To the Graduate Council:

I am submitting herewith a thesis written by Amy W. Farley entitled "Prevalence of *Dirofilaria immitis* in cats in eastern Tennessee and associated risk factors." I have examined the final copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Comparative and Experimental Medicine

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PREVALENCE OF *DIROFILARIA IMMITIS* IN CATS IN EASTERN TENNESSEE AND ASSOCIATED RISK FACTORS

A Thesis
Presented for the
Master of Science Degree
The University of Tennessee, Knoxville

Amy Whetstone Farley
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This thesis is dedicated to my mother,

Jane Finley Whetstone,

and to my husband,

Gary N. Farley

Their patience is endless.

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ABSTRACT

The objectives of this research were to estimate the prevalence of *Dirofilaria immitis* infection in cats in eastern Tennessee, and to identify risk factors associated with this infection. Blood samples were collected from a convenience sample of 125 cats authorized for euthanasia at a local animal shelter and both heartworm antibody and antigen tests were run. The cats were then necropsied and examined for *D immitis* Heartworms were found in 3.2% (4/125) of cats Of the 125 serum samples, 2 4% (3/125) were antigen positive, and 7.2% (9/125) were antibody positive Heartworm serology was performed on 803 feline serum samples submitted to the University of Tennessee College of Veterinary Medicine Parasitology laboratory between January 1994 and December 1998. A heartworm antigen test was performed on each sample. Of the 803 samples, yearly prevalence of heartworm antigen positive cats ranged from 2 6% to 10 3% with an overall prevalence of 5.5% A correlation was found between yearly heartworm prevalence in cats and yearly precipitation in eastern Tennessee. Serum samples were collected from cats seen at veterinary clinics in Knox county whose owners agreed to participate in the study Serum was tested for both *D immitis* antibody and antigen Of the 560 samples, 20 (3 6%) were antigen positive, 32 (5 7%) were antibody positive, and 48 (8.6%) were positive on at least one of the two tests Owners were asked to fill out questionnaires regarding physical and behavioral characteristics of the cat. Statistical analyses were performed to determine the association between possible risk factors and *D immitis* infection in cats Characteristics of cats from the animal shelter, banked serum samples, and cats seen at veterinary clinics were analyzed No statistical difference was found between sex, neuter status, time of day spent outdoors, amount of

time spent outdoors, or fur length and heartworm infection status. There was also no association found between clinical signs and heartworm infection status in cats.

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CHAPTER I.

LITERATURE REVIEW

The first paper in the United States describing heartworms found on necropsy in a dog was published in 1847 (Osborne, 1847). Originally called *Filaria immitis*, the genus *Dirofilaria* was introduced in 1911. It is believed that heartworms were not endemic to America but rather were introduced from abroad, probably when infected dogs were brought from Spain or France (Roncalli, 1998) Heartworms in the U.S were originally confined to the south, particularly the southeastern coastline, but today have been reported in all 50 states (Heartworm Society, 1998) Thomas Lane Bancroft finalized the dog-mosquito-dog lifecycle of heartworms in 1903, but it was not until 1947 that the arsenical compound, thiacetarsamide, was first tested as a treatment for *Dirofilaria immitis* infection in dogs (Bancroft, 1903; Otto, 1947) The concept of a heartworm prophylaxis that would kill larval stages deposited by a mosquito before they matured to adult worms was introduced in 1962 with the use of a daily dose of diethylcarbamazine (Kume, 1955) Change to monthly prophylaxis came later with the introduction of ivermectin in 1987, milbemycin oxime in 1990, moxidectin in 1998, and selemectin in 1999 (Roncalli, 1998)

The first method used for clinical diagnosis of *D immitis* was the detection of microfilariae in the blood, either by direct smear or by concentration In 1981, the first ELISA (enzyme-linked immunosorbant assay) test was developed for the detection of circulating antibodies produced against *D immitis* (Grieve et al., 1981) This

development led to the introduction of serological testing for detection of *D immitis* antigen, which is routinely used for detection of heartworm infection in dogs today.

The first report of *Dirofilaria immitis* in a cat in the U.S. occurred in Virginia in 1922 when two adult heartworms were found in the right ventricle (Riley, 1922) In recent years, infection with *D immitis* has become more recognized as a cause of disease in cats. Prevalence parallels that of dogs in heartworm endemic areas, but is usually lower in cats (International Feline Heartworm Disease Council, 1995). Prevalence may be underrepresented because nonspecific clinical signs can lead to misdiagnosis. Vomiting, coughing, dyspnea, anorexia, lethargy, and weight loss are all common nonspecific complaints (McCall, Calvert et al., 1994) Acute respiratory distress caused by pulmonary lesions resulting from heartworm infection is frequently diagnosed as asthma. Also, general respiratory clinical signs may be confused with lungworms or cardiomyopathy (Calvert et al., 1982).

D immitis infection in cats usually involves fewer worms compared to infections in dogs. Cats usually harbor six or fewer worms, but one or two is very common (IFHDC, 1998) Some experimentally infected cats have been found to have significantly heavier worm burdens In one study, an experimentally infected male cat harbored 65 worms at necropsy (McCall, McTier et al., 1994) It has also been found that *D immitis* infections in male cats involve more worms than infections in female cats (Mansour, 1995). This is also true with *D immitis* infections in dogs Naturally infected male dogs harbor twice as many worms as female dogs (Dzimianski et al , 1989). *D immitis* adult worms in cats are often smaller than worms found in dogs. Female worms removed from cats have measured 19 5 to 25.8cm, and male worms have measured 9.6 to 16.5cm

(McCall, et al., 1992) In dogs, male worms measure 12-20cm and female worms measure 25-31cm (Levine, 1968).

The lifecycle of *D. immitis* differs somewhat in cats and dogs. In the dog/mosquito lifecycle, a mosquito bites an infected dog and takes up circulating microfilariae, the microfilariae develop in the mosquito to the infective 3rd stage larvae, the mosquito then bites another dog and the 3rd stage larvae enter the bite wound. These larvae develop and molt in the connective tissues and the immature adults migrate to the right side of the heart and mature The adults then produce microfilariae that circulate in the blood. This entire cycle in dogs take about six to seven months In cats, the time from the bite of an infected mosquito until mature adults are in the heart or pulmonary arteries is about eight months (Wong et al., 1983). Also, because infections in cats rarely produce microfilariae, a reservoir of infected dogs with circulating microfilariae is necessary for transmission to cats (Donahoe, 1976) Adult heartworms in infected cats are reported to live only about two to three years compared to adult worms in dogs which may live up to seven years (Wong et al , 1983) These differences in the biology of infection in cats and dogs is partly because the dog is the normal host for *D immitis* and the cat is only partially adapted (IFHDC, 1998)

There is not sufficient data to determine which mosquito species are significant vectors of heartworm infection in cats In order for a mosquito to be a competent vector for *D immitis*, it must support the development of the microfilariae to the infective 3rd stage larvae. For the mosquito to transmit the infection to cats, it must ingest microfilariae from an infected dog, then bite a cat to transmit the infective stage, so the mosquito must readily feed on both dogs and cats Many mosquito species have been

found to preferentially feed on dogs over cats This may be one reason why prevalence is lower in cats. (Genchi et al , 1992) There have been 13 species of mosquito implicated as possible vectors of *D immitis* in the southern U.S., among them, the Asian Tiger mosquitoes (*Aedes albopictus*), a recent introduction to the country and an important vector for heartworm infection in dogs in Japan (Scoles, 1998). In Knox county, Tennessee, 12 species of mosquito have been found to feed on dogs. The most numerous species collected were *Aedes triseriatus* and *Ae trivittatus*, both known to be competent vectors for *D immitis* (Hribar and Gerhardt, 1986, Scoles, 1998).

