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Nationwide Seroprevalence of *Dirofilaria immitis* Antigen and Antibodies to *Borrelia burgdorferi*, *Anaplasma* spp., and *Ehrlichia* spp. in Shelter Cats in the United States, 2007–2011

Rachel C. Smith [1], Lindsay A. Starkey [1,*], Joy V. Bowles [2], Jamie M. Butler [2], Jane Mount [2], Tracy M. Land [2] and Byron L. Blagburn [2,†]

[1] Department of Pathobiology, College of Veterinary Medicine, Oklahoma State University, Stillwater, OK 74078, USA; rachel.c.smith@okstate.edu

[2] Department of Pathobiology, College of Veterinary Medicine, Auburn University, Auburn, AL 36832, USA

* Correspondence: lindsay.starkey@okstate.edu

† Author is deceased.

Abstract: Vector-borne infections persist as a significant issue in both human and animal health. Many of the most common vector-borne infections in the USA, especially tick-borne infections, are known to be zoonotic, including Lyme disease, anaplasmosis, and ehrlichiosis, and these infections may also negatively impact the health of infected animals. Convenient patient-side assays for the detection of antibodies to *Borrelia burgdorferi*, *Anaplasma* spp., and *Ehrlichia* spp., and antigen of *Dirofilaria immitis* have allowed for the generation of robust and large-scale prevalence data in dogs. Data of similar scale and distribution are not available in cats, and most feline prevalence studies have evaluated a small sample size with limited geographic distribution. The objective of this study was to evaluate the prevalence of antibodies to *B. burgdorferi*, *Anaplasma* spp., and *Ehrlichia* spp., and *D. immitis* antigen among shelter cats across the USA, a population that is presumably at high risk for ectoparasitism and, consequently, exposure to vector-borne infections. In total, 2232 whole blood samples were collected from shelter cats across four regions of the USA—South, Northeast, Midwest, and West—and were evaluated using the Idexx SNAP® 4Dx® Test. Ectoparasites were also opportunistically collected from cats during blood collection and morphologically identified. The prevalence of at least one vector-borne infection was 2.60%, and the nationwide prevalence was 1.88% for *B. burgdorferi*, 0.54% for *Anaplasma* spp., 0.09% for *Ehrlichia* spp., and 0.55% for *D. immitis*. A total of 1120 ectoparasites were collected from 423 cats, including 27 ticks and 1093 fleas. Although the overall prevalence of the pathogens in this survey is relatively low, we observe that there is an increased exposure risk regionally for some agents, with geographic distributions in this study mostly coinciding with established human and canine distributions. Understanding these findings in an assumed non-protected population of cats allows us to extrapolate the risk to pet cats if they are not provided routine veterinary care, including a broad-spectrum parasite prevention program.

Keywords: *Anaplasma*; antibodies; antigen; *Borrelia burgdorferi*; *Dirofilaria immitis*; *Ehrlichia*; seroprevalence; shelter medicine

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1. Introduction

Vector-borne infections are a significant burden on both human and animal health across the globe. In the United States, the incidence of vector-borne disease in people, particularly tick-borne diseases, continues to rise as vector and reservoir populations increase and the geographic distributions of vectors expand [1]. Among the vector-borne infections that are nationally notifiable in the USA, Lyme disease caused by *Borrelia burgdorferi* is by far the most common, followed by granulocytic anaplasmosis caused by *Anaplasma phagocytophilum*, babesiosis, and ehrlichiosis caused by *Ehrlichia chaffeensis* and *Ehrlichia*

ewingii [2]. Many vector-borne infections impacting human health are zoonotic, and companion animals can play an important role in the epidemiology and expanding distribution of some zoonotic vector-borne diseases [3]. Domestic dogs have been suggested as an effective sentinel species for evaluating local and regional risk for Lyme disease and ehrlichiosis [4,5]. Widespread circulation of zoonotic vector-borne infections among companion animals could have substantial public health implications; furthermore, many of these infections can produce clinical disease in pets. For both of these reasons, there is significant interest in evaluating the prevalence of these infectious agents, particularly *B. burgdorferi*, *Anaplasma* spp., and *Ehrlichia* spp. among companion animals. The availability and convenience of multi-target, point-of-care serological assays that are labeled for use in dogs have led to the generation of vast amounts of data on the canine prevalence of antibodies to *B. burgdorferi*, *Anaplasma* spp., *Ehrlichia* spp., and antigen of *Dirofilaria immitis*, also known as heartworm [6–8]. The Companion Animal Parasite Council (CAPC) gathers canine prevalence data based on the results of these patient-side assays and maintains continuously updated prevalence maps through their website [9].

Although there are numerous large-scale prevalence studies of these agents in domestic dogs, investigation into the prevalence of these same agents in domestic cats is more limited. The majority of studies determining the prevalence of *B. burgdorferi*, *Anaplasma* spp., and *Ehrlichia* spp. among cats in the USA have tested a sample size with limited geographic distribution (Table 1).

Table 1. Chronological summary of previously published studies on the prevalence of *B. burgdorferi*, *Anaplasma* spp., and *Ehrlichia* spp. in cats in the USA: antibody (Ab) detection, polymerase chain reaction (PCR), enzyme-linked immunosorbent assay (ELISA), and immunofluorescence assay (IFA).

