



Research Article

# Meta-Analysis of the Prevalence of *Dirofilaria immitis* in Dogs from Grenada, West Indies

Camille-marie Coomansingh<sup>1</sup>, Michael Yabsley<sup>2</sup>, Nannette Wagner<sup>3</sup>, Rhonda Pinckney<sup>4</sup>, Muhammad I. Bhaiyat<sup>5</sup>, Alfred Chikweto<sup>6</sup>, Calum Macpherson<sup>7</sup> and Paul J. Fields<sup>8</sup>

<sup>1,2,3,4,5,6,7</sup>School of Veterinary Medicine, St. George's University, True Blue, Grenada, West Indies

<sup>8</sup>Windward Islands Research and Educational Foundation, St. George's University, Grenada, West Indies

Correspondence should be addressed to: Camille-marie Coomansingh; ccoomansingh@sgu.edu

Received date: 3 August 2014; Accepted date: 23 October 2014; Published date: 24 July 2015

Academic Editor: Alparslan Yildirim

Copyright © 2015. Camille-marie Coomansingh, Michael Yabsley, Nannette Wagner, Rhonda Pinckney, Muhammad I. Bhaiyat, Alfred Chikweto, Calum Macpherson and Paul J. Fields. Distributed under Creative Commons CC-BY 4.0

## Abstract

A comparison of several individual studies was made in order to determine the statistical significance for the prevalence of *Dirofilaria immitis* in dogs (n=1245) in Grenada, using necropsy and different ELISA test kits. The overall prevalence was estimated to be 17% in a study of live dogs, 12% in a study of necropsied dogs, and 14% when combining the studies using meta-analysis. There was no evidence of infection in dogs less than 6 months of age and no statistical difference (P>0.50) between male and female dogs. The prevalence reported is the cause for concern particularly since *Dirofilaria immitis* in dogs were found in all parishes in Grenada, favorable climatic conditions exist for vector-borne transmission, there is only sporadic use of preventatives, and there is an almost complete lack of public knowledge of the zoonotic potential of this filarial parasite in Grenada.

**Keywords:** *Dirofilaria immitis*, Meta-analysis, Prevalence, Grenada.

## Introduction

*Dirofilaria immitis* is the cause of heartworm disease in many domestic animal species but primarily in dogs. The parasite is restricted geographically mainly to regions with warm climatic conditions including Curacao,

Trinidad, Brazil and Malaysia as 130 growing degree days (GDD) is required for L3 development which is demonstrated to take 8-10 days at 28-30°C, 11-12 days at 24°C, 16-20 days at 22°C and arrested development below 14°C (Cancrini and Gabrielli, 2007; Genchi et al., 2009).

---

**Cite this Article as:** Camille-marie Coomansingh, Michael Yabsley, Nannette Wagner, Rhonda Pinckney, Muhammad I. Bhaiyat, Alfred Chikweto, Calum Macpherson and Paul J. Fields (2015), " Meta-Analysis of the Prevalence of *Dirofilaria immitis* in Dogs from Grenada, West Indies", International Journal of Veterinary Medicine: Research & Reports, Vol. 2015 (2015), Article ID 429690, DOI: 10.5171/2015.429690

However with the increase of worldwide surface temperature of an average 0.74°C, temperate regions which were once considered free of *D. immitis* are now observing infections during the summer months (Vezzani et al., 2006; Kronefeld et al., 2014; Genchi et al., 2005; Morchón et al., 2012). Several species of mosquitoes are now found to be vectors of *D. immitis* including: *Culex* spp, *Aedes* spp, *Anopheles* spp, *Armigeres* spp, *Myzorrhynchus* series of *Anopheles* and *Aedes Taeniorhynchus* spp (Anderson and Davis, 2014; Vezzani et al., 2011; Manrique-Saide et al., 2010). Dogs with low worm burdens normally exhibit no clinical signs, however as the number of worms increase and blood flow within the heart is inhibited, the following clinical signs are manifested: respiratory distress, pulmonary hypertension, peripheral edema, congestive heart failure and death.