Cats have a more severe response than dogs to *D immitis* infection One reason for this is their smaller body size. In a cat, a one or two worm infection can be considered life-threatening (IFHDC, 1998) Severe host response often leads to significant pulmonary arterial changes. (McCracken and Patton, 1993). Most severe clinical signs occur at 5-6 months post infection when immature adults reach the pulmonary arteries and again when thromboembolism occurs after death of mature worms (Ralston et al , 1998) This is often the time when sudden death from disintegrating worms is likely to occur Some cats can withstand the infection with few or no clinical signs, while other cats have chronic problems throughout the course of the infection Unfortunately, when sudden death occurs, it may be the first and only clinical sign (IFHDC, 1998) It is important to accurately diagnose *D immitis* infection in cats because even a single worm infection can be fatal (McCracken and Patton, 1993).

Correct diagnosis of *D immitis* infection is dependent, in part, on how well available serologic tests perform. Knowledge of the sensitivity and specificity of a particular test is important for interpretation of the test result Sensitivity is the

probability that a test will correctly identify infected animals, and specificity is the probability the test will correctly identify negative animals A highly sensitive test will produce few false negative results while a specific test will produce few false positive results. It would be ideal for a test to be both highly sensitive and specific. This is difficult to achieve because there is sometimes a tradeoff between the sensitivity and specificity of a test in that as one increases, the other decreases To calculate the sensitivity and specificity of a test, some "gold standard" or definitive proof of infection must be used for comparison of test results (Fletcher et al , 1996). In the case of *D immitis* infection in cats, this is usually necropsy Tests used to detect *D immitis* antigen in cats are very specific, usually 100%, meaning there are few times when the test is positive and the cat is not infected (McCall et al , 1998) However, the sensitivities of antigen tests are lower Single worm infections, which are common in cats, may not produce enough antigen to be detected by the test. Also, male worms produce much less antigen than female worms and single, male worm infections are rarely identified by antigen testing. In these cases, an antigen test may be negative in the presence of infection. Because specific tests rarely produce false positive results, antigen tests can be useful in ruling in heartworm infection as a cause of clinical signs (Fletcher et al., 1996).

Tests to identify antibody to *D immitis* are highly sensitive and, therefore, rarely produce false negative results Antibody tests for *D immitis* infections are less specific, meaning they misclassify negative animals as positive (Fletcher et al., 1996). Because antibody is produced in response to migrating, larval stages that have not yet reached the heart or pulmonary arteries, a test may be positive when there are no worms in the heart

These are not truly "false positive" results since the test is actually detecting antibody to *D immitis* larvae but, because heartworm disease is a result of worms in the heart or pulmonary arteries, a positive antibody test in a cat with clinical signs does not necessarily confirm the *D immitis* infection as the cause of disease. An antibody test then may be used to rule out the possibility of heartworm disease as a cause of the cats clinical signs, since they produce few false negative results (McCall et al., 1998). Antibody tests may also be used to detect prevalence of infection in specific geographic areas because presence of circulating antibodies indicates transmission has occurred (IFHDC, 1998). Also, a positive antibody test in an asymptomatic cat may be detecting 4th stage larvae that have not yet entered the heart, and may be useful for anticipation of acute illness which may occur later as a result of the infection (Bestul et al , 1998).

The available serodiagnostic heartworm antigen tests detect antigen produced mostly by the adult female *D immitis* (Weil et al., 1985). There are currently two commercially available antigen tests approved for use in cats; the Dirochek™ (Synbiotics Corp., San Diego, CA), a qualitative ELISA microwell test, and the Snap™ (Idexx Laboratories, Westbrook, MA), a semiquantitative ELISA test Both use a monoclonal antibody for detection of antigen produced specifically by *D immitis* adults The specificity of antigen tests in cats is reported to be 100%, and antigen tests will reliably detect heartworms at approximately six to eight months post infection when worms have reached the heart and matured (McCall et al., 1998) The sensitivity has also been reported up to 100%, but has been shown to decrease proportionally with the number of female worms (Ryan et al., 1995). Infections with one female worm, immature worms, or only male worms may not produce enough detectable antigen and can be missed (Atkins

et al., 1995) In a recent study, the Dirochek™ test was only able to identify four of 12 cats with necropsy confirmed heartworm infections In a similar study, the sensitivity for the Dirochek™ test was calculated to be 79% (Genchi et al , 1998, Nelson and Young, 1998).

Antibody tests are more sensitive than antigen tests but lack some of the specificity and are more difficult to interpret. Antibody may be present for infections in which the animal's immune system was able to clear the infection before worms reached the heart or infections in which the worms have already died (Bestul et al., 1998) There are two commercially available feline antibody tests: the Assure/FH™ (Synbiotics) and the SoloStep FH™ (Heska Corp , Ft Collins, CO). Heska reports in their literature that the Solostep FH™, a lateral flow immunoasay, has a sensitivity and specificity of 98% and 92% respectively for both natural and experimental infections as well as for all single worm infections. In one study in which 12 cats had necropsy confirmed *D immitis* infections, 11 tested positive using the SoloStep FH™ antibody test The test was also able to detect three of four single worm infections (Genchi et al., 1998) The Assure/FH™ feline antibody test uses a single antigen (DiT33) found in adult male and female worms, 4th stage larvae, and microfilariae for the detection of antibody It is a qualitative ELISA test with a color change indicating a positive result (Bestul et al , 1998) In a recent study of 19 cats with experimental *D immitis* infections, one was positive with the Assure/FH™ at one month post infection, 18 tested positive at three months, and all 19 tested positive at five months post infection. Six of the 19 cats had single worm infections at necropsy performed at eight months post infection, and all six were positive for antibody using the Assure/FH™ at three months post infection. Only

one of those six was antigen positive at eight months post infection (Bestul et al., 1998) Earlier antibody tests showed possible cross reaction to other parasites because of the use of multiple antigens (McCall, Supakorndef, Ryan et al., 1995). The Assure/FH™ uses a single defined antigen specific for *D immitis* and no cross-reaction has been found to common gastrointestinal parasites, Feline Corona Virus (FcoV), Feline Immunodeficiency Virus (FIV), or Feline Leukemia Virus (FeLV) (Bestul et al., 1998) Both Heska Corporation and Animal Diagnostics (St. Lousi, MO) each have a quantitative heartworm antibody test Samples must be sent to their diagnostic laboratories to be tested (Dillon et al., 1998).

