5-2021

Determination of Ownership Practices and Cytauxzoon felis Prevalence in Domestic Felines of Northwest Arkansas

Daniella M. Duran
University of Arkansas, Fayetteville

Determination of Ownership Practices and *Cytauxzoon felis* Prevalence in Domestic Felines of Northwest Arkansas

College of Veterinary Medicine
1800 Denison Avenue
Manhattan, KS 66056

Department of Animal Science
1120 West Maple St.
Fayetteville, AR 72701

Student Investigator:
Daniella Duran
University of Arkansas
Classification: Senior
Area of Study: Animal Science (Pre-Professional)
Email: dmduran@uark.edu

Project Leader:
Dr. Kathryn Reif
MSPH, Ph.D
Assistant Professor
College of Veterinary Medicine
Kansas State University
Email: kreif@vet.k-state.edu

Mentor:
Dr. Lauren Thomas
Clinical Assistant Professor and DVM
Department of Animal Science
University of Arkansas
Email: lrt03@uark.edu

Table of Contents

Abstract

Cytauxzoon felis is a protozoan pathogen that causes the infectious disease, cytauxzoonosis in wild and domestic felines. The natural host of this pathogen is the bobcat (*Lynx rufus*), which is why this disease is also commonly referred to as "bobcat fever". The *C. felis* pathogen infects wild and domestic cats upon transmission by infected ticks. *Amblyomma americanum*, also known as the Lone Star tick, the primary transmission vector for *C. felis*. This tick species is largely populated throughout the state of Arkansas, making *C. felis* a large threat to wild and domestic felines in these areas. Cytauxzoonosis is commonly thought to lead to acute death in domestic cats because of its short and aggressive path of infection. However, recent studies have indicated a higher prevalence of asymptomatic 'carrier' cats that remain persistently infected and serve as transmission reservoirs in nature. Evidently, *C. felis* exhibits more complexity than what is commonly appreciated, perhaps due to the possible existence of various strains with different virulence characteristics. This study intended to determine the prevalence of persistently infected cats in northwest Arkansas along with potential risk factors for *C. felis*. Blood samples from eligible cats were collected by regional veterinarians and tested for the prevalence of *C. felis* DNA using genomic DNA extraction and PCR assay protocols. We also incorporated the use of surveys to identify general trends for the physical attributes, lifestyles, and health conditions of outdoor cats in northwest Arkansas. It was discovered that 18.2% of the sampled population tested positive for *C. felis* infections, proving that this pathogen is prevalent in domestic cats of northwest Arkansas and putting naïve feline populations at risk of infection. In conclusion, *C. felis* persists as an aggressive, disease-causing agent that has devastating impacts on domestic cat health, so it is crucial that prevalence rates continue to be investigated and communicated to cat owners and feline care providers.

Introduction and Justification

Cytauxzoonosis is the most grievous of the vast range of diseases transmitted to cats via tick vectors (Nagamori & Reichard, 2015). In veterinary medicine, cytauxzoonosis cases are among the most difficult to diagnose and treat in a timely manner to improve survival chances. Current treatments with antiprotozoal therapy, atovaquone and azithromycin, provide the highest survival rate of only 60% in clinically ill, naturally infected cats (Birkenheuer, Brunker, Cohn, & Craig, 2011). Effective treatment using atovaquone and azithromycin also requires an early diagnosis, intense treatment, and extensive supportive care around the clock (Wang *et al*., 2017). Therefore, advancements in research on the aggressive causative agent, *C. felis*, need to be made to discover more successful control methods for the protection and promotion of health among domestic and wild felines.