Human infections have resulted in adult worms being found in the heart and inferior vena cava (Takeuchi et al., 1981). More commonly observed symptoms from the somatic migration of third stage larvae leads to fever, cough and pneumonitis (Robinson et al., 1977; Theis, 2005) and also granuloma formation that calcify and form what are known as 'Coin Lesions', which can be misdiagnosed as cancerous (Monchy et al., 1993; Miliaras et al., 2010).

Due to the significant health risk to dogs and potentially to humans of *Dirofilaria immitis*, nine separate studies were conducted in dogs in Grenada over an eight-year period spanning 2002 to 2009. A meta-analysis combining the results of these studies provided a more precise estimate of the prevalence of heartworms in Grenadian dogs.

### Materials and Methods

Nine independent studies were conducted in dogs in Grenada from 2002 through 2009 by four scientists. In four live dog studies, blood samples were collected from 579 live dogs from villages throughout Grenada by convenience sampling. All animals

were outdoor dogs which had spent all their lives in Grenada and were not on preventatives. In five studies, necropsy examination was performed on 666 dogs submitted by private veterinarians to the St. George's University School of Veterinary Medicine. None of the animals utilized in these studies had a history of being on preventives or travelled outside of Grenada.

### Live Dog Studies

2002 (Wagner): 224 dogs from five parishes; St George, St. David, St. Andrew, St. Mark and

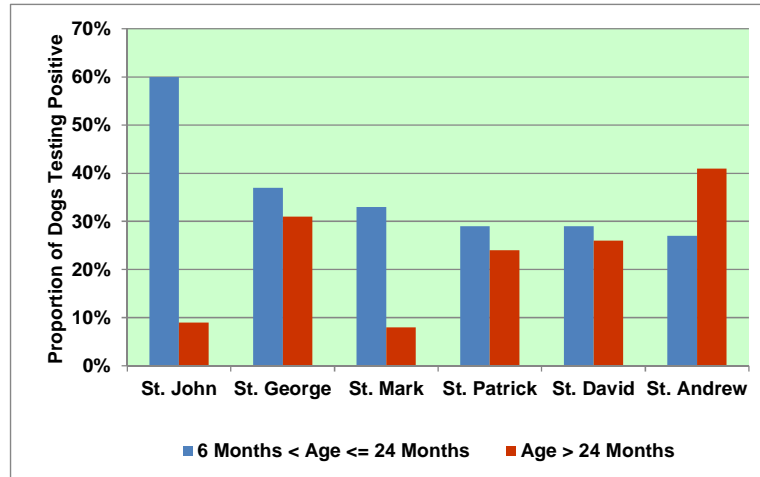
St. John, were tested using the *DiroCHEK*® Antigen Test by Synbiotics, California, USA (sensitivity 97%, specificity 90%).

2004 and 2006 (Yabsley): 177 dogs from five parishes; St. George, St. David, St. Andrew, St. John and St. Patrick, were tested using *3DX*® ELISA Snap Test by IDEXX, Maine, USA (sensitivity 98.7%, specificity 99.95%)

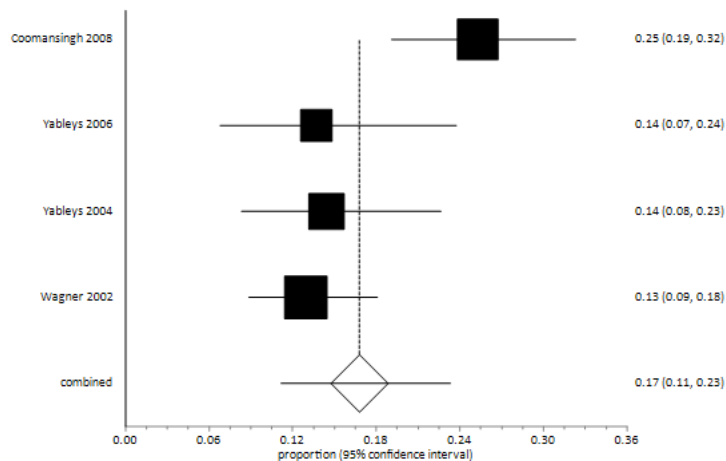
2007 (Coomansingh): 178 dogs from all six parishes; St. George, St. David, St. Andrew, St. Patrick, St. Mark and St. John, were tested using Antigen Rapid *Dirofilaria immitis*

Ag Test kit® by Animal Genetics, Korea (sensitivity 94.4%, specificity 99.95%), and positives were re-tested using the Modified Knott's Test to detect circulating microfilaria (sensitivity 91.8%, specificity 100%) (Martini et al., 1991).

