Short Communication

Molecular and antigen detection of *Dirofilaria immitis* infection in client-owned cats in Bangkok, Thailand

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Abstract

Feline heartworm disease, caused by *Dirofilaria immitis* infection, can be potentially fatal to cats. Screening for the infection in cats is important in endemic areas. Clinical utility of molecular techniques for diagnosis of feline heartworm disease is not clearly determined. The objective of this study was to evaluate whether a polymerase chain reaction (PCR) assay could be used for detection of *D. immitis* infection in cats that were concurrently tested for *D. immitis* antigen. Blood samples were collected from 100 client-owned cats in a veterinary teaching hospital. Whole blood samples were tested for *D. immitis* antigen and extracted for total DNA. Presence of *D. immitis* DNA was analyzed using a PCR assay. Overall, there was one cat (1.0%) positive for *D. immitis* antigen. None of the cats were positive for *D. immitis* DNA. The results demonstrated very low prevalence of *D. immitis* infection in client-owned cats from a veterinary teaching hospital in Bangkok. However, the prevalence might be underestimated because of small sample size of the cats. In this study, the PCR assay was not shown to be superior to the antigen test for detection of *D. immitis* infection in cats. *Dirofilaria immitis* infection could not be ruled out based on a negative PCR test result. The clinical utility of PCR assay for diagnosis of feline heartworm disease is limited.

Keywords: *Dirofilaria immitis*, DNA, Antigen, Cat, Bangkok

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Introduction

Dirofilaria immitis infection is the etiologic agent of heartworm disease (HWD) that is recognized in domestic and wild canids and felids, as well as ferrets (McCall *et al*., 2008). Heartworm disease is much less common in cats than dogs, and clinical manifestations of feline HWD may differ from those of dogs (Lee and Atkins, 2010). Heartworm associated respiratory disease (HARD), caused by immature adult worms is the unique manifestation in cats (Venco *et al*., 2015). Clinical signs associated with HARD are respiratory or gastrointestinal signs, such as dyspnea, tachypnea, coughing, and intermittent vomiting unrelated to eating (Lee and Atkins, 2010). Because of overlapping clinical signs with feline asthma or allergic bronchitis, feline HWD may be overlooked. Feline HWD can be fatal even with light infection of mature adult worms resulting in sudden death due to pulmonary thromboembolism from fragments of dead worms (Litster and Atwell, 2008). Aberrant migration to the body cavities and the central nervous system was reported from cats (Favole *et al*., 2013). *D. immitis* infection in dogs and cats is common in tropical and subtropical regions where there are high density populations of canine reservoirs and mosquito vectors. Seroprevalence in cats ranging from 2.6% to 4.6% was reported in Asia (Liu *et al*., 2005; Sukhumavasi *et al*., 2012; Lu *et al*., 2017).

Diagnosis of *D. immitis* infection in cats is challenging. False-negative results can occur due to variations in sensitivity of tests that are used for different stages of *D. immitis*, such as a direct blood smear, a modified Knott test, and antigen and antibody testing (Lee and Atkins, 2010, Dillon *et al*., 2014; Little *et al*., 2014; Venco *et al*., 2015; Gruntmeir *et al*., 2017). Polymerase chain reaction (PCR) assays were developed for identification of filarial nematodes in blood samples of dogs (Mar *et al*., 2002; Rishniw *et al*., 2006; Albonico *et al*., 2014; Silbermayr *et al*., 2014; Ferreira *et al*., 2017), but they are not widely used in cats (Liu *et al*., 2005, Park *et al*., 2014). In addition, clinical utility of a PCR assay for diagnosis of *D. immitis* infection in cats is not clearly understood. The objective of this study was to determine whether a PCR assay was able to be used for screening *D. immitis* infection in cats that were concurrently tested for *D. immitis* antigen.

Materials and Methods

The study was performed at the Small Animal Teaching Hospital, Chulalongkorn University from October 2016 to October 2017. The protocol was approved by the Chulalongkorn University Laboratory Animal Care and Use Committee (Protocol No. 1631054) with a written consent signed by the cat's owner. One hundred client-owned cats were included in the study. Demographic data and medical records were obtained from each cat.