The progression of effective treatment methods for cytauxzoonosis has led to much higher chances of survival for naturally infected cats. However, there remains a large grey area in our knowledge of how the feline immune system responds to *C. felis* (Wang *et al*., 2017). Clinical signs and symptoms displayed by sick animals are clues that veterinarians use to determine a patient's diagnosis and the necessary treatments. The difficulty with cytauxzoonosis in cats is that there are no distinct clinical signs or symptoms associated with this disease and there are various ways in which infected cats respond to *C. felis* infection (Wang *et al*., 2017). This makes it very difficult for owners to recognize sick cats, for veterinarians to diagnose infected cats, and for treatment to begin in time for their improved survival. Typically, it takes anywhere from five days to two weeks after a cat has been infected for them to start showing symptoms, with the most common ones being lack of appetite and depression (Wang *et al*., 2017). Especially in young cats with no history of health complications, this may not seem too

concerning to owners at first. However, due to the rapid pace of this aggressive infection it does not take long for these seemingly mild symptoms to progress into pyrexia, gastrointestinal upset resulting in vomiting and/or diarrhea, anemia, labored breathing, painfulness, and/or irregular vocalizations. This state is extremely difficult to recover from even with aggressive treatment, so many cats are either euthanized or succumb to the final stages of the disease and death.(Wang *et al*., 2017). Clearly, cytauxzoonosis can have devastating impacts on infected cats and can kill even the healthiest of cats in a matter of days. This highlights the importance of continuing to learn about the feline immune response to *C. felis* for the development of more efficient diagnosis and treatment strategies.

The life cycle of *C. felis* is one of very high complexity (Figure 1). The hematoparasitic protozoan parasite is found in the blood of its natural host, the bobcat, along with other feline survivors, or 'carriers', of the disease that serve as transmission reservoirs. When ticks feed on these 'carrier' animals, the parasite begins sexual reproduction within the gut of the tick vector and develops into sporozoites. When the tick later feeds on a naïve feline host, these sporozoites are released from the salivary glands of the tick into the host's bloodstream where they invade macrophages and multiply (Li *et al,.* 2017). These infected macrophages mature into schizonts, and it is at this stage of infection that causes clinical illness and acute death. The widespread formation of these schizonts block tissue vasculature within the host, leading to rapid organ failure throughout the body (Cohn & Sherrill, 2015). In the late-stage of the disease, the schizonts eventually rupture, releasing thousands of merozoites that infect the host's red blood cells (Lloret *et al*., 2015). The parasite reproduces within the erythrocytes and causes them to eventually rupture, so merozoites are continuously released into the bloodstream during this stage of infection. These hosts remain persistently infected even after recovery and result in the

host joining the population of transmission reservoirs for *C. felis*. Regardless if infected cats manage to survive cytauxzoonosis on their own or with treatment by a veterinarian, most remain persistently infected, increasing the risk of infection for naïve hosts.

It is generally believed that infection with *C. felis* negates the possibility of secondary infection. However, there have been cases where repeat infection leading to clinical illness due to *C. felis* have occurred if a secondary strain is present or enough time has passed for the cat's immunity to fade (Birkenheuer, Cohn, Shaw, & Shoemake, 2020). Consequently, felines that have been infected with *C. felis* not only put other cats at risk by serving as transmission reservoirs, but also remain at risk themselves due to the possibility of infection by a different strain. It has also been proven that bobcats, the natural host for *C. felis*, also remain chronically infected after the initial transmission of the pathogen and indefinitely remain at risk of reinfection (Zeiman, Nielson, & Agustín Jiménez, 2018). In a research effort based in southern Illinois, an area with high rates of *C. felis* infection in domestic and wild felines, five bobcats were annually captured and tested for *C. felis* infection. All five subjects tested positive after their initial capture and remained infected with their original strain of *C. felis* in their following reevaluations over the course of three and a half years. In addition to the initial strain, one subject was found to be infected with a new strain after each of the remaining two captures. Although it was not proven whether the new strains resulted from reinfection during the release period or mutation of the original strain, the study provided evidence towards the existence of multiple *C. felis* strains and the possibility of subsequent *C. felis* strain for bobcats (Zeiman, Nielson, & Agustín Jiménez, 2018). For domestic cats in areas of high exposure incidence, this not only means that they are at risk of primary *C. felis* infection, but they also potentially remain at risk of reinfection due to strain diversity (Zeiman, Nielson, & Agustín Jiménez, 2018). Among

other reasons, these circumstances support the need for further exploration of the complexities and uncertainties associated with *C. felis* ecology and cytauxzoonosis epidemiology.