Dogs testing positive in the 2007 Coomansingh study were found in all parishes in Grenada with no evidence of infection in animals less than 6 months of age (Figure 1). There was evidence that younger dogs had a higher prevalence of *Dirofilaria immitis* infection than older dogs, and particularly higher in St. John and St. Mark parishes ( $P < .05$ , using a Z-test for difference in proportions). No evidence was found of a difference in the proportion of positive tests between male and female animals ( $P > .50$ , using a Z-test for difference in proportions). All test results were confirmed by microscopic examination at 400x in the laboratory.



**Figure 1: Proportion of Dogs Testing Positive for *Dirofilaria immitis* by Age and Parish in Grenada (Coomansingh, 2007)**



**Figure 2: Proportion of Live Dogs Testing Positive for *Dirofilaria immitis* in Four Studies in Grenada from 2004 to 2007 and Combined Using Meta-Analysis**

***Necropsy Dog Studies:***

2005-2009 (Bhaiyat et al., 2009): 666 dogs from six parishes; St. George, St. David, St. Andrew, St. Patrick, St. Mark and St. John, were examined for *Dirofilaria immitis* in the necropsy studies by opening the thoracic and peritoneal cavities and dissecting the heart,

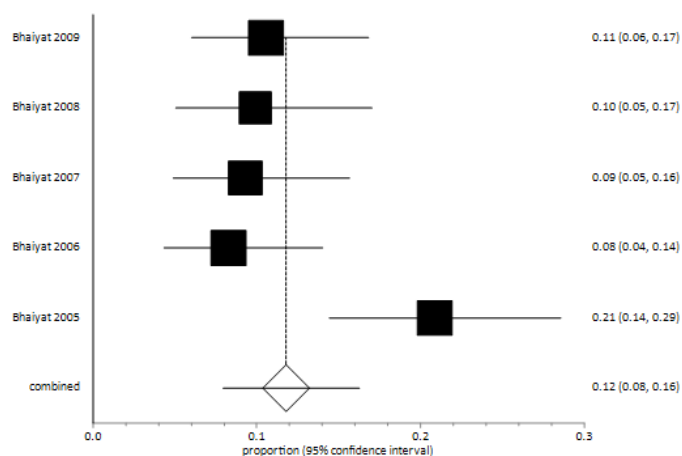
lungs, pulmonic trunk and vena cava. Other lesions related to heartworm infestation were also noted.

**Results**

Using meta-analysis to combine the results for the four studies on live dogs, 16.8% (95%

confidence interval 11.2% to 23.3%) tested positive for *Dirofilaria immitis* (Figure 2). Similarly, combining the results for the five studies on necropsied dogs, 11.8% (95%

confidence interval 7.9% to 16.3% ) were identified as positive for *Dirofilaria immitis* (Figure 3).



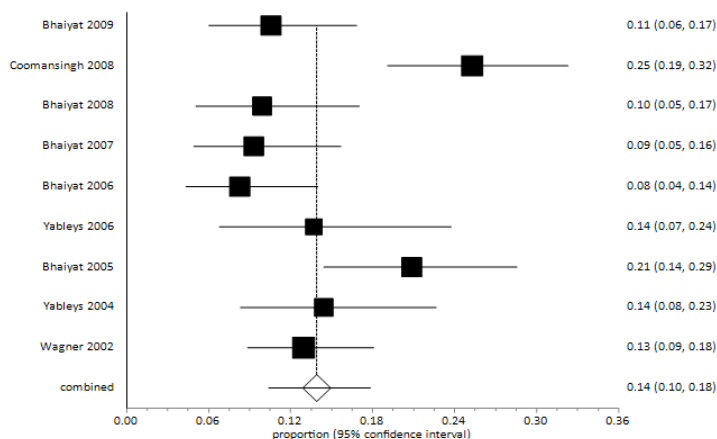
**Figure 3: Proportion of Necropsied Dogs Identified as Positive for *Dirofilaria immitis* in Five Studies in Grenada from 2005 to 2009 and Combined Using Meta-Analysis**