Tests used to detect microfilariae in dogs are ineffective in cats because of same sex infections, single worm infections, and immune mediated response eliminating microfilaria (IFHDC, 1998). Only 20% of experimentally infected cats are positive for microfilariae, and even those are only positive for a short time (Donahoe et al., 1976). When microfilaremia does occur in cats, it does not continue for more than 7 5 months after worms have matured to adults. It appears that the cat's immune response is able not only to suppress microfilariae production by the female worms, but also to clear any circulating microfilariae (IFHDC, 1998)

Echocardiography is another diagnostic procedure currently being investigated for its usefulness in heartworm detection in cats A recent study showed echocardiography was able to detect heartworm infections in 11 of 12 necropsy confirmed cases (Genchi et al., 1998) It has been speculated that because worms often reside in the extremities of the pulmonary arteries in cats, infections would be hard to visualize by echocardiography In one recent study of 11 cats with echocardiography confirmed *D immitis* infections, six

cats were identified that only had worms in the pulmonary trunk or arteries (Venco et al., 1998). It is possible that with careful examination, sensitivity of echocardiography for detection of *D immitis* infection in cats can be increased, but because of the cost of echocardiography equipment, this may not be a feasible screening tool for most clinicians.

Thoracic radiography can be used to detect enlargement of main caudal pulmonary arteries, a characteristic radiographic finding of heartworm disease in cats. (McCall, Calvert et al., 1994) In a recent study, radiography correctly diagnosed six of nine infected cats that had cardiopulmonary abnormalities. (Atkins et al., 1998). However, "many heartworm-infected cats with cardiopulmonary signs show subtle radiographic changes" (McCall et al., 1994) While thoracic radiography can be a valuable tool for assessing severity and progression of heartworm disease, it is not a practical screening technique because radiographic findings are rare in asymptomatic cats. (Atkins et al., 1995)

Cats are infected with heartworms worldwide but prevalence varies geographically The prevalence of heartworm infection in cats is the proportion of cats infected at a single point in time, as opposed to incidence, which is the proportion of cats initially free of infection that become infected within a specified time period (Fletcher et al., 1996) Within the U S., results of necropsy and serologic testing show prevalence of *D immitis* infection in cats to vary geographically Prevalence has been reported from 26% in Oklahoma, to 10% in Louisiana, and 9% in Texas (Fox et al., 1998; Guererro et al , 1992, Nelson and Young , 1998) In eastern Tennessee, data collected between 1985 and 1990 showed prevalence of heartworm infection in shelter cats to be 2.5% and

prevalence in cats seen at a veterinary teaching hospital to be 9.47% (Patton and McCracken, 1991).

There are no products currently labeled for use as adulticide therapy in cats. Thiacetarsamide, one drug available for adulticide treatment in dogs, has been used in cats at the dog dosage of 2.2mg/kg of body weight twice daily for two consecutive days The threat of thromboembolism occurring as worms die following therapy "can be expected in approximately one third of cats treated" (IFHDC, 1995). Also, conflicting reports have been made regarding the toxicity and efficacy of thiacetarsamide use in cats Because of this risk, adulticide therapy is not recommended for use in subclinical cases (IFHDC, 1995) Prednisone has been found effective for treatment of cases in which there is evidence of interstitial lung disease or in cases of antigen or antibody positive cats with clinical signs. The recommended prednisone regimen is 2mg/kg of body weight per day decreasing to 0.5mg/kg over two weeks and discontinued after two more weeks (IFHDC,1998) Acute cases are also treated with supportive care depending on clinical signs Because of the difficulties in treating heartworm disease in cats, prevention must be emphasized.

The increased awareness of *D immitis* infection as a potential cause of disease in cats has led to the introduction of new prophylactic compounds There are two products labeled for prevention of heartworm infection in cats Heartguard™ for Cats (Merial Corp., Iselin, NJ) is a chewable tablet containing ivermectin administered by manufacturers direction at a monthly dosage of 55 mcg for cats up to five pounds, and 165 mcg for cats five to 15 pounds. Revolution™ (Pfizer Corp., New York, NY) is a topical solution of selemectin applied monthly by manufactures directions at a dosage of

15mg for cats up to five pounds and 45mg for cats 5 1 to 15 pounds The prophylactic drugs are the same as used for dogs and work by killing larvae introduced by an infected mosquito before they molt to adulthood (Blair et al., 1980)

With new prophylaxic compounds now available, practitioners are in need of accurate information to educate and objectively inform their clients about prevention of this disease. The association between suspected risk factors for *D immitis* exposure, and infection in cats is unclear. It is suggested that cats living outdoors are at higher risk for infection than indoor cats, but reports have shown infection in cats said to live exclusively indoors (Ryan et al., 1995) It is speculated that male cats are at higher risk for infection than females because of behavioral patterns, such as roaming farther from home. However, male cats that are experimentally infected also show higher worm burden than female cats, which indicates more than a behavioral factor. (McCall et al., 1992).

Because there are diagnostic limitations in using serologic tests in cats that are effective for detection of *D immitis* infection in dogs, and because clinical signs of *D immitis* infection in cats are usually nonspecific and often misdiagnosed, cases of *D immitis* infection in cats may not be found until the animal is showing advanced clinical signs of the disease Accurate diagnosis and prevention is important to the client/clinician relationship as well as to the health of pet cats More research needs to be done to clearly understand the relationship between suspected risk factors and prevalence of *D immitis* infection in cats This information will help clinicians inform clients about which cats are at increased risk for acquiring *D immitis* infection and the magnitude of the problem in a given area A cat that tests heartworm positive, even in the absence of

clinical signs, is at risk for further infection, which may lead to clinical disease, therefore, prophylaxis may be advisable. In this project, the association between suspected risk factors such as sex, neuter status, time spent outdoors, and heartworm infection in cats will be evaluated. The estimation of prevalence and identification of risk factors will improve the ability to diagnose and prevent heartworm disease in cats.

CHAPTER II.

MATERIAL AND METHODS

Shelter Cats

A convenience sample consisted of 139 cats authorized for euthanasia at a local animal shelter between June 1998 and August 1999 Cats were not specifically euthanized for this project. Cats were estimated by shelter staff to be at least six months old Blood samples were collected in Vacutainer® tubes containing no additive by shelter staff at the time of euthanasia. Cats were brought to the University of Tennessee within six hours of euthanasia and stored at 5 °C. The blood samples were centrifuged upon receipt and serum was transferred to 1 0 ml tubes for storage at 5 °C. All tests were performed within 48 hours of receipt of sample. After testing, samples were stored at – 20 °C.