As previously stated, the primary transmission vector for the *C. felis* pathogen is *Amblyomma americanum*, or more commonly referred to as the Lone Star tick (Allen, Thomas, Wohltjen, & Reichard, 2019). Free-roaming domestic and wild felines contract the aggressive disease, cytauxzoonosis, via the bite of an *A. americanum* vector infected with *C. felis* (Wang *et al*., 2017). Activity levels of these vectors tend to fluctuate throughout the year, causing cytauxzoonosis cases to drastically increase in the months of April, May, and June (Reichard, Baum, Cadenhead, & Snider, 2008). Cases tend to subside for a short period after these months, then have a smaller spike in the months of August and September, and finally decrease progressively throughout the remaining months of the year (Reichard, Baum, Cadenhead, & Snider, 2008). *A. americanum* has been discovered to gravitate towards wooded environments and less crowded residential areas, causing cases of cytauxzoonosis to be significantly higher in these locations (Reichard, Baum, Cadenhead, & Snider, 2008). Given this location preference for the primary transmission vector, cytauxzoonosis cases are most commonly associated with the southeast and south-central regions of the United States (Wikander, Anantatat, & Reif, 2020; Reichard, Baum, Cadenhead, & Snider, 2008). In a study conducted by Oklahoma State University in 2016, 673 cats throughout Oklahoma and southern Kansas were captured and examined for flea and tick infestations. Ticks were found on 18.7% of the sampled cat population with the most prominent species being *A. americanum* (65.9%) (Thomas, Staubus, Goolsby, & Reichard, 2016). Considering the proximity to Oklahoma and southern Kansas, there is a high probability that an abundance of *A. americanum* inhabits the northwest Arkansas area.

Therefore, it is important to gain a better understanding of the exposure incidence and risk of *C. felis* infection for the free-roaming feline population in northwest Arkansas.

A common means of protection against ticks and tick-borne infections for domestic cats is the use of flea/tick prevention products. Although these products may aid in the prevention of tick infestations, advancements towards more efficient tick control has proven to be much more difficult in comparison to flea control (Dryden, 2009). A significant reason for this difficulty is because unlike fleas, most tick species reproduce on wildlife hosts, so it is nearly impossible to gain control over tick populations (Dryden, 2009). This disadvantage has resulted in the common use of tick-killing pesticides, or acaricides, as tick prevention for dogs and cats (Dryden, 2009). Unfortunately, the use of acaricides on cats is much more limited than dogs with only five approved options available in the United States (Reichard *et al,* 2019). Recent advancements in research, however, have led to discoveries of effective preventatives for tick-borne *C. felis* infections in domestic cats. The combination product of sarolaner (an approved acaracide for cats) and selamectin (commonly known as Revolution Plus and used for treatment/prevention of fleas, ear mites, heartworm, and some intestinal parasites) into a topical solution was shown to be an effective preventative against *C. felis* infections in cats after exposure to *A. americanum* (Reichard *et al.,* 2019). In this study, a group of cats were randomly assigned to treatment and control groups and then exposed to *A. americanum* infected with *C. felis*. It was discovered that the sarolaner/selamectin combination had a 90.4% success rate after the initial treatments and a 94.7% success rate after the third treatments in preventing *C. felis* infections (Reichard *et al.,* 2019). In another study, the use of an imidacloprid 10%/flumethrin 4.5% collar (commonly known as a Seresto collar) was proven to be 100% successful in preventing *C. felis* transmission by *A. americanum* (Reichard *et al.*, 2019). Although these are significant discoveries towards

controlling *A. americanum* infestations, these products cannot be provided to all the cats at risk of *C. felis* infections. Therefore, further advancements towards effective tick population control methods are still necessary for the treatment and prevention of harmful tick-borne diseases in domestic cats.