Geographic Distribution	Sample Source	Detection Method	Prevalence	Reference
B. burgdorferi				
Northeast	Owned pet cats	Ab detection	14.1% (10/71)	[10]
Northeast	Owned pet cats	Ab detection	47.31% (44/93)	[11]
Nationwide	Submitted for Dx at NCSU-VBDDL	Ab detection	5.45% (39/715)	[12]
MD	Owned pet cats	Ab detection PCR	28.00% (7/25) 0.00% (0/49)	[13]
ME	Owned pet cats	Ab detection	18.23% (29/159)	[14]
MA	Feral cats	Ab detection	24.00% (42/175)	[15]
***Anaplasma* spp.**				
FL	Free roaming cats	PCR	0.00% (0/484)	[16]
Northeast	Owned pet cats	Ab detection (IFA and ELISA)	37.63% (35/93) 30.11% (28/93)	[11]
AL, MD, TX	Owned pet and sheltered cats	PCR	0.00% (0/92)	[17]
IL, OK, CO, MD OR, NY	Blood donor cats	PCR	0.00% (0/146)	[18]
Unreported	Anemic and healthy owned cats	PCR	0.00% (0/176)	[19]
AL, FL, CA WI, MI, RI	Owned pet and sheltered cats	Ab detection	4.30% (20/460)	[20]

Table 1. *Cont.*

Geographic Distribution	Sample Source	Detection Method	Prevalence	Reference
Nationwide	Submitted for Dx at NCSU-VBDDL	Ab detection and PCR	*Anapl* genus Ab 0.70% (5/715) *A. phag* Ab 1.80% (13/715) *A. platys* Ab 0.00% (0/715) *A. phag* PCR 0.68% (5/737) *A. platys* PCR 0.41% (3/737)	[12]
MD	Owned pet cats	Ab detection	4.00% (1/25)	[13]
SD	Owned pet cats	PCR	0.00% (0/39)	[21]
MA	Feral cats	Ab detection PCR	9.71% (17/175) 6.94% (12/173)	[15]
***Ehrlichia* spp.**				
FL	Free roaming cats	PCR	0.00% (0/484)	[16]
IL, OK, CO, MD OR, NY	Blood donor cats	PCR	0.00% (0/146)	[18]
AL, MD, TX	Owned pet and sheltered cats	PCR	0.00% (0/92)	[17]
Unreported	Anemic and healthy owned cats	PCR	0.00% (0/176)	[19]
Gulf Coast Region	Unknown history following natural disaster	PCR	0.00% (0/47)	[22]
Nationwide	Submitted for Dx at NCSU-VBDDL	Ab detection PCR	*Ehrl* genus Ab 0.70% (5/715) *E. canis* Ab 0.70% (5/715) *E. ewingii* Ab 0.14% (1/715) *E. chaffeensis* Ab 0.28% (2/715) *E. canis* PCR 0.14% (1/737) *E. ewingii* PCR 0.41% (3/737) *E. chaffeensis* PCR 0.27% (2/737)	[12]
MD	Owned pet cats	Ab detection	12.00% (3/25)	[13]
SD	Owned pet cats	PCR	0.00% (0/39)	[21]

Within geographic regions of the USA where Lyme disease and anaplasmosis are endemic, feline antibody prevalence has been reported to be as high as 47% and 37% for *B. burgdorferi* and *Anaplasma* spp., respectively [11]. This is remarkably high exposure considering that the sample population in that study was owned pet cats. Other studies testing samples from both owned and feral cats have found a substantially lower feline antibody prevalence (Table 1). Evidence of *Ehrlichia* spp. infection has rarely been reported in cats in the USA, and the reported prevalence is much lower compared to *B. burgdorferi* or *Anaplasma* spp. (Table 1). Only one study has been published that investigated the prevalence of these agents in a large population of cats with nationwide geographic distribution [12]. To date, there are no published studies on the prevalence of *B. burgdorferi*, *Anaplasma* spp., and *Ehrlichia* spp. that have specifically surveyed a large population of shelter cats with nationwide distribution despite this being a population that is presumably

at high risk for exposure to vector-borne infections. There are a number of factors that likely contribute to limited data on cats compared to dogs. Currently, available point-of-care assays that detect antibodies to these agents are only labeled for canine use [23–25], and this may deter some clinicians from using these tests for feline patients. There remain questions regarding the pathogenicity of *B. burgdorferi*, *Anaplasma* spp., and *Ehrlichia* spp. in cats. Experimental infection of cats with *B. burgdorferi* and *A. phagocytophilum* has resulted in infection and seroconversion in the absence of overt disease [26], and attempted infection of cats with *Ehrlichia canis* was unsuccessful [27]. Nonetheless, clinical signs consistent with tick-borne disease have been reported in some naturally infected cats, although the majority of naturally infected cats do not demonstrate signs of disease at the time antibodies are detected. Despite these factors, the significance of these tick-borne pathogens in cats and the epidemiological contribution of cats can only be accurately assessed through a large-scale investigation into the prevalence of these infections in feline populations of various lifestyles and risks.