Necropsies were performed on each cat at the University of Tennessee College of Veterinary Medicine within one week of receipt. The hearts, lungs, and major vessels were examined for *Dirofilaria immitis* The cats and their corresponding serum samples were given research numbers (1-139) in the order they were tested. The serum samples were tested with both the Dirochek™ antigen test and the Assure/FH™ antibody test (both Synbiotics Corp, San Diego, CA) according to manufacturers directions Cats were removed from the study if either no blood sample was taken or if cats were not available for necropsy, leaving a total of 125 cats in the study. At the time of necropsy, data were recorded for each cat including sex, neuter status, and length of fur (ie short or long) These characteristics were analyzed with SAS (SAS Institute Inc., Cary, NC) for

association with heartworm infection status using either a chi-square or a Fisher's exact test A p value less than 0.05 was considered significant.

Banked Samples

The UTCVM parasitology laboratory's banked serum collection contains serum of any species submitted to the laboratory for any reason. All banked serum is stored at –70 °C in 1.0 ml cryovials The database for these samples was searched and domestic feline serum submitted between January 1994 and December 1998 were selected to be included in the study. Any samples submitted from outside of eastern Tennessee were removed from the study leaving only samples that were submitted by the University of Tennessee Veterinary Teaching Hospital staff or by area veterinary clinics. Also, if any antigen positive serum was submitted from the same cat more than once, all but one sample was removed from the study Samples used in the study were moved to –20 °C storage and were allowed to thaw in 5 °C refrigeration immediately before testing. Dirochek™ antigen tests were performed in batches of 22 samples within 48 hours of refrigeration, and samples were returned to –20 °C storage immediately following testing.

The 803 serum samples were also categorized according to reason for original submission to the parasitology laboratory Temperature and precipitation data for eastern Tennessee was taken from the National Climatic Data Center website for correlation to yearly percentage of antigen positive cats Sex, neuter status, and breed information was available for 278 of the 803 cats in the University of Tennessee Veterinary Teaching Hospital records database. The parasitology laboratory's database was also searched for Dirochek™ results for all dogs submitted for heartworm testing during the same time-period and were compiled for comparison to the cats Statistical analyses of these data

were performed using SAS. Correlation coefficients were also calculated using SAS Correlation coefficients range from –1, indicating an inverse, linear relationship, to 1, indicating a direct, linear relationship between the two variables tested with 0 showing no relationship

Veterinary Clinic Samples

Clients bringing a cat to one of 19 participating veterinary clinics in Knox County for any reason during the study period (May-August 1999) were asked to participate in the study The minimum age for inclusion in the study was six months. Blood samples were collected by clinic staff in non-additive, gel serum separator tubes. The serum was separated by centrifugation and the tubes stored in refrigeration. Each client was given a questionnaire regarding physical and behavioral characteristics of their cat for completion during the office visit (see appendix p.49). Serum samples and questionnaires were collected from each clinic weekly. Serum was transferred to 1.0 ml cryovials and refrigerated at 5 °C until tested. Dirochek™ antigen and Assure/FH™ antibody tests were performed within seven days of pickup, and samples were transferred to –20 °C storage Questionnaire results were compiled in EpiInfo (CDC,USA); chi-square or Fisher's exact tests were performed using SAS

CHAPTER III.

RESULTS

Shelter Cats

A total of 125 cats euthanized by the animal shelter were collected, 25.6% (32) were intact males, 7 2% (9) neutered males, 54 4% (68) intact females, and 12 8% (16) spayed females. No cat appeared to be a specific breed, and all cats were estimated to be over six months old. *Dirofilaria immitis* adults were found in the heart or pulmonary arteries of four cats (3 2%). Of those four cats, there were two domestic short hair, intact females, one domestic short hair, intact male, and one domestic long hair, intact male Of the 125 cats, three (2.4%) were antigen positive, and nine (7.2%) were antibody positive. There were 11 (8.8%) cats positive on at least one of the three diagnostic tests (Table 1).

Table 1 Heartworm serology and necropsy results from cats obtained from animal shelter

Diagnostic Test	Number positive/total	% positive
Necropsy	4/125	3.2
Dirochek™ antigen	3/125	2 4
Assure/FH™ antibody	9/125	7 2

Of the four cats in which worms were found on necropsy, a single, non-gravid, female worm measuring 21 8 cm was found in one cat that also tested positive for antigen and antibody. Two cats each contained a single, male worm, one measuring 12.8 cm and the other 13.2 cm Both cats were antigen negative and antibody positive The fourth cat contained one male and one non-gravid female worm and tested antigen positive but antibody negative. Both worms were cut during extraction from the heart and were unable to be accurately measured In an additional cat that tested positive for antigen, no worms were found in the heart or lungs (Table 2) This cat also tested antibody negative. For analysis, a cat was considered to be heartworm positive if heartworms were found at necropsy, or if the cat tested antigen or antibody positive There was no statistical significance ($p>0$ 05) between sex, neuter status or fur length and heartworm infection status using a Fisher's exact test (Table 3)

Banked Samples

There were 803 feline serum samples from the parasitology laboratory tested for heartworm antigen. A total of 44 (5 5%) tested heartworm antigen positive Yearly prevalence ranged from 2.6% in 1995 to 10 3% in 1994 and showed no overall increase or decrease in prevalence over the five year period

The percentage of antigen positive cats were compared to the percentage of antigen positive dogs that were submitted to the parasitology laboratory during the same years Yearly prevalence of dogs and cats were not parallel. The percentage of antigen positive dogs ranged from 3 9% in 1996 to 6 9% in 1998 with a total prevalence of 5 0% (Table 4)

Table 2. Shelter cats positive for *D immitis* antigen, antibody or necropsy.

Shelter cat number	Necropsy	Dirochek™ antigen	Assure/FH™ antibody
28	1 female worm	Pos	Pos
72	1 male worm	Neg	Pos
87	1 male worm	Neg	Pos
136	1 male and 1 female	Pos	Neg
34	Neg	Neg	Pos
39	Neg	Neg	Pos
45	Neg	Neg	Pos
46	Neg	Neg	Pos
120	Neg	Neg	Pos
133	Neg	Neg	Pos
139	Neg	Pos	Neg

Table 3. Characteristics and heartworm prevalence of cats from animal shelter

Characteristic	Number heartworm positive/total	% Heartworm positive
Male	3/41	7.3
Female	8/84	9 5
Male intact	3/32	9 4
Male neutered	0/9	0 0
Female intact	8/68	11.8
Female spayed	0/16	0.0
Male/female intact	11/100	11 0
Neutered/spayed	0/25	0 0
Long fur	2/18	11 1
Short fur	9/105	8 6

Table 4. Heartworm antigen test results for banked feline and canine serum samples from 1994-1998.

Year	Number antigen positive/total number cats	% antigen positive cats	Number antigen positive/total number dogs	% antigen positive dogs
1994	12/117	10.3	29/652	4 4
1995	4/156	2 6	38/698	5 4
1996	13/168	7.7	23/596	3 9
1997	5/164	3 1	28/649	4 3
1998	10/198	5 1	45/654	6 9
Overall	44/803	5 5	163/3249	5 0

There was no statistical significance between the average yearly prevalence of dogs and cats.