A meta-analysis was performed to combine the results of all nine studies in Grenada from 2002 to 2009. The Stuart-Ord inverse double arcsine square root transformation was used to stabilize the variance among the studies. Cochran's Q ( $P < .001$ ) was used to test for heterogeneity and the  $I^2$  statistic (73%, 95% confidence interval 37% to 85%) was used to test for inconsistency. These tests indicated the presence of statistical heterogeneity. The random effects model is used to synthesize heterogeneous research study results by determining a weighted average of the effect sizes of the studies. So, the random effects model was used to perform the meta-analysis in this study. The DerSimonian-Laird method was used for estimating the variance component in the model.

In addition, the Begg-Mazumdar test with Kendall's tau ( $P = .180$ ), the Egger test ( $P = .136$ ) and the Harbord test ( $P = .561$ ) were used to test for bias. These tests indicated no evidence of bias among the study results.

Based on the meta-analysis results for the nine heartworm studies combined, the

estimated overall proportion of dogs with *Dirofilaria immitis* infection from 2002 to 2009 was found to be 13.9% with a 95% confidence interval of 10.4% to 17.9% (Figure 4). The purpose of meta-analysis is to improve the precision of the estimate of a population parameter – in this study the prevalence of *D. immitis* in Grenadian dogs as measured by the proportion of positive cases. By combining the statistical evidence from multiple studies, the confidence interval around the prevalence estimate will become narrower than the confidence interval for a single study. In fact, the confidence interval will be narrower than for any one of the studies included in the analysis. The narrower confidence interval thereby gives a more precise estimate of the prevalence of a disease. We performed meta-analysis combining the evidence from nine studies each of which had measured the proportion of cases judged to be positive for *D. immitis* infection within a sample of dogs drawn from the population.



**Figure 4: Proportion of All Dogs Determined to be Positive for *Dirofilaria immitis* in all Nine Studies in Grenada from 2002 to 2009 and Combined Using Meta-Analysis**

## Discussion

Combining the nine studies produced a total sample size of 1245 dogs and incorporated information about the prevalence in both living and necropsied dogs. The process produced an overall sample which was far more representative of the population of dogs in Grenada than any single study.

The proportions of *Dirofilaria immitis* infection in live and necropsied dogs are similar to those reported for live dog studies in other Caribbean countries which share the same climate, including Curacao, 10% (Hesselink, 1988), Trinidad, 17% (Ali, 1994 Unpublished data) and the Dominican Republic, 18% (Duran-Struuck et al., 2005). The prevalence found was much lower when compared to that found in countries of similar climates but which are not small island nations, such as 26% in Malaysia (Retnasabapathy and San, 1976) and 54% in Brazil (Garcez et al., 2006). The reasons for the lower small-island prevalence rates requires further study.

The proportion of positive test results found in the 2005 and 2007 study on necropsied

and living dogs may be higher than in the other studies in Grenada due to more far-reaching sampling methods, as all the parishes on the island were included in those studies but not in the other studies.

Since the *Aedes aegypti* mosquito has been found to be a major potential vector of transmission (Tiawsirisup et al., 2005; Serrão et al., 2001), and since fewer fogging programs for eradication have been performed in Grenada on an island-wide basis, the prevalence of *Dirofilaria immitis* estimated in this research could be expected to increase in the future.

## Conclusion

These results show that one in seven dogs in Grenada is likely to be infected with *Dirofilaria immitis*. Dogs should therefore be kept on year-round heartworm prophylaxis regimens as mosquitoes are common throughout the year. Long term integrated vector control program would be useful as *Dirofilaria immitis* infections have been shown to be developing resistance to some drugs (Blagburn, 2011).