In comparison with common tick-borne infections, there is far more investigation into the prevalence of *D. immitis* in felines. This is likely in part due to the availability of diagnostic tests approved for use in cats. Because cats rarely develop patent heartworm infection [28,29], infection is usually self-limited, and felines do not contribute substantially to the infection of vectors or the spread of heartworm disease. Regardless, heartworm can cause serious and fatal diseases in cats that become infected. Heartworm diagnosis is complex in cats, and it has been shown that a multimodal approach combining antigen testing, antibody testing, and ancillary testing increases diagnostic accuracy in cats [30,31]. Antigen detection alone must be interpreted cautiously in cats, as it almost certainly underestimates the true burden of feline heartworm disease. However, antigen detection for *D. immitis* is conveniently included alongside antibody detection of common tick-borne pathogens in multiple canine point-of-care assays. The purpose of this study was to evaluate the infection prevalence of four vector-borne agents—*Borrelia burgdorferi*, *Anaplasma* spp., *Ehrlichia* spp., and *D. immitis*—among shelter cats from across the USA. When possible, ectoparasites were also collected from sampled cats and morphologically identified in order to estimate the frequency and diversity of ectoparasites on shelter cats and, consequently, potential exposure to vector-borne infections.

2. Results

A total of 2232 whole blood samples and 1120 ectoparasites were collected between 2007 and 2011 from individual shelters (n = 115). All four regions of the USA, with a total of 46 states, were represented. The most samples were submitted from the Midwest, with n = 756 samples originating from 32 shelters, followed by the Northeast, with n = 637 samples originating from 26 shelters, the West with n = 484 samples originating from 27 shelters, and the South with n = 355 samples originating from 30 shelters. Samples were solicited but not received from Alaska, Arkansas, Maine, and Rhode Island. The average age for the sample population was 1.89 $\pm$ 2.37 years among cats for which an age estimate was reported (n = 1866). The total population consisted of 1188 females, 986 males, and 58 cats for which no sex was reported (n = 2232). Among the samples submitted, 2.60% (58/2232) [95% CI: 1.96–3.37%] demonstrated evidence of infection with at least one vector-borne agent, with the highest prevalence occurring in the Northeast at 6.91% (44/637) [95% CI: 5.11–9.23%], followed by the Midwest 1.19% (9/756) [95% CI: 0.58–2.33%], South 0.85% (3/355) [95% CI: 0.22–2.66%], and West 0.41% (2/484) [95% CI: 0.07–1.65%] (Table 2).

Table 2. Prevalence of antibodies to *B. burgdorferi*, *Anaplasma* spp., and *Ehrlichia* spp., and antigen of *D. immitis* in shelter cats, USA, 2007–2011.

	B. burgdorferi	***Anaplasma* spp.**	***Ehrlichia* spp.**	***D. immitis***
Total	1.88% (42/2232)	0.54% (12/2232)	0.09% (2/2232)	0.55% (8/1457)
Northeast	5.34% (34/637)	1.10% (7/637)	0.31% (2/637)	1.29% (5/389)
Midwest	0.66% (5/756)	0.53% (4/756)	0.00% (0/756)	0.00% (0/518)
South	0.56% (2/355)	0.00% (0/355)	0.00% (0/355)	0.64% (2/312)
West	0.21% (1/484)	0.21% (1/484)	0.00% (0/484)	0.42% (1/238)

2.1. B. burgdorferi

The nationwide prevalence of antibodies to *B. burgdorferi* was 1.88% (42/2232) [95% CI: 1.38–2.56%], with the highest prevalence in the Northeast, followed by the Midwest, South, and West (Figure 1). The prevalence of *B. burgdorferi* was the highest among the infectious agents surveyed in this study and the only agent for which every region had at least one positive. Region was observed to have a significant effect on the prevalence of *B. burgdorferi* ($p < 0.05$), and the prevalence in the Northeast was significantly higher compared to the West ($p < 0.05$), Midwest ($p < 0.05$), and South ($p < 0.05$). Season was also observed to have a significant effect on the prevalence of *B. burgdorferi* ($p < 0.05$), but seasonal prevalence only differed significantly between fall and winter ($p < 0.05$).