The percentage of antigen positive cats was then compared to the average yearly temperature and precipitation in eastern Tennessee (Table 5). The yearly precipitation ranged from 49 3 to 62.6 inches with a five-year average of 55 0 inches. A significant correlation coefficient of 0.976 was calculated between yearly precipitation and percentage of antigen positive cats The average yearly temperatures ranged from 54 9 °F to 56 6 °F with an average temperature of 55 9 °F over the five-year period. The correlation coefficient of 0.053 was calculated between average yearly temperature and percentage of positive cats. Figures 1 and 2 show the antigen positive cats each year, the yearly precipitation, and average yearly temperature respectively

Of the 803 serum samples, 709 (88.3%) were originally submitted for *Toxoplasma gondii* testing, with 5.1% (36) of those positive for heartworm antigen. There were 87 (10 8%) samples originally submitted for heartworm testing, with 9 2% (8) testing antigen positive There were seven samples submitted for both heartworm and *T gondii* testing and none were heartworm antigen positive (Table 6) A chi-square analysis showed no statistical significance (p= 0 365) between heartworm positive cats whether they were originally submitted for *T gondii* or heartworm testing.

There were 278 of the 803 cats for which information was available on sex, neuter status, and breed (Table 7) Fisher's exact or chi-square tests showed no statistical association (p>0.05) between each characteristic and heartworm positive status

Table 5 Percent cats positive for heartworm antigen, and average temperature and precipitation in eastern Tennessee from 1994-1998

Year	% Antigen positive	Yearly precipitation (inches)	Average temperature (°F)
1994	10 3	62 6	56 6
1995	2 6	49 3	56.5
1996	7.7	57.6	55 3
1997	3.1	52.5	56 0
1998	5 1	52.8	54 9

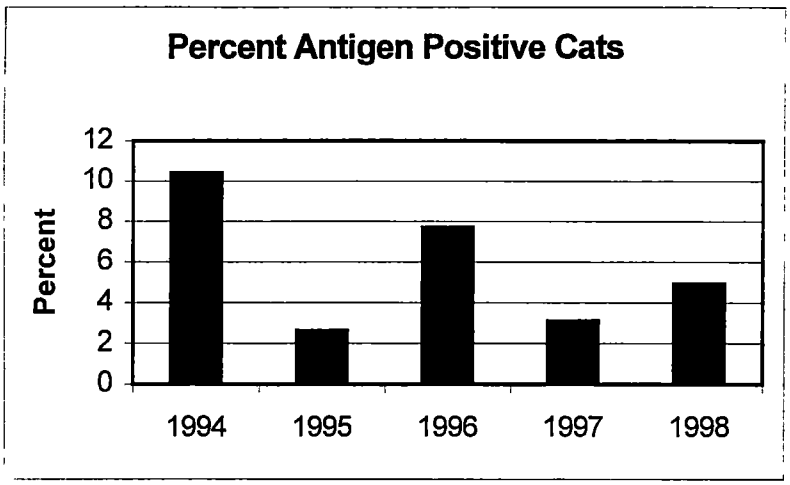

Figure 1. Percent antigen positive banked feline serum1994-98

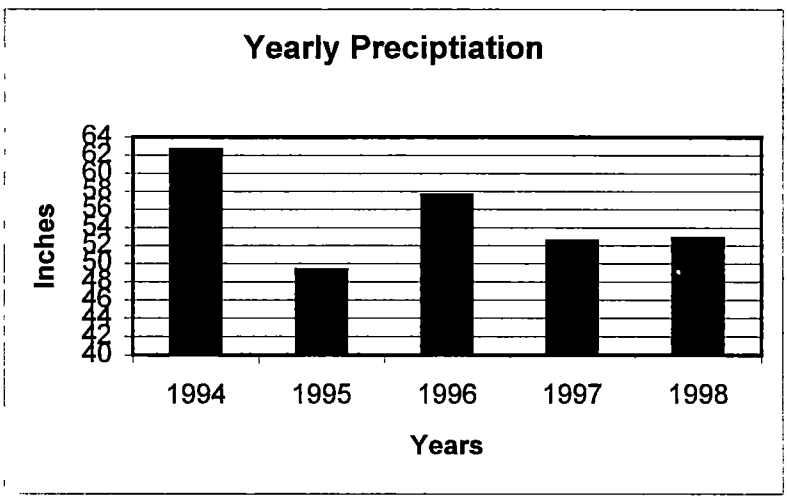

Figure 2 Yearly precipitation in eastern Tennessee 1994-98

Table 6. Antigen positive banked feline serum samples by reason for original submission to parasitology laboratory.

Reason for original submission	Number submitted/ total	% Submitted/ total	Number antigen positive/# submitted	% Positive
Toxoplasma gondii titer	709/803	88 3	36/709	5 1
Heartworm testing	87/803	10 8	8/87	9 2
Both tests requested	7/803	0 87	0/7	0.0

Table 7 Characteristics of 278 banked serum samples.

Characteristic	Number heartworm positive/ total	% Heartworm positive
Male	9/158	5 7
Female	6/119	5.0
Male intact	2/24	8 3
Male neutered	7/134	5.2
Female intact	1/15	6 7
Female spayed	5/104	4 8
Male/female intact	3/39	7 7
Spayed/neutered	12/238	5 0
Specified breed	2/42	4 8
No specified breed	13/235	5 5

Veterinary Clinic Samples

A total of 560 feline serum samples were collected from local veterinary clinics Of those samples, 20 (3.6%) tested antigen positive, and 32 (5 7%) tested antibody positive. There were 48 (8 6%) samples that tested positive on at least one of the two tests (Table 8) Sixteen samples tested positive for antigen but negative for antibody, 28 tested positive for antibody but negative for antigen, and four tested positive for both antigen and antibody (Table A-1)

A total of 560 questionnaires were compiled for statistical analysis (Table A-2 and A-3) A cat was considered positive for heartworm infection if it tested positive on at least one of the two tests performed. The following associations were tested, male vs. female, neutered or spayed vs intact, neutered males vs intact males, any specific breed vs no breed, long fur vs short fur, time of day spent outdoors, number of hours spent outdoors (0 hours vs >0 hours), cats brought to clinic for respiratory problems or vomiting vs. cats brought in for all other reasons (Tables 9-10) Data on flea control are in Table A-3

Table 8 Results of heartworm serology for veterinary clinic samples

	Assure/FH™ antibody	Dirochek™ antigen	Assure/FH™ and/or Dirochek™
# positive/total (%)	32/560 (5.7)	20/560 (3 6)	48/560 (8 6)

Table 9 Characteristics of heartworm positive cats seen at veterinary clinics.