Cytauxzoon felis infection in domestic cats has been identified in 17 states, with Arkansas having one of the highest reported prevalence rates of 28% in healthy feral cats (Cohn & Sherrill, 2015). In a more recent study, the prevalence of *C. felis* was tested in 33 healthy feral cats residing in the Arkansas River Valley. Using PCR and cytology diagnostic testing on each cat, it was discovered that 13% of the sample population exhibited subclinical *C. felis* infection (Jacobs, 2018). In an alternate study, 902 blood samples were tested for prevalence of *C. felis* from domestic cats residing in Arkansas, Oklahoma, and Missouri. The results demonstrated that domestic cats from the state of Arkansas exhibited the highest infection rate of 15.3%, resulting in the conclusion that domestic cats from Arkansas are more likely to test positive for C. *felis* than cats from the other two sampled states (Birkenheuer *et al*., 2015). It is evident that there is a higher prevalence of C. *felis* in the state of Arkansas than what cat owners and veterinarians may be aware of. Furthermore, it crucial that prevalence rates are further investigated and awareness of the risk is communicated to cat owners and feline care providers.

Objectives

This project was a collaborative effort between the University of Arkansas Department of Animal Science and Kansas State University's College of Veterinary Medicine. The primary objective of this project was to determine the prevalence of *Cytauxzoon felis* in domestic cats across the northwest Arkansas region because of the large population of transmission vectors. In

doing so, this study identified the percentage of 'carrier' cats that are serving as transmission reservoirs in the area.

The first steps were applying for research grant funding from the University of Arkansas Honors College and approval from the University of Arkansas Institutional Review Board (IRB) and Institutional Animal Care and Use Committee (IACUC). The project received grant funding from the Honors College and the required regulatory approvals were obtained to conduct the objectives of this project. The next step was spreading awareness of this research opportunity to small animal hospitals and shelters across the northwest Arkansas area and encouraging participation from veterinarians. There were many veterinarians that expressed interest in this study and generously agreed to aid in the project's purpose to determine the risk of exposure to *C. felis* for outdoor cats in northwest Arkansas. Once the participating clinics were identified, these veterinarians could participate in three different ways:

1. Veterinarians collected opportunistic leftover blood samples from eligible cats undergoing blood draws NOT for the purpose of this study.
2. Veterinarians could also purposefully collect blood samples from eligible cats that were not undergoing blood draws for alternate reasons.
3. In addition to collecting blood samples, participants were also asked to help broadcast an online survey intended for outdoor (or partially outdoor) cat owners that lived in northwest Arkansas.

Once the collection and storage of blood samples from eligible cats was initiated, the samples were retrieved from participating clinics and sent to the Reif Lab at Kansas State's College of Veterinary Medicine in Manhattan, Kansas. Once the blood samples reached the Reif Lab, they were tested for the presence of *C. felis* DNA through PCR assay. The data retrieved

from the lab testing allowed us to draw conclusions on *C. felis* prevalence among the sampled cats.

The final objective of this study is to inform regional veterinarians of our data and conclusions on *C. felis* prevalence in our sampled population. Distributing these findings along with the data from the broadcasted outdoor cat owner survey will generate more awareness of the prevalence and risk factors associated with *C. felis*.

Methods and Materials

Institutional Approvals

Since this project involved both human and animal subjects, formal approval was needed from appropriate regulatory offices at the institutions leading the study. As such, approval was obtained from the Kansas State University Institutional Animal Care and Use Committee (IACUC). Additionally, approval from the Kansas State University and University of Arkansas Institutional Review Boards (IRB) were submitted. Once the IRB approvals were obtained, the pursuit of this project's objectives could begin.