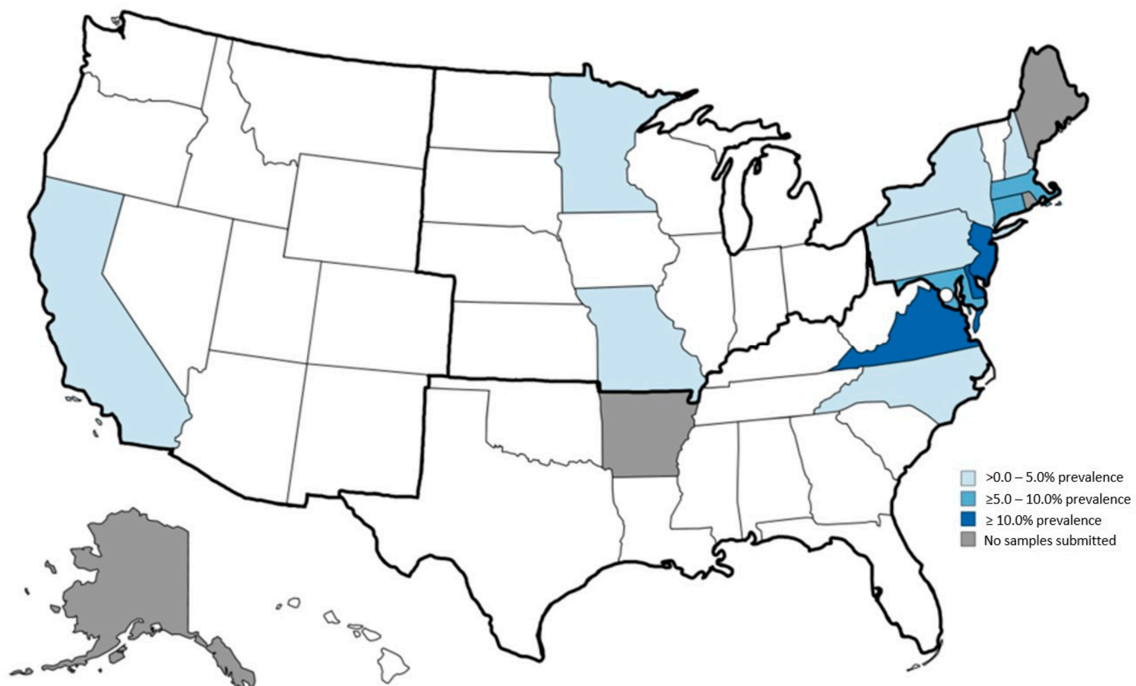

Figure 1. Prevalence of antibodies to *Borrelia burgdorferi* in shelter cats, USA, 2007–2011.

2.2. Anaplasma spp.

The nationwide prevalence of antibodies to *Anaplasma* spp. was 0.54% (12/2232) [95% CI: 0.29–0.97%] (Figure 2), with the highest regional prevalence occurring in the Northeast, followed by the Midwest, West, and South (Table 2). Neither region ($p = 0.11$) nor season ($p = 0.24$) were found to have a significant effect on prevalence. Evidence of co-exposure to *B. burgdorferi* was observed in 50.0% (6/12) of cats that had antibodies to *Anaplasma* spp.

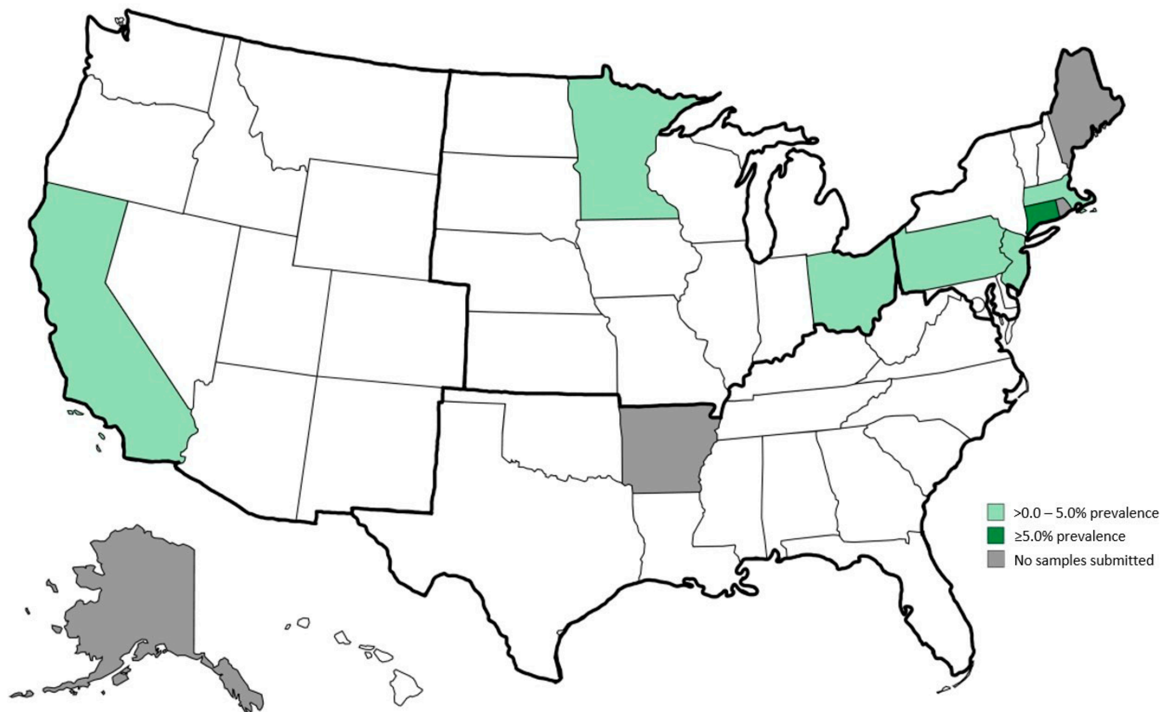

Figure 2. Prevalence of antibodies to *Anaplasma* spp. in shelter cats, USA, 2007–2011.

2.3. *Ehrlichia spp.*

The nationwide prevalence of antibodies to *Ehrlichia* spp. was 0.09% (2/2232) [95% CI: 0.02–0.36%], with both positives originating from the Northeast (Figure 3). This was the lowest prevalence among the infectious agents surveyed in this study (Table 2). Neither region ($p = 0.22$) nor season ($p = 0.48$) were found to have a significant effect on prevalence.