Characteristic	Number positive for antigen or antibody	Total number	% Positive
Male	21	264	8.0
Female	27	290	9 3
Intact male and female	3	19	15.8
Neutered/spayed	44	535	8 2
Intact male	1	8	12.5
Neutered male	20	256	7 8
Intact female	2	11	18 2
Spayed female	25	279	9 0
No specified breed	40	496	8.1
Specified breed	7	57	12 3
Long hair	14	155	9 0
Short hair	35	397	8 8
0 hours outside	23	264	8 7
> 0 hours outside	25	283	8 8
Respiratory signs	3	20	15.0
Vomiting	1	15	6 7

Table 10. Number of heartworm positive cats seen at veterinary clinics based on time of day spent outdoors

Time of day	Always out* Positive/total (%)	Sometimes out* Positive/total (%)	Never out* Positive/total (%)
9am-3pm	9/132 (6 8)	8/110 (7 3)	29/298 (9 7)
3pm-9pm	11/116 (9 5)	4/158 (2.5)	23/269 (8 6)
9pm-3am	4/75 (5.3)	6/106 (5 7)	33/351(9 4)
3am-9am	4/77 (5 2)	6/92 (6 5)	34/361 (9 4)

* Owners were asked to indicate how often their cat is outside during each six-hour time period

Fisher's exact or chi-square tests showed no statistical significance between any characteristics analyzed and heartworm infection status ($p>0.05$)

Combined Analyses

Characteristics of shelter cats, cats for which banked serum was available, and veterinary clinic cats were combined for analysis of possible risk factors. A cat was considered positive for heartworm infection if worms were found at necropsy, or if the cats tested antigen or antibody positive. Sex, neuter status, and breed were known for cats taken from the animal shelter, banked samples, and veterinary clinics. Fur length was known for cats from the animal shelter and veterinary clinics (Table 11). Fisher's exact or chi-square analysis showed no significant association between any characteristic and heartworm infection status.

Table 11. Combined characteristics from animal shelter, banked samples, and veterinary clinics.

Characteristic	Number heartworm+/ total	% Heartworm positive
Male	33/436	7 1
Female	41/493	8.3
Male intact	6/64	9 4
Male neutered	27/399	6 8
Female intact	11/94	11.7
Female spayed	30/399	7 5
Male/female intact	17/158	10 8
Neutered/spayed	56/798	7 1
Specified breed	9/99	9.1
No specified breed	64/856	7 5
Long fur	16/173	932
Short fur	44/502	8 8

CHAPTER IV.

DISCUSSION

Shelter Cats

In 125 cats from the Knox County animal shelter that were necropsied, four had *Dirofilaria immitis* in the heart or pulmonary arteries Of those four, one cat contained a single female worm, two cats each contained a single male worm, and one cat contained one female and one male worm (Table 2)

The two cats with male only infections tested antigen negative The serum antigen test for heartworm infection detects antigen secreted by the adult *D immitis* (McTier et al., 1995) Infections with female worms are more often detected because the female produces more antigen than the male *D immitis* (Weil et at, 1985) The two infections involving only single male worms may not have produced enough antigen to be detected by the test The two in which female worms were found both tested antigen positive

An additional cat tested positive for antigen but no heartworms were found in the heart or lungs At necropsy, the pulmonary arteries were dilated indicating a possible *D immitis* infection It is possible that the heartworm(s) had died and enough antigen was still present to be detected by the test. The lungs also appeared abnormal with congestion, inflammation, and nodules present. Many *Aelurostrongylus abstrusus* larvae were found on a sucrose flotation performed on feces from this cat The Dirochek™ heartworm antigen test is an ELISA test that uses monoclonal antibodies for the detection of antigen, therefore it is unlikely that a cross-reaction to the *A abstrusus* infection would occur It is possible that the *D immitis* infection was ectopic and the condition of the

lungs was unrelated to the *D immitis* infection An ectopic infection may produce circulating antigen and would result in a positive antigen test (McCall, Supakorndej, Dzimianski et al., 1995).

There were nine (7.2%) shelter cats positive for *D immitis* antibody Heartworms were found in three of the nine antibody positive cats. One cat in which heartworms were found tested antibody negative. Also, the cat that tested antigen positive in which no worms were found tested antibody negative. In these two cases, it is possible that because of individual variation in antibody production, not enough antibody was produced to be detected by the test. Another possibility is that the cats were immunosuppressed by another factor such as FIV or FeLV infection, and as a result were unable to produce enough antibody to be detected In one study, a cat with heartworm infection confirmed by necropsy tested negative for antigen and antibody, but was FeLV positive (Nelson and Young, 1998) Another study using echocardiographically confirmed cases of heartworm infection reported that a higher percentage of infected cats showing clinical signs consistent with heartworm disease tested antibody positive than infected cats with no signs (Genchi et al., 1998). This suggests that asymptomatic cats may have less circulating antibody

There were six cats that tested antibody positive that were both antigen negative and in which no worms were found The antibody test is highly sensitive for the detection of *D immitis* infection because it detects host antibody produced against both larval and adult *D immitis* The antibody test may be positive for infections in which the migrating worms have not yet reached the heart or died before reaching the heart (Bestul et al., 1998) The test may also be positive if adult worms in the heart have already died but

antibody is still present in the circulation. In a recent study in which heartworms were surgically removed from cats, one cat remained antibody positive until 18 months after surgery (Bestul et al., 1998). It is possible that the inconsistencies between necropsy and serologic testing could have been a result of test failure.

A similar study of cats collected from animal shelters in eastern Tennessee between 1985 and 1990 reported that 2.5% (3/122) of cats had worms in the heart at necropsy, and 0.82% (1/122) were positive for *D immitis* antigen (Patton and McCracken, 1991) These results are not significantly different from necropsy and antigen test results of shelter cats in this study, showing no change in prevalence over the time period

Banked Samples

There were 803 banked feline serum samples tested for heartworm antigen. There was no pattern of increase or decrease in yearly prevalence over the five-year period. The prevalence of infection in cats was higher than the prevalence in dogs tested, but the difference was not statistically significant It is probable that many cats were submitted for either *T gondii* or heartworm testing because they were showing some clinical signs and some disease was suspected This would result in a population of "sick cats" rather than of healthy cats whose serum was submitted for routine screening. Conversely, the majority of dogs were submitted for yearly routine testing and it is expected that many were on heartworm prophylaxis The yearly prevalence of heartworm infection in cats did not appear to parallel that of dogs. With prevalence, it is impossible to know when initial infection occurred It would be necessary to know how many cats and dogs became infected each year, or the incidence of infection, to show this association.