Public health officials and veterinarians should collaborate in educating the general public, and the local physicians, about the potential health issues which can arise from heartworm infection in dogs and the need for prophylactic care due to the possibility of zoonotic transmission. This would need to be a vertical program with community-based participation, as studies in the USA suggest that only one third of veterinarians regularly discuss zoonotic parasites or methods of reducing risks with their clients (Allert, 1995). In Grenada, very few animal owners take their pets to the veterinarian and therefore public health education programs could assist in explaining the dangers of parasitic zoonoses and the benefits of deworming protocols for dogs.

### Acknowledgements

The author is indebted to Dr. Sachin Kumthekar and Dionne Gittens for their help with this investigation; and Animal Genetics Inc for providing the test which was used in 2007 live dog study. Funding was provided by the SRGI of the St Georges University.

Please note that the format of the article should be considered. Spacing should be considered, too. Paragraphs justified and margins adjusted.

### Notes

<sup>1</sup>Information on statistical test used can be found in references: Kulinskaya (2008); Leandro (2004); Lipsey (2000); Sutton (2000)

### References

1. Allert, C., 1995. "Reducing risk of zoonotic diseases- a shared responsibility. Roundtable: Veterinarians, physicians jointly responsible". *Journal of the American Veterinary Medical Association*, 207 (4) 403-404.
2. Anderson, E.M., Davis, J.A., 2014. "First record of *Armigeres malayi* and *Armigeres milnensis* in Timor-Leste". *Journal of the American Mosquito Control Association*. 30 (1) 51-53.
3. Blagburn, B.L., Dillon, A.R., Arther, R.G., Newton, J.C., 2011. "Comparative efficacy of four commercially available heartworm preventive products against the MP3 laboratory strain of *Dirofilaria immitis*". *Veterinary Parasitology* 176(2-3): 189-194
4. Cancrini, G., Gabrielli, S., 2007. Vectors of *Dirofilaria* nematodes: Biology, behaviour and host/parasite relationships. In: Genchi, C., Rinaldi, L., Cringoli, G. (Eds.), *Dirofilaria immitis* and *D. repens* in Dogs and Cat and Human infections. Naples, Italy
5. Duran-Struuck, R., Jost, C., Hernandez, A., H., 2005. "Dirofilaria immitis prevalence in canine population in the Samana Peninsula (Dominican Republic)- June 2001". *Veterinary Parasitology*. 133 (4) 323-327.
6. Garcez, L.,M., de Souza, N., F., Mota, E., F., Dickson, L.,A., Abreu, W., U., Cavalcanti Vde, F., Gomes, P., A., 2006. "Focus on canine heartworm disease in Majoró Island, North of Brazil: A risk factor for human health". *Revista Da Sociedade Brasileira De Medicina Tropical* 39 (4) 333-