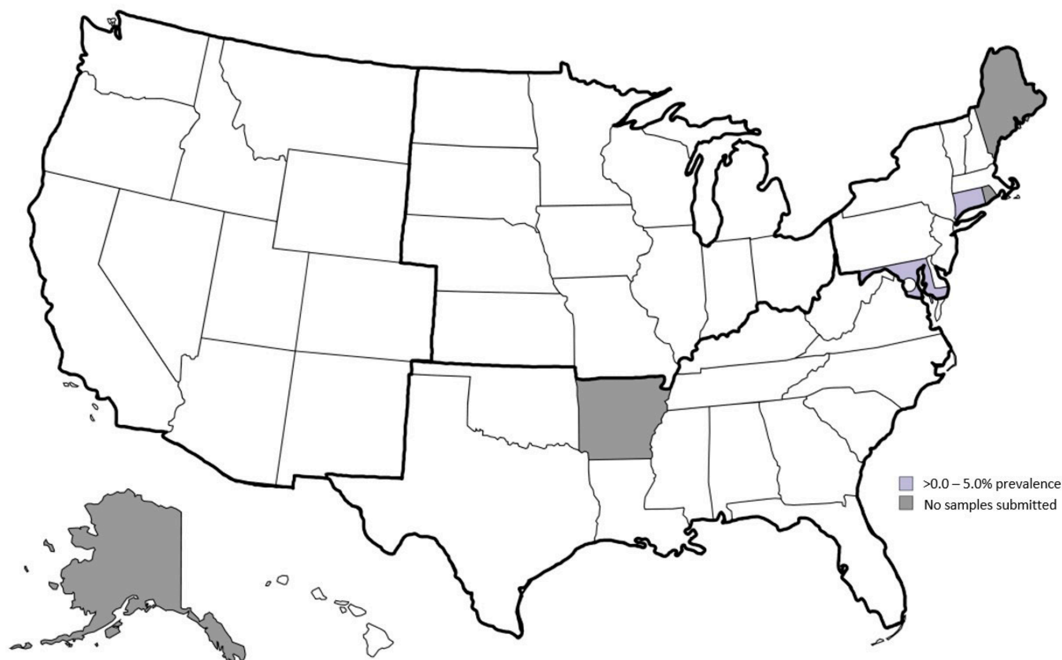

Figure 3. Prevalence of antibodies to *Ehrlichia* spp. in shelter cats, USA, 2007–2011.

2.4. Dirofilaria immitis

The nationwide prevalence of *D. immitis* antigen was 0.55% (8/1457) [95% CI: 0.26–1.12%] among cats greater than or equal to 6 months of age (Figure 4). The highest prevalence occurred in the Northeast, followed by the South, Midwest, and West (Table 2). Neither region ($p = 0.14$) nor season ($p = 0.09$) were found to have a significant effect on prevalence.

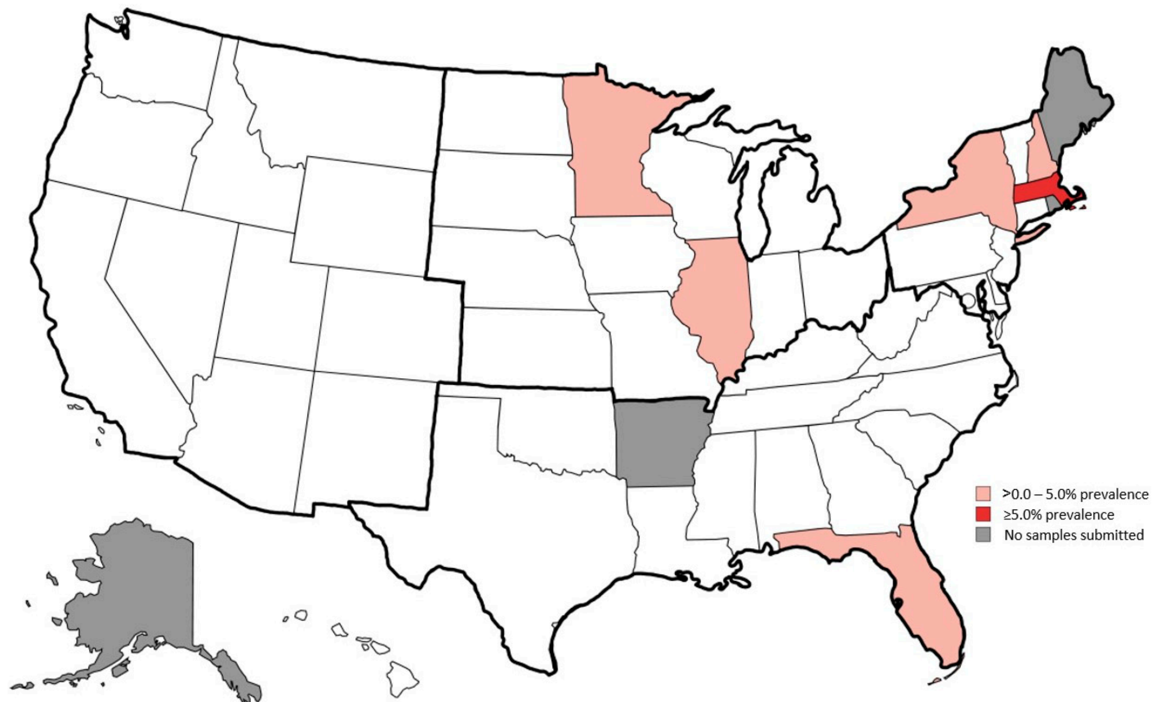

Figure 4. Prevalence of *Dirofilaria immitis* antigen in shelter cats, USA, 2007–2011.