A correlation was found between the average yearly precipitation in eastern Tennessee and the percentage of heartworm antigen positive cats. The correlation coefficient (r=0.976) shows a direct, linear relationship with a correlation coefficient of 1.0 being a perfect direct, linear relationship Because some mosquito species rely on standing water for breeding purposes, it is speculated that the more water present, the more successful mosquito breeding will be. Because it takes 6-8 months for the worms to mature and be detected by an antigen test after a cat is bitten by an infective mosquito, it would be expected that an increase in heartworm infection would occur at least one year after the increase in the mosquito population occurred. When the prevalence of infection in cats was offset from the precipitation by one year, ie precipitation in 1994 paired with prevalence in 1995, the correlation coefficient showed an indirect, linear relationship (r= -0 93) Again, it would be more useful to know how many cats became infected each year to show the relationship between precipitation and heartworm infection. A correlation was not found between average yearly temperature in eastern Tennessee and the yearly percentage of heartworm antigen positive cats (r=0 053)

A chi-square analysis showed no statistical difference between antigen positive cats submitted for *T gondii* or heartworm testing (p=0.37) This means a cat was equally likely to be heartworm antigen positive, regardless of which test was requested Cats with respiratory signs resulting from heartworm infections may initially be suspected as having toxoplasmosis, since respiratory signs associated with both *T gondii* and heartworm infections are similar However, the presence of a *T gondii* titer may indicate a chronic, rather than an acute infection, with any present respiratory problems being unrelated to the *T gondii* infection

In a similar study in which cats seen at the University of Tennessee Veterinary Teaching Hospital between 1985 and 1990 were tested for heartworm antigen, 9 5% (9/95) were positive for antigen (Patton and McCracken, 1991) This is not significantly different from the overall percentage of antigen positive cats from 1994 to 1998 This indicates that there has been no overall increase or decrease in prevalence of *D immitis* infection in cats in eastern Tennessee over this time period.

Veterinary Clinic Samples

Of the 560 serum samples collected from local veterinary clinics, 8.6% (48) were positive for either heartworm antigen or antibody, or both This is similar to the 8 8% positive found in the shelter cats in this study Many studies to determine prevalence of heartworm infection in cats have used shelter cats exclusively because of the ability to necropsy each cat for definitive proof of infection. The prevalence found in shelter cats has been used to estimate the prevalence in pet cats in the same area. It is thought that the prevalence in shelter cats would be higher than that of pet cats because they are often free-roaming and would have had more contact with potentially infective mosquitoes However, this study has shown the prevalence is similar in both populations This is possibly an effect of age. The older a cat, the more time it has had to contact infective mosquitoes. Client questionnaires of the pet cats sampled showed an age range from six months to 19 years with an average age of seven years While the actual ages of the shelter cats are unknown, it is probable that because many were ownerless and roaming in areas where they were likely to be taken in by animal control, that the average age was much lower than that of the pet cats The age difference in shelter and pet cats, then, would average the number of exposures with pet cats being exposed less often but over a

longer period of time. Another possibility for the similarity in prevalence is that free-roaming cats may actually become infected with heartworms more often than pet cats, but die from the disease before they are brought to shelters. This would mean that the prevalence calculated by necropsy of shelter cats would underestimate the prevalence in free-roaming cats. Also, many shelter cats may have been pet cats surrendered to the animal shelter by their owners and would, therefore, have a similar prevalence of heartworm infection to the pet cats seen at veterinary clinics.

There were 16 samples positive for antigen but negative for antibody. Recent studies show evidence that antibody concentration may rise and fall during the course of an infection (Genchi et al., 1998; Dillon et al., 1998). Most studies to determine the performance of antibody tests use experimentally infected cats and rarely exceed nine months in duration. (McCall et al., 1998; Bestul et al., 1998). These studies give no information about what happens to antibody concentration over the entire course of the infection. Dillon and colleagues studied pet cats and reexamined them three times over several months. They found that antibody status of infected cats changed from both positive to negative, and negative to positive over the three examinations (Dillon et al , 1998) It is possible that antibody concentration is detectable during the two times when signs of disease are present, when immature adults first reach the heart and again as the adult worms die. But during the middle of the infection, when cats are often asymptomatic, little antibody is produced (Genchi et al., 1998) During these times of low antibody production, antigen produced by adult worms may be detectable

Risk Factor Analysis

In this study, no association was found between male and female cats and heartworm infection status. There were 74 heartworm antigen, antibody or necropsy positive cats in the 956 cats for whom sex was known. The percentage of infected females was slightly higher with 8.3% compared to males with 7.1%, but with no significant difference However, it is often reported that male cats have higher heartworm infection rates than female cats in both experimental and natural infections One study using cats from a county animal shelter reported finding that male cats appeared to be at a higher risk for infection than female cats (Nelson and Young, 1998). This study found 25 heartworm infected cats out of 259 necropsied, with male and female infection rates to be 12.3% and 7 6% respectively However, my analysis of their data showed no significant difference ($p=0.204$) between males and females. In another study, infection rates were found to be 82% and 67% for experimentally infected male and female cats respectively but, there was no statistical significance in their data (McTier et al., 1992). Although McTier and collegues made no statement in their study about the difference in infection between males and females, this study has been cited by others to show a higher infection rate in male cats It is possible that infection rates of these and similar studies could be misinterpreted and lead to misconceptions regarding sex as a risk factor for heartworm infection in cats.

It has been speculated that long fur has a protective effect against heartworm infection by providing a physical barrier against mosquito bites In this study, the percentage of positive long hair and short hair cats was similar with no significant difference It is possible that mosquitoes most frequently bite cats around the ears, so that fur length

would not be a factor in determining risk of infection. These data provide evidence that long fur is not a protective characteristic against heartworm infection in cats.

It is thought that intact cats are at an increased risk for heartworm infection because behavioral characteristics, such as roaming, would allow them more exposure to potentially infective mosquitoes. In this study, no statistical difference was found between neuter status and heartworm infection in cats. However, when all three groups were combined, analysis resulted in a p value of 0.11, which was the smallest p value of any analysis in this study. It may be that intact male cats are more likely to roam than intact female cats, so an association is only seen in males When only male cats whose neuter status was known were analyzed, however, no statistical significance was found. This may be because the effect of the behavior of intact cats is negligible and other factors such as proximity to mosquito breeding habitats are more indicative of risk of exposure.

It is expected that the less time a cat spends outdoors, the less likely they are to be exposed to infective mosquitoes, so that being totally indoors should have a protective effect against heartworm infection. In this study, similar percentages of positive indoor and outdoor cats were found with no statistical significance. This has been reported in other studies in which cats reported to live exclusively indoors had a slightly higher infection rate than cats who lived exclusively outside (Dillon et al., 1998). The reason these findings have not shown the expected effect being totally indoors should have on heartworm infection rate could be because of the owner's perception of their cats time spent inside. It is possible that owners perceive their cat as living exclusively inside, but actually allow them onto a porch or deck. It is also possible that owners intend their cat to

be kept inside, but they are accidentally let outside for short periods. Another possibility is that owners have adopted a stray cat and have kept it exclusively indoors for the time they have owned it, and any infection it may have acquired would have occurred during its time as a stray. These factors could overestimate the prevalence of heartworm infection in indoor cats, but these data do provide evidence that being a totally indoor cat does not exclude it from the possibility of infection

It is unknown what species of mosquito is responsible for transmitting heartworm infection to cats. In an attempt to implicate a vector species, days were subdivided into four, six hour segments so that the dawn hours and the dusk hours would each be in a segment. Owners were questioned as to which segments of time their cats spent outdoors Because many mosquito species feed only at specific times of day, such as dusk or dawn, if significantly more cats reported to be outdoors during a particular time segment were positive, then that would help limit the number of possible vectors to those who are known to feed at that time No statistical difference was found between time of day spent outside and heartworm infection status This could mean that several species of mosquito feeding at different times of day are responsible for transmission or that the species involved feeds throughout a 24-hour day. It is also possible that a mosquito species responsible for transmission of heartworm infection to cats will come inside homes to feed.