7. Genchi, C., Rinaldi, L., Cascone, C., Mortarino, M., Cringoli, R., 2005. "Is heartworm disease really spreading in Europe?" *Veterinary Parasitology*. 133 (2-3) 137-148.
8. Genchi, C., Rinaldi, L., Mortarino, M., Genchi, M., Cringoli, G., 2009. "Climate and *Dirofilaria* infection in Europe." *Veterinary Parasitology*. 163 (4) 286-292.
9. Hesselink, J., W., 1988. "The prevalence of heartworm (*Dirofilaria immitis*) in dogs of Curacao". *Tijdschrift voor Diergeneeskunde*. 113 (15-16) 853-859.
10. Knonefeld, M., Kampen, H., Sassnau, R., Werner, D., 2014. "Molecular detection of *Dirofilaria immitis*, *Dirofilaria repens* and *Setaria tundra* in mosquitoes from Germany" *Parasites and Vectors*. 7: 30.
11. Kulinskaya, E., Morgenthaler, S., and Staudte, R.G., 2008. *Meta-analysis: A guide to calibrating and combining statistical evidence*. Wiley, U.S.A.
12. Leandro, G., 2004. *Meta-analysis in Medical Research: The Handbook for the Understanding and Practice of Meta-analysis*. Wiley, USA.
13. Lipsey, M.W., and Wilson, D. B., 2000. *Practical Meta-analysis*. Sage Publications, U.S.A.
14. Manrique-Saide, P., Escobedo-Ortegón, J., Bolio-González, M., Sauri- Arceo, C., Dzib- Florez, S., Guillermo-May, G., Ceh-Pavía, E., Lenhart, A., 2010. "Incrimination of the mosquito, *Aedes taeniorhynchus*, as a primary vector of heartworm, *Dirofilaria immitis*, in coastal Yucatan, Mexico". *Medical and Veterinary Entomology* 24 (4), 456-460.
15. Martini, M., Poglayen, G., Capelli, G., Roda, R., 1991. "Diagnosis of canine filariosis: Relative sensitivity and specificity of some haematological techniques". *Angewandte Parasitologie*. 32(3) 133-136.
16. Miliaras, D., Meditskou, S., Kelekis, A., Papachristos, I., 2010. Human pulmonary dirofilariasis: One more case in Greece suggests that *Dirofilaria* is a rather common cause of coin lesions in the lungs in endemic areas of Europe. *International Journal of Immunopathology and pharmacology* 23 (1) 345-348.
17. Monchy, D., Levenes, H., Guegan, H., Poey, C., Dubourdieu, D., 1993. Pulmonary dirofilariasis. *Médecine tropicale: revue du Corps de santé colonial* 53 (3) 366-371.
18. Morchón, R., Carretón, E., González-Miguel, J., Mellado-Hernández, I., 2012. Heartworm disease (*Dirofilaria immitis*) and their vectors in Europe- New distribution trends. *Frontiers in Physiology* 3:196.
19. Retnasabapathy, A., San, K.T., 1976. Incidence of canine heartworm (*Dirofilaria immitis*) in Malaysia. *The Veterinary Record* 98 (4) 68-69.
20. Robinson, N.B., Chavez. C.M., Conn, J.H., 1977. Pulmonary dirofilariasis in man: a case report and review in literature. *Journal of Thoracic and Cardiovascular Surgery* 74 (3) 403-408.
21. Serrão, M.L., Labarthe, N., Lourenço-de-Oliveira, R., 2001. Vector competence of

*Aedes aegypti* (Linnaeus 1752) Rio de Janeiro strain, to *Dirofilaria immitis* (Leidy 1856).

*Memórias do Instituto Oswaldo Cruz.* 96: (5) 593-598.

23. Sutton, A. J., Sutton, A. J., Abrams, K. R., Jones, D.R., Sheldon, T.A., and Song, F., 2000. *Methods for Meta-analysis in Medical Research.* Wiley, U.S.A.

24. Takeuchi, T., Asami, K., Kobayashi, S., Masuda, M., Tanabe, M., Miura, S., Asakawa, K., Murai, T., 1981. *Dirofilaria immitis* infection in man: report of a case of the infection in heart and inferior vena cava from Japan. *American Journal of Tropical Medicine and Hygiene* 30 (5) 966-969.

25. Theis, J.H., 2005. Public health aspects of *Dirofilaria immitis* in the United States. *Veterinary Parasitology* 133(2-3) 157-180.

26. Tiawsirisup, S., Khlaikhayai, T., Nithiuthai, S., 2005. A preliminary study on in vitro transmission of *Dirofilaria immitis* infective larvae by *Aedes aegypti* (L.) (Diptera: Culicidae). *The Southeast Asian Journal of Tropical Medicine and Public Health* 36 Suppl 4: 86-89.

27. Vezzani, D., Eiras, D.F., Wisnivesky, C., 2006. *Dirofilaria immitis* in Argentina: Historical Review and first report of *Dirofilaria immitis* in a natural mosquito population. *Veterinary Parasitology* 136 (3-4) 259-273

28. Vezzani, D., Mesplet, M., Fontanarrosa, M.F., Schnittger, L., 2011. PCR detection of *Dirofilaria immitis* *Aedes aegypti* and *Culex pipiens* from urban temperate Argentina. *Parasitology Research.* 108 (4) 985-989.