Interestingly, among the eight cats that tested positive for heartworm antigen, three originated from the same shelter in Massachusetts within Worcester County and were submitted during June 2011.

2.5. Ectoparasites

In total, 1120 ectoparasites ($n = 27$ ticks and $n = 1093$ fleas) were collected from 423 individual cats. Adult ticks were recovered from 15 cats, with the number of ticks collected from individual cats ranging from one to five. Among the ticks collected, the most commonly identified species was *Ixodes scapularis* ($n = 11$), followed by *Dermacentor variabilis* ($n = 5$) and *Amblyomma americanum* ($n = 5$), *Otobius megnini* ($n = 3$), *Rhipicephalus sanguineus* ($n = 1$), *Amblyomma maculatum* ($n = 1$), and one that was too damaged to be morphologically identified. All ticks collected in this study fell within previously described geographic distribution ranges (Figure 5). Fleas were collected from 411 cats with the number of fleas collected from individual cats ranging from 1 to 20. Among the fleas collected, the most commonly identified species was *Ctenocephalides felis* ($n = 1056$), followed by *Ctenocephalides canis* ($n = 23$), *Cediopsylla simplex* ($n = 11$), *Pulex irritans* ($n = 1$), *Echidnophaga gallinacea* ($n = 1$), and one that was too damaged to be morphologically identified. Flea and tick co-infestations were observed in three cats, consisting of *I. scapularis* and *C. felis* in two cats, and *D. variabilis* and *C. felis* in one cat.

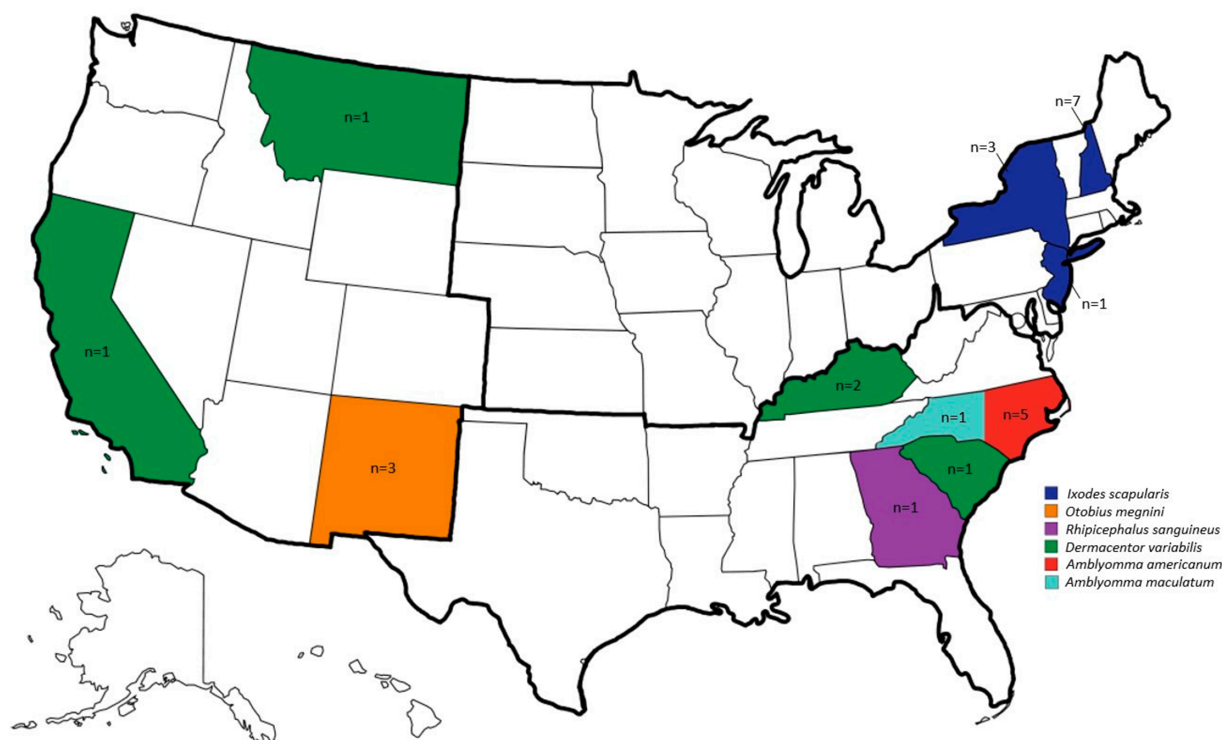

Figure 5. Identification and distribution of tick species collected from shelter cats, USA, 2007–2011.