Whole blood was collected from cats, and placed in EDTA tubes, and stored at -20°C. Fresh blood samples were tested for circulating antigen by a commercially available test (SNAP Feline Triple Test, IDEXX Laboratories Inc, Westbrook, ME). All stored blood samples were extracted for total DNA using a commercially available kit (NucleoSpin Tissue Kit, Macherey-Nagel, Germany), following the manufacturer's protocol. The PCR protocol was followed that of the previous study (Rishniw *et al*., 2006). All PCRs were performed in Agilent SureCycler 8800 thermocycler (Agilent, UK) with the same conditions previously described (Rishniw *et al*., 2006). DNA from a known *D. immitis* positive dog was used as a positive control and water as a negative control. PCR products were identified in a 1.5% agarose gel by electrophoresis and visualized under ultraviolet light. Positive samples were identified as an expected band of approximately 542 bp on the gel.

Prevalence of *D. immitis* antigen and DNA was defined as the proportions of cats with positive results for the antigen test and the PCR assay, respectively. Pearson's χ^2 and Fisher's exact test were used to evaluate the difference between the prevalence of *D. immitis* infection detected by the antigen and the PCR assay. All statistical analyses were performed with standard software. Statistical significance was considered at P-value < 0.05.

Results and Discussion

Of 100 cats, there were 18 intact and 33 castrated males, and 16 intact and 33 spayed females. The median age of the cats was 5 years (range; 1 to 17 years). Breeds of cats included Domestic Shorthair (n=87), Persian (n=8), American Shorthair (n=2), Siamese (n=1), Maine Coon (n=1), and Scottish Fold (n=1). Most cats (60.0%) were allowed an outdoor access, and the others (40.0%) were strictly indoor. Seventy-eight percent of the cats lived in multi-cat households and the others (22.0%) were raised in single-cat households. Monthly heartworm preventives using topical products were administered in 27.0% of the cats. Overall, cats were presented at the veterinary hospital due to clinical illness (84.0%), purposes of wellness examination (9.0%), vaccination (6.0%), and neutering (1.0%). Of 84 cats with clinical illness, diseases were identified in 49 cats that included respiratory disease (17.0%), nephro-urinary disease (10.0%), abscess or bite wound (7.0%), oral disease (6.0%), orthopedic disease (6.0%), reproductive disease (2.0%), and hepatobiliary disease (1.0%). Of the 100 cats, 14.0% and 22.0% were positive for feline leukemia virus (FeLV) antigen and feline immunodeficiency virus (FIV) antibodies, respectively. The overall antigen-positive rate of *D. immitis* was 1.0% (1/100), but none of the cats were positive for PCR assay (Fig. 1).

In this study, prevalence of *D. immitis* infection detected by the antigen test (1.0%) and the PCR assay (0%) was very low in client-owned cats from a veterinary teaching hospital in Bangkok. The rate of prevalence in our study was much lower than that were found in some areas of Thailand (Sukhumavasi *et al*., 2012), South Korea (Liu *et al*., 2005) and Taiwan (Lu *et al*., 2017). Lower prevalence of this study could reflect the cat population sampled and geographical variations. The cat population in this study might be at low risk of exposure to *D. immitis*. Our study primarily included only client-owned cats and 40% of them were strictly indoor. These risk factors may have contributed

to the low prevalence of *D. immitis* infection. In addition, the different rate of prevalence might be influenced by the small sample size of the cats, and differences in techniques of DNA extraction and PCR assay.

There was only one cat positive by antigen test. Antigen test kits are provided globally and frequently used because of excellent specificity and moderate to good sensitivity (Snyder *et al*., 2000; Genchi *et al*., 2018). Because false-positive antigen test results are uncommon, a positive result generally indicates a current infection (Snyder *et al*. 2000). However, test kits for dogs have potential cross-reactions with *Angiostrongylus vasorum* and *Spirocerca lupi* (Schnyder and Deplazes, 2012; Aroch *et al*., 2015). The current status of the lung worm and the esophageal worm in cats in Thailand is unknown, so that cross-reactivity between *D. immitis* and other feline nematodes in commercially available antigen test kits is not clearly explained. False-negative antigen test results occur more frequently due to light infections or only infection of only male worms (Snyder *et al*., 2000). Other circumstances, including early infection with immature female worms, aberrant infection, and formation of antigen-antibody complexes also result in antigen-negative test results (Little *et al*., 2014; Venco *et al*., 2015; Gruntmeir *et al*., 2017). Therefore, exclusion of *D. immitis* infection might not solely rely on antigen-negative result.

