recorded for each carcass.

The *D. immitis* infection status of each wild canid was determined at the time of necropsy by examining for the presence of *D. immitis*. This method is considered the gold standard for *D. immitis* diagnosis (Atkins, 2003). Starting at the caudal vena cava, the heart was dissected following the blood flow; the right atrium and the right ventricle were dissected along the septum into the pulmonary artery (Henry et al., 2018). Dissection continued following major pulmonary arterial branches and terminated at the branching of the left and right lobar arteries (Henry et al., 2018). Worm burdens were quantified when the parasites were not damaged by the hunting process. Wild canids were excluded from the study if the heart was destroyed by the hunting process.

2.2. Statistical analyses

Confidence intervals (CI) for *D. immitis* prevalence were estimated using the Agresti-Coull CI method (Agresti and Coull, 1998). Resultant diagnostic data were plotted on a map of southern Ontario according to the latitude and longitude of each *D. immitis*-positive and -negative wild canid. Graphic displays were produced via QGIS 2.14.3 (Quantum GIS Development Team; http://www.qgis.org). All statistical analyses were conducted using Stata/SE 15.1 (StataCorp, College Station, Texas, USA; http://www.stata.com).

3. Results

Of the 310 wild canids collected, 290 were suitable for examination. These included 91 (86 coyotes and 5 foxes) collected in 2016 and 199 (187 coyotes and 12 foxes) collected in 2017. Wild canids were collected between January and May each year; 92% were collected between January and March.

Dirofilaria immitis infections were identified in 4.8% (14/290; 95% CI 2.8–8.0%) of the wild canids examined. Among coyotes, 5.1% (14/273; 95% CI 3.0–8.5%) were positive; no evidence of *D. immitis* parasites were found in the 17 foxes examined (95% CI 0–21.6%). Of the 14 *D. immitis* infections identified, worm burden was assessed for 9 infections. The median worm burden for the infections that were assessed was 5 adult parasites (range 3 to 27) (Table 1); no immature parasites were detected. *Dirofilaria immitis* infections were found primarily in carcasses from two regions of southern Ontario: 12 of the infections were detected in census subdivisions in the south-western region and two were detected in the eastern region (Fig. 1).

Table 1
Median number and range of *Dirofilaria immits* parasites identified in the heart and pulmonary arteries from 9 wild canids in southern Ontario, from 2016 to 2017.

D. immitis parasites	Median	Range
Total	5	3–27
Female	2	1–8
Male	3	1–19

4. Discussion

To the best of the authors' knowledge, this is the first survey of *D. immitis* infection in wild canids in southern Ontario. Overall, *D. immitis* infection was detected in 4.8% (14/290) of the wild canids examined. The overall prevalence is low compared to studies in the south-eastern United States that have reported prevalence estimates ranging from 16% to 100% in wild canids, depending on geographic area and age class considered (Custer and Pence, 1981; Nelson et al., 2003). However, the latter region is a major endemic focus for *D. immitis* infection in North America (Bowman et al., 2009) because the warmer, more humid, climate creates environments conducive to faster parasite development in the vector host and longer transmission periods (Bowman and Atkins, 2009). In contrast, the cooler climate in southern Ontario hinders *D. immitis* development for parts of the year and limits the transmission period of the parasite from June to October (Slocombe et al., 1995).

Notably, *D. immitis* infection was not detected in any of the 17 foxes examined in the present study. It is possible this reflects a lack of power as a result of the small number of foxes sampled. Alternatively, our negative findings may support work which suggests that foxes are unimportant reservoir hosts for *D. immitis*, largely due to low burdens of adult *D. immitis* that are typically found in surveyed fox populations (Simmons et al., 1980; King and Bohning, 1984; McCall et al., 2008). Nevertheless, although we did not find evidence of the parasite in the foxes we examined, a large confidence interval associated with our small sample size makes it difficult to draw any reasonable conclusions concerning the parasite in Ontario foxes.

The prevalence of infection in wild canids in southern Ontario observed in the present study is considerably higher than the estimated 0.12% prevalence in dogs from 2008 to 2015, as determined by antigen testing of dogs across this region (Evason et al., 2019). However, that prevalence estimate for dogs is likely biased by dogs on chemoprophylaxis and does not indicate the true risk for dogs in the province (Herrin et al., 2017; Evason et al., 2019). Thus, it would be more appropriate to compare infection in wild canids to infection in unprotected dogs since neither population is protected by chemoprophylaxis (Wang et al., 2014). Unfortunately, recent data pertaining to *D. immitis* infection in unprotected dogs in Ontario are currently limited.