3. Discussion

To the authors' knowledge, this is the largest and most geographically diverse survey for antibodies to *B. burgdorferi*, *Anaplasma* spp., *Ehrlichia* spp., and antigen of *D. immitis* using the Idexx SNAP® 4Dx® Test in shelter cats in the USA. Although data for this study were originally collected more than 10 years ago, there have been no published reports on the prevalence of these select vector-borne infections for a feline population of similar size, distribution, and risk. Among the tick-borne pathogens surveyed in this study, we detected antibodies to *B. burgdorferi* most frequently, followed by *Anaplasma* spp., and antibodies to *Ehrlichia* spp. were detected least frequently. We found that evidence of exposure to *B. burgdorferi* and *Anaplasma* spp. was most common in the Northeast region, which is consistent with findings of a previous nationwide feline survey as well as canine and human data [7,12,32,33]. Although the geographic distributions of *B. burgdorferi* and *Anaplasma* spp. exposure in this study were consistent with expected trends, the overall and Northeast regional prevalence of these agents was shockingly low compared to some previous regional and nationwide surveys (Table 1). Only one study has previously conducted a nationwide survey on cats for antibodies to *B. burgdorferi*, *Anaplasma* spp., and *Ehrlichia* spp., and the antibody prevalence observed in our study was slightly lower for all pathogens than reported in this previously published study [12]. It is important to note that the sample population of that previous report were feline samples submitted to the North Carolina State University Vector-Borne Disease Diagnostic Lab (NCSU-VBDDL). While the cats were likely to be owned and under veterinary care, they were also likely submitted to the NCSU-VBDDL for suspicion of vector-borne infection based on risk or presence of clinical signs. Although the samples used in the present study originated from shelter animals, which are presumably at greater risk for vector-borne infection exposure than well-cared-for pets, there was no reason to suspect vector-borne infection in the shelter cats sampled. Ectoparasite surveys have previously found *I. scapularis*, the primary vector of both *B. burgdorferi* and *A. phagocytophilum*, to be among the most common tick species found on owned and feral cats [34,35]. Presumably, upon intake, the majority of shelter-housed animals have had a lapse in parasite prevention, if they have any history of parasite prevention at all, and are likely to have spent extended time in environments with a higher

risk of ectoparasites than owned pets. One possible explanation for the low antibody prevalence in this study is that the longevity of antibodies to *B. burgdorferi*, *Anaplasma* spp., and *Ehrlichia* spp. are not well described in cats and likely vary between individuals. The duration of time between initial intake and sampling of cats for this study is not known, and this is a limitation. Detectable antibodies are likely to wane over time, and it is possible that prevalence would be higher if cats were sampled within a few weeks of initial intake. The overall prevalence of antibodies to *Ehrlichia* spp. in this study, 0.09%, was very low; however, this does not drastically differ from other published reports (Table 1). Cats appear to either be infrequently exposed or somewhat refractory to infection with *Ehrlichia* spp. circulating in the USA. *Ehrlichia* spp. infection is most often attributable to *E. chaffeensis* in humans and *E. ewingii* in dogs, with incidence concentrated in the Central and Mid-Atlantic USA, and less often by the more pathogenic *E. canis* in dogs, with incidence concentrated in the South-Central and Southeastern USA [4]. Regarding vectors of *Ehrlichia* spp., cats are more commonly parasitized by *A. americanum*, the primary vector of *E. chaffeensis* and *E. ewingii*, and are rarely parasitized by *R. sanguineus*, the primary vector of *E. canis* [34,35]. The two cats in this study with antibodies to *Ehrlichia* spp. originated from shelters in Maryland and Connecticut, and we speculate that they had most likely been exposed to *E. chaffeensis* or *E. ewingii*.

The overall prevalence of *D. immitis* antigen in this study, 0.55%, is low. At the time these data were collected, the pitfalls of heartworm antigen testing alone in cats were not as well understood as they are currently [29,31], and this is a limitation of the present study. Incorporating multimodal diagnostic strategies to include antibody testing and immune-complex dissociation antigen testing on the samples would have been desired. However, a much larger sample volume and budget would have been needed. However, when the present study is compared to other large-scale antigen detection studies with nationwide distribution, the overall prevalence in this study is higher but does not differ drastically from antigen prevalence previously reported in owned cats, ranging from 0.3 to 0.4%, and sheltered cats, 0.4% [36,37]. Unexpectedly, the majority of positive cats in this study originated from the Northeast, with three of the eight positives originating from the same shelter and submission time. Geographic clustering of vector-borne infections, including *D. immitis*, can be caused by infected reservoirs in the same area, causing increased vector infection and, consequently, increased local transmission and prevalence. Interstate movement of dogs, particularly transportation of animals from the southern USA to regions of low heartworm prevalence, can potentially cause increased heartworm prevalence in regions where infection is less commonly reported [38]. Complete histories were not reported for cats included in this study. Therefore, it is not known if the heartworm-positive cats in the Northeast could have been imported from a heartworm-endemic region. Alternatively, the cats may have been housed at a shelter facility with dogs imported from a region where heartworm is more prevalent. Lack of access to or knowledge of this information is a limitation of this study. Overall, the prevalence of infection with or exposure to vector-borne agents was relatively low in this study, which is somewhat surprising given the probable risk of the sample population. If the samples could be tested with currently available, improved assays and diagnostic approaches, we speculate that the true prevalence of these infections might be higher than what we report. Furthermore, although blood samples were tested within 48 h of receipt by Auburn University, an unknown time lapse between sample collection by shelters and receipt of samples for testing is a limitation of this study, which may have had an impact on antibody degradation, resulting in false negative results and under-diagnosis. However, even with the acknowledged limitations, these data still present valuable information on the prevalence of these agents among a high-risk population.