Vomiting has been reported in cats with heartworm infection In a recent study of 94 antibody positive cats with signs thought to be consistent with heartworm infection, 24% presented with vomiting (Dillon, 1998) In this study, 15 cats brought to participating veterinary clinics were reported to be vomiting Only one of those 15 was positive for

heartworm infection. It is possible that significant numbers of cats with reported vomiting testing heartworm positive are only found when the study is restricted to cats with clinical signs thought to be consistent with heartworm disease Also, it is possible that some cats in this study had a history of vomiting, but were not brought to clinics for that reason.

Cats that were reported to have respiratory signs seen at veterinary clinics had the highest percentage of heartworm infection with 15%, however this was not significantly higher than the percentage of heartworm positive cats brought to clinics for any other reason. Respiratory signs have been found to occur more often in cats with heartworm infection. In a recent study, dyspnea and cough were found to occur significantly more often in cats with heartworm infections than any other clinical sign observed (Atkins et al , 1998). In this study, three out of 20 cats with respiratory signs were heartworm positive It is possible that there were too few cats with respiratory signs in this study to show an association

Conclusion

Cats in eastern Tennessee are infected with *D immitis* with an overall prevalence of 8 8% in cats obtained from the Knox county animal shelter, 5 5% in banked samples collected from 1994 to 1998, and 8.6% in pet cats seen at veterinary clinics No characteristic analyzed was found to be significantly associated with heartworm infection in cats This finding indicates that all cats are at equal risk for heartworm infection Because of the limitations in heartworm diagnostic tests and the complexity of the disease in cats, these data suggest that the use of both antigen and antibody tests to detect heartworm infections in cats is necessary.

These results will help clinicians give clients accurate information about the scope of heartworm infection in cats in this area. Knowledge of local prevalence can be used along with diagnostic tests to aid in diagnosis of heartworm disease by putting into perspective the likelihood of infection.

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APPENDIX

QUESTIONNAIRE

1 Which county do you live in? _____

2. How many years and/or months have you lived in this county? _____years_____months

3. What is the birth date of your cat? _____month _____year

4 How many years and/or months have you owned your cat? _____years _____months

5 During a typical day (24 hour period), how many hours does your cat spend outside? _____hours

Please indicate how often your cat is outside during each of the following time periods

Time period	Always or almost always	Sometimes	Never
9am-3pm			
3pm-9pm			
9pm-3am			
3am-9am			

6 Is your cat (circle one of the following) A male, neutered B male, not neutered C female, spayed D female, not spayed

7 Is your cat a particular breed (ie Siamese, Persian, etc)? _____yes _____no if yes, what breed?_____

8. Which best describes the overall length of your cats fur? (circle one) A short B long

9 Is your cat on Heartguard for cats? (a monthly, chewable heartworm preventive) _____yes _____no

10 What is the reason for your clinic visit today? (please list ALL reasons)

Check how often you use the following products **on your cat**

Product	Often	Sometimes	Never
Flea/tick collar/medallion			
Flea shampoo/dip			
Flea/tick spray/powder			
Spot on flea control (liquid applied to back of neck)			
Oral flea control (liquid mixed with food or given orally)			
Spray insect repellant (ie Off)			
Please list below anything else you use to control insect pests on your cat.⬇			

Table A-1 Results of the 48 heartworm positive cats seen at veterinary clinics

Cat ID #	Dirochek antigen	Assure/FH antibody	Cat ID #	Dirochek antigen	Assure/FH antibody
8	-	+	156	+	-
25	+	-	166	-	+
28	+	-	168	+	-
37	-	+	190	-	+
41	-	+	192	-	+
43	+	-	200	-	+
50	+	+	221	+	-
51	+	-	222	-	+
56	-	+	228	-	+
66	+	+	252	-	+
75	-	+	253	-	+
81	-	+	322	-	+
87	-	+	327	-	+
89	+	-	348	-	+
91	-	+	363	-	+
111	-	+	372	+	-
113	+	+	414	+	-
124	+	-	433	-	+
125	-	+	468	+	-
130	+	-	472	-	+
134	+	+	494	+	-
136	+	-	510	-	+
148	-	+	535	-	+
153	-	+	556	+	-

Table A-2. Results of client questionnaires for cats seen at veterinary clinics.

Characteristic	#/total*	% of total	# positive	% positive
No breed	496/553	89 7	40	8 1
Yes breed	57/553	10.3	7	12 3
Abyssinian	4/56	7 1	0	0
British Blue	1/56	1.8	0	0
Burmese	1/56	1 8	1	100
Himalayan	11/56	19 6	2	1 8
Maine Coon Cat	7/56	12.5	1	14 3
Manx	3/56	5 4	1	33 3
Ocicat	1/56	1 8	1	100
Persian	9/56	16 1	1	11 1
Siamese	12/56	21 4	0	0
Scottish Fold	2/56	3 6	0	0
Snow shoe	1/56	1.8	0	0
Oriental shorthair	3/56	5 4	0	0
Ragdoll	1/56	1.8	0	0
Long fur	155/552	28 1	14	9.3
Short fur	397/552	71 9	35	8 8
Outside 0 hrs	264/547	48 3	23	8 7
Outside > 0 hrs	283/547	51.7	25	8.8
Male	264/554	47.7	21	8 0
Female	290/554	52.3	27	9 3
Spayed or neutered	535/554	96 6	44	8.2
Intact male/female	19/554	3.4	3	15 8
Male neutered	256/554	46 2	20	7 8
Male intact	8/554	1 4	1	12.5
Female spayed	279/554	50 4	25	9 0
Female intact	11/554	2 0	2	18 2
Heartworm preventive	19/554	3 4	2	10 5
No heartworm preventive	535/554	96 6	46	8.6
Reason for visit:				
Respiratory	20/539	3 7	3	15 0
Vomiting	15/539	2 8	1	6.7
Vaccinations	282/539	52 3	28	9 9

Table A-2 continued

Reason for visit:	**#/total**	**% of total**	**# positive**	**% positive**
Treatment	99/539	18 4	7	7 1
Other sick	40/539	7 4	2	5 0
Heartworm study	96/539	17 8	10	10 4
Spay/neuter	9/539	1 7	1	11.1
Other surgery	7/539	5 0	0	0
Other	43/539	8 0	5	11.6

* total= number of clients answering the question

A-3 Results of client questionnaires on use of flea control products in cats seen at veterinary clinics.

Flea control	Often Number positive/total	Sometimes Number positive/total	Never Number positive/total
Collar or medallion	1/17	2/37	42/427
Shampoo/dip	1/5	6/101	38/376
Spray/powder	0/5	1/31	43/440
Spot on	13/164	12/151	22/216
Oral	0/4	1/12	44/463
Insect repellant	0/2	0/11	45/464
Other	0/6		

VITA

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