Research Design

This project utilized a quantitative correlational research design in the primary objective of identifying the prevalence of domestic cats who are *C. felis* carriers in the northwest Arkansas area. To elaborate, this design is used for research purposes that focus on two variables and investigate the relationship between them. For this specific study, these variables consisted of *C. felis* infections and domestic cats in the northwest Arkansas area. The relationship between our variables was investigated by collecting blood samples from outdoor (or partially outdoor) domestic cats in the northwest Arkansas area and testing them for infection with *C. felis.* This

project also utilized a qualitative research component in the objective of broadcasting an anonymous survey intended for outdoor cat owners across the northwest Arkansas area.

Sample Collection

To accomplish the goal of determining C. *felis* prevalence in outdoor cats from northwest Arkansas, we needed the assistance of regional veterinarians with a feline medicine component for the collections of blood samples. Initially, we reached out to local small animal practices by phone calls and emails to inquire if their veterinarians would be interested in participating in the study and open to further discussion of details and instructions. As we collected responses from interested clinics, we held meetings with their veterinarians to provide them with the project-related materials and instructions for sample collections. In the end, there were 5 hospitals and 1 shelter with locations in the cities of Fayetteville, Springdale, and Bentonville that agreed to participate the objectives of this study. The responsibilities of participating clinics included the following:

1. Having a representative from each facility sign our 'clinic consent from' which gave their permission for us to retrieve their collected blood samples and other project-related materials and use them towards our research objectives.
2. Collecting opportunistic leftover blood samples from eligible cats (of one year of age and spend time outdoors) that were undergoing blood draws for alternate reasons including, but not limited to: pre-surgical screenings for procedures such as spay/neuter, routine bloodwork, or diagnostic testing. These instances did not require owner consent and consisted of the veterinarian either saving leftover blood from the initial blood draw or drawing 1-2mls of extra blood from each cat. These samples were placed in ethylenediamine tetraacetic (EDTA) tubes with the corresponding patient ID's and stored

in the freezer at each clinic. To keep track of these leftover sample collections, participants recorded the collection date, patient ID, age, gender, and county of residence for each cat if the information was accessible.

3. Collecting purposeful blood samples from eligible cats (of one year of age and spend time outdoors) that did not undergo blood draws for alternate reasons. These instances required signed owner consent to the 'owner consent form' that authorized the veterinarians to draw 1-2mls of blood from their cat for the purposes of this study. Actively participating owners also filled out a paper survey intended to acquire general information on cats undergoing purposeful blood sample collections. These samples were also placed in ethylenediamine tetraacetic (EDTA) tubes with the corresponding patient ID's and stored in the freezer at each clinic.
4. Posting the link to our anonymous online survey intended for outdoor cat owners in northwest Arkansas on their websites, social media platforms such as Facebook, and/or other outdoor cat forums.

For actively participating clinics that agreed to pursue purposeful blood sample collections, we offered to provide supply kits with the materials needed for the collection and storage of samples. These kits were intended to prevent participating veterinarians from using excessive amounts of their own materials for the blood draws purposed for this study. The materials offered included: a box for opportunistic leftover samples, a box for actively collected samples, EDTA sample tubes, disposable needles, and disposable syringes. Identifiable information for the patients and clients that participated in sample collections were kept and remain confidential.

Sample Testing

The leftover and purposefully collected blood samples along with the project-related consent forms, surveys, and collection logs were retrieved from the participating clinics and shipped to the Reif Lab at Kansas State University. To prevent the blood samples from thawing, they had to be transferred from the clinic freezers directly into Styrofoam containers with ice blocks and overnight shipped to the lab in Manhattan, Kansas. It was at this location where the collected samples were tested for the presence of *C. felis* DNA and our desired data from the collected samples was obtained.