4. Materials and Methods

4.1. Sample Collection and Processing

Individual shelters from across the USA were contacted via telephone or email for interest in participation in this study. Shelters willing to participate were mailed a sample

collection kit to use at their discretion, which consisted of a Styrofoam cooler box for climate-controlled shipping, an information survey form, participation instructions, fecal specimen cups (to be used in a separate endoparasite survey study), EDTA blood collection tubes, syringes, and zippered plastic bags for collection of ectoparasites. Information, including the name, county, state of the submitting shelter, the identification number, species, estimated age, sex, and reproductive status corresponding to each animal sampled, was provided by the submitting shelter on the survey form. Sampled animals were housed and cared for by participating shelters, and the decision to collect and submit samples from an animal was carried out at the discretion of shelter personnel and in accordance with individual shelter policies. Participating shelters were requested to collect samples only from new-intake animals that had not yet been treated with parasiticides. Shelters were also requested to ship blood samples as soon as possible following collection, but not exceeding 48 h post-collection with refrigeration or 24 h post-collection if no refrigeration was available. Collected samples were return-shipped with cold packs to Auburn University College of Veterinary Medicine via overnight courier with completed survey forms. Upon receipt, blood samples were either refrigerated or tested immediately, and all blood samples were completely processed within 48 h of receipt. Plastic bags containing ectoparasites were immediately placed in a −20 °C freezer for a minimum of 3 h to ensure killing or immobilization of all ectoparasites prior to morphological identification. For identification of ticks, specimens were placed into a petri dish and identified using a tick identification key [39] under a dissecting microscope. For the identification of fleas, specimens were transferred to a glass slide with lactophenol underneath a coverslip. Fleas were then identified using a reference key [40] and compound microscope. Information from the survey forms, assay results, and the number and identification of ectoparasites from individual animals were compiled into a master Microsoft Excel (version 1808) spreadsheet along with the date of sample receipt.

4.2. Serological Assay

The Idexx SNAP® 4Dx® Test (IDEXX Laboratories, Inc., Westbrooke, ME, USA) is a point-of-care ELISA, which is for use in dogs to detect circulating *D. immitis* antigen and antibodies to *B. burgdorferi*, *A. phagocytophilum*, and *E. canis* [41]. It is important to note that the assay used in this study, the SNAP® 4Dx® Test, preceded subsequent versions of the assay, the first and second generations of the Idexx SNAP® 4Dx® Plus Test, which were released in 2012 and 2022, respectively [23,42]. These newer versions of the assay include additional peptides, resulting in earlier detection of antibodies to *A. phagocytophilum* and *E. canis*, as well as enhanced detection of *A. platys* and *E. ewingii* compared to the original SNAP® 4Dx® Test. The synthetic peptide included in the SNAP® 4Dx® Test for the detection of antibodies to *A. phagocytophilum* has demonstrated some cross-reactivity with *A. platys* [41]. The synthetic peptides included for the detection of *E. canis* have limited cross-reactivity with some strains of *E. chaffeensis* but did not cross-react with *E. ewingii* [43]. The first-generation SNAP® 4Dx® was validated in dogs with reported sensitivities for *D. immitis* (99.2%), *B. burgdorferi* (98.8%), *A. phagocytophilum* (99.1%), and *E. canis* (96.2%), and specificities of 100.0% for each target organism, relative to results of indirect fluorescent antibody (IFA) tests for the same targets [41]. The extra-label use of the SNAP® 4Dx® and SNAP® 4Dx® Plus tests in other hosts, including cats and horses, has previously been published [12,14,44,45]; however, the assays have not been validated or optimized for these hosts.

4.3. Data and Statistical Analysis

The geographic distribution of samples was divided into four regions (Northeast, Midwest, Southeast, and West) as previously described [46]. Seasonal distribution was evaluated according to the following categories: summer (June, July, August), fall (September, October, November), winter (December, January, February), and spring (March, April, May). All statistical analyses were performed using RStudio version 2024.4.1.748 (RStudio

Inc., Boston, MA, USA). The prevalence of each infectious agent was calculated along with corresponding 95% confidence intervals. Due to many of the contingency table cells having small values ($x \leq 5$), Fisher's Exact Test was performed to determine the independence of factors, with significance designated at p-value < 0.05. Factors analyzed for significant effect on infectious agent prevalence included region and season. When a significant effect was observed for a factor, a post hoc Pairwise Fisher's Test with Bonferroni adjustment was performed to determine which factor levels had a significant effect on prevalence. For analysis of *D. immitis*, all data were removed for cats < 6 months of age to account for the average time of infection development, during which heartworm antigen cannot be detected.

5. Conclusions

In this study, shelter-housed cats from across the USA were surveyed for infection with select vector-borne agents of veterinary and zoonotic importance. To date, this is the largest survey of select pathogens using the Idexx SNAP® 4Dx® Test in shelter cats with nationwide distribution. Although the overall prevalence of the pathogens in this survey is relatively low, we observe that there is increased exposure risk regionally for some agents, with geographic distributions in this study mostly coinciding with established human and canine distributions. Prevalence studies underscore the importance of routine veterinary care, including the compliant and continuous use of parasite preventives. Although there was a relatively low risk of exposure to or infection with vector-borne infection for this sample population, shelters should still consider vector-borne infections during or following an intake of cats that have consistent clinical signs. Further validation is warranted, but extra-label use of canine-approved point-of-care assays may be a justifiable measure in cats with suspected vector-borne infection.

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Data Availability Statement: The original contributions presented in the study are included in the article. Further inquiries can be directed to the corresponding author.

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