Lastly, it should be mentioned that 12 of the 14 *D. immitis* infections were detected in wild canids within the south-western region of southern Ontario (Fig. 1). As previously mentioned, this region is considered an endemic focus for *D. immitis* infection in dogs in the province (McGill et al., 2019); the concentration of *D. immitis*-positive wild canids in this area suggests that spatial patterns of infection may be similar among wild canids and dogs in the western region of southern Ontario. Additionally, two of the 14 *D. immitis* infections in wild canids were identified in the eastern region of the study area, another endemic focus for *D. immitis* infection in dogs (McGill et al.,

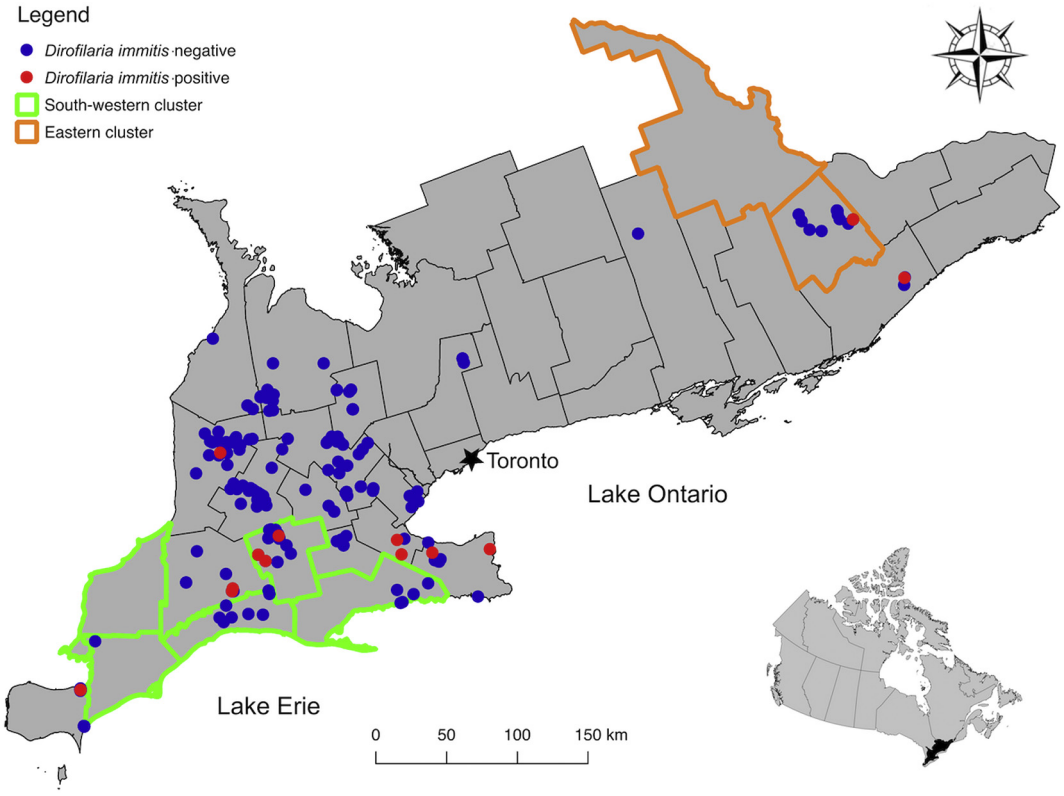

Fig. 1. Map of southern Ontario with the locations of necropsy-confirmed *Dirofilaria immitis*-positive (red) and -negative (blue) wild canids from 2016 to 2017. The south-western and eastern infection clusters of *Dirofilaria immitis* infection in dogs, identified by McGill et al. (2019), are indicated by the green and orange boundaries respectively. The inset shows the location of southern Ontario within Canada. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2019). However, it should be noted that while the aforementioned data for dogs were obtained throughout southern Ontario, the data in this study were sparsely distributed as we obtained wild canids mainly from the western and eastern regions of southern Ontario. The spatial gaps in our sample distribution make it difficult to compare spatial patterns of infection between dogs and wild canids in the region. It is therefore unclear how the spatial pattern of infection in wild canids relates to those observed in dogs.

5. Conclusion

Our findings provide preliminary insights into the prevalence and geographical distribution of *D. immitis* in coyotes and foxes in southern Ontario. However, sample collection focused on two discreet areas which limited our ability to compare spatial patterns of infection between wild canids and domestic dogs across the region. Future investigations that focus on a direct comparison between *D. immitis* infection in wild canids and unprotected dogs across southern Ontario from the same geographic areas would help elucidate the relationship between these two populations.

Ethical statement

Prior to the onset of this study, The University of Guelph Animal Care Committee was queried regarding the need for an Institutional Animal Care and Use Protocol. Carcasses were obtained from hunters/trappers and the carcasses were a product of the normal hunting/trapping activities, i.e. in no way are we encouraging people to hunt/trap coyotes or foxes for the purpose of this study. As such, an approved protocol was not required, per direction of the Committee.