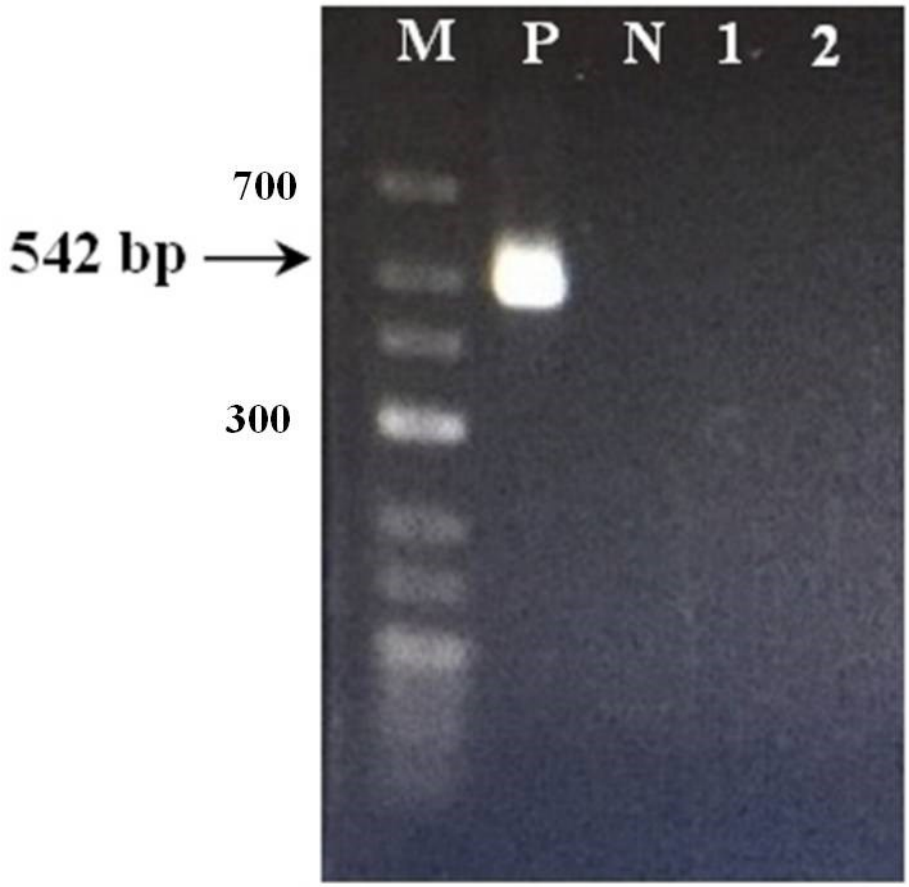

Figure 1 Gel electrophoresis of *D. immitis* PCR products. Lane M, GeneRuler Low Range DNA ladder (Thermo Scientific); lane P, positive control; lane N, negative control; lane 1, blood sample from a cat without circulating antigen; lane 2, blood sample from a cat with positive circulating antigen.

Antibody tests for *D. immitis* antibodies are currently used in most countries (Snyder *et al*., 2000; Kramer and Genchi, 2002; Levy *et al*., 2003; Berdoulay *et al*., 2004; Dunn *et al*., 2011; Genchi *et al*., 2018), but they are unavailable in Thailand. In cats, antibody tests can detect *D. immitis* infection since 2 months postinfection, therefore they can be used as early screening for infection (Browne *et al*., 2005; Dillon *et al*., 2014). However, seropositivity indicates a previous exposure to *D. immitis*, but worms may be not currently existed or may be recently cleared from the circulation (Browne *et al*., 2005). In addition, the sensitivity of antibody tests varies with time as the worms mature (Dillon *et al*., 2014). False-negative antibody test results could be detected in cats that had positive necropsy results (Snyder *et al*., 2000; Berdoulay *et al*., 2004). Discrepancy between results of antigen and antibody tests were previously documented (Curtis *et al*., 2017; Lu *et al*., 2017). Due to limited performances of antigen and antibody testing, current guidelines for feline heartworm infection recommend to include both antigen and antibody testing in order to determine the index of suspicion in cats (Jones *et al*., 2014).

All cats in this study had negative PCR test results. A discordant result was found in a cat that was positive by antigen test and negative by PCR assay. Failure to detect *D. immitis* DNA could be explained by absence of microfilariae or fragments of worms in blood circulation of the cats (Pereira *et al*., 2018). Otherwise, *D. immitis* DNA in the PCR reaction might be lower than the limit of detection in the assay (Rishniw *et al*., 2006). We suggest that *D. immitis* infection might not be ruled out based on a negative PCR test result alone. Some limitations were found in our study. We recruited only cats that were presented to a veterinary hospital and this cannot be considered random sampling from client-owned cats in Bangkok. In conclusion, the prevalence of *D. immitis* infection was very low, based on the antigen test and the PCR assay in a small group of client-owned cats in this area. The PCR assay was not shown promising to detect *D. immitis* infection in cats.

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