Declaration of Competing Interest

The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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References

Agresti, A., Coull, B.A., 1998. Approximate is better than “exact” for interval estimation of binomial proportions. Am. Stat. 52, 119–126.
Atkins, C.E., 2003. Comparison of results of 3 commercial heartworm antigen test kits in dogs with low heartworm burdens. J. Am. Vet. M. Assoc. 222, 1221–1223.
Bowman, D.D., Atkins, C.E., 2009. Heartworm biology, treatment, and control. Vet. Clin. North Am. Small Anim. Pract. 39, 1127–1158.
Bowman, D.D., Little, S.E., Lorentzen, L., Shields, J., Sullivan, M.P., Carlin, E.P., 2009. Prevalence and geographic distribution of Dirofilaria immitis, Borrelia burgdorferi, Ehrlichia canis, and Anaplasma phagocytophilum in dogs in the United States: results of a national clinic-based serologic survey. Vet. Parasitol. 160, 138–148.
Brown, H.E., Harrington, L.C., Kaufman, P.E., McKay, T., Bowman, D.D., Nelson, C.T., Wang, D., Lund, R., 2012. Key factors influencing canine heartworm, Dirofilaria immitis, in the United States. Parasit. Vectors 5, 245–254.
Custer, J.W., Pence, D.B., 1981. Ecological analyses of helminth populations of wild

canids from the gulf coastal prairies of Texas and Louisiana. J. Parasitol. 67, 289–307.
Evason, M., Stull, J.W., Pearl, D.L., Peregrine, A.S., Jardine, C., Buch, J.S., Lailer, Z., O'Connor, T., Chandrashekar, R., Weese, J.S., 2019. Prevalence of Borrelia burgdorferi, Anaplasma spp., Ehrlichia spp. and Dirofilaria immitis in Canadian dogs, 2008 to 2015: a repeat cross-sectional study. Parasit. Vectors 12, 64.
Henry, L.G., Brunson, K.J., Walden, H.S., Wenzlow, N., Beachboard, S.E., Barr, K.L., Long, M.T., 2018. Comparison of six commercial antigen kits for detection of Dirofilaria immitis infections in canines with necropsy-confirmed heartworm status. Vet. Parasitol. 254, 178–182.
Herrin, B.H., Peregrine, A.S., Goring, J., Beall, M.J., Little, S.E., 2017. Canine infection with Borrelia burgdorferi, Dirofilaria immitis, Anaplasma spp. and Ehrlichia spp. in Canada, 2013–2014. Parasit. Vectors 10, 244.
King, A.W., Bohning, A.M., 1984. The incidence of heartworm Dirofilaria immitis (Filarioidea), in the wild canids of Northeast Arkansas. Southwest. Nat. 29, 89–92.
Klotins, K.C., Martin, S.W., Bonnet, B.N., Peregrine, A.S., 2000. Canine heartworm testing in Canada: are we being effective? Can. Vet. J. 41, 929–937.
Kotwa, J.D., Isaksson, M., Jardine, C.M., Campbell, G.D., Berke, O., Pearl, D.L., Mercer, N.J., Osterman-Lind, E., Peregrine, A.S., 2019. Echinococcus multilocularis, southern Ontario, Canada. Emerg. Infect. Dis. 25, 265–272.
McCall, J.W., Genchi, C., Kramer, L.H., Guerrero, J., Venco, L., 2008. Heartworm disease in animals and humans. Adv. Parasitol. 66, 193–285.
McGill, E., Berke, O., Peregrine, A.S., Weese, J.S., 2019. Epidemiology of canine heartworm (Dirofilaria immitis) infection in domestic dogs in Ontario, Canada: geographic distribution, risk factors and effects of climate. Geospat. Health 14, 17–24.
Nelson, T.A., Gregory, D.G., Laursen, J.R., 2003. Canine heartworms in coyotes in Illinois. J. Wildl. Dis. 39, 593–599.
Simmons, J.M., Nicholson, W.S., Hill, E.P., Briggs, D.B., 1980. Occurrence of (Dirofilaria immitis) in gray fox (Urocyon cinereoargenteus) in Alabama and Georgia. J. Wildl. Dis. 16, 225–228.
Slocombe, J.O.D., 1978. Heartworm in dogs in Canada in 1977. Can. Vet. J. 19, 244–247.
Slocombe, J.O.D., 2002. Heartworm in dogs and cats in Ontario in 2001. [Unpublished]. Available from. http://ovc.uoguelph.ca/doc/heartworm/2001/Ontario01.pdf (Last accessed February, 2019).
Slocombe, J.O.D., 2011. Heartworm in Canada in 2010 with Comments on Ontario. [Unpublished]. Available from. http://ovc.uoguelph.ca/doc/heartworm/2010/Ontario.pdf (Last accessed February, 2019).
Slocombe, J.O.D., McMillan, I., 1980. Heartworm in dogs in Canada in 1979. Can. Vet. J. 21, 159–161.
Slocombe, J.O.D., Villeneuve, A., 1993. Heartworm in dogs in Canada in 1991. Can. Vet. J. 34, 630–633.
Slocombe, J.O.D., Bhartendu, S., Surgeoner, G.A., 1995. The transmission period for heartworm in Canada. In: Proceedings of the Heartworm Symposium, 31 March-2nd April, 1995. (pp. 43–48). Alabama, USA.
Wang, D., Bowman, D.D., Brown, H.E., Harrington, L.C., Kaufman, P.E., McKay, T., Nelson, C.T., Sharp, J.L., Lund, R., 2014. Factors influencing US canine heartworm (Dirofilaria immitis) prevalence. Parasit. Vectors 7, 264.