Within 72 hours after arrival at the Reif Lab, the samples were set at room temperature and spun in a vortex to mix the thawed contents. Once they were ready for testing, the EDTA whole blood samples underwent genomic DNA extractions and Quantitative Real-Time PCR (qPCR) assay procedures to determine whether or not *C. felis* DNA was present. At this point in the *C. felis* life cycle, the parasite is in the merozoites stage and resides within the host's red blood cells. For the DNA extraction, 0.1 ml of whole blood from each sample were broken open and Quick-DNA™ Miniprep Kits protocols (Zymo Research, Irvine, CA, USA) were used to extract nucleic acid from each sample. The extracted nucleic acid samples contained combinations of feline and *C. felis* DNA for those that were infected, so the next step was to specifically amplify the *C. felis* DNA by using primers that match a portion of a specific gene in the *C. felis* genome. The target was a 92 bp region *C. felis* cytochrome c oxidase subunit III (Cox3) gene because it has been proven an efficient target for *C. felis* detection in PCR sample processing methods (Wikander, Anantatat, & Reif, 2020; Schreeg, *et al.*, 2016). These primers, along with the other necessary reagents, were included in the PCR mastermix and the target *C.*

felis gene sequences were amplified. Finally, the positive signals could then be determined and presence of *C. felis* in each of the samples was determined.

For more information on the sample testing procedures for this study, refer to the study entitled "Prevalence of *Cytauxzoon felis* Infection-Carriers in Eastern Kansas Domestic Cats" conducted in 2020 by Yvonne M. Wikander, Tippawan Anantatat, Qing Kang, and Kathryn E. Reif. Sample testing for this study occurred in the same location and utilized the same methods and materials.

Survey Development and Distribution

Since this research project's proposal and objectives were approved by the IRB, we were able to utilize the Qualtrics online survey platform through the University of Arkansas for the purposes of the survey. This component of the project was used to determine *C. felis* risk factors by asking for general information about outdoor cat ownership practices. Additionally, it offered to provide owners with more information about cytauxzoonosis if they were interested. The survey consisted of twenty questions (see appendix) that owners were asked to answer based on the cats they currently own/care for on their property. There were four primary objectives for the survey in which the questions were based:

1. Determine the predominant physical attributes of cats in the area by asking questions concerning gender, age, and breed.
2. Determine the predominant living environments/lifestyles of cats in the area by asking questions considering the number of cats owned, amount of time spent outdoors, ownership status, and state/county of residence.
3. Determine the predominant health conditions of cats in the area by asking questions concerning spay/neuter tendencies, vaccination practices, Feline Immunodeficiency Virus

(FIV)/Feline Leukemia Virus (FeLV) status, use of flea/tick prevention, and tick observation frequency.

4. Determine extent of owner awareness in regards to cytauxzoonosis and compare different owner experiences with cytauxzoonosis.

Once the survey was created in Qualtrics, the anonymous link and fliers with a QR code were provided to participating and regional veterinarians to help with broadcasting the survey across northwest Arkansas and maximizing the number of responses. The survey consisted of 20 questions that were primarily formatted as multiple choice, in which many allowed participants to select multiple answers to account for all the cats they own or care for on their property. In addition, we formatted a couple questions as free response. The questions and answer options for the Qualtrics survey used in this study may be found in the appendix (page 25).

Results

Sample Testing Results

In total, 22 whole blood samples from eligible cats were collected between November 2020 and April 2021. Davis Animal Hospital (Springdale, AR/Washington County), Crossover Veterinary Clinic (Fayetteville, AR/Washington County), Animal Medical Center- Pinnacle Hills (Rogers, AR/Benton County), and Fayetteville Animal Services (Fayetteville, AR/Washington County) were responsible for the sample contributions. The collected samples were shipped to the Reif Lab at Kansas State University on April 6th, 2021, arrived at the lab on April 7th, 2021, and were promptly tested for *C. felis* infections using the respective genomic DNA extraction and PCR assay protocols. All 22 samples met the criteria for testing and the results were received on April 8th, 2021. Of the 22 sampled cats, 4 cats were from Benton County, Arkansas and the remaining 18 cats were from Washington County, Arkansas. The sampled population ranged

from 1 year to 18 years of age, consisting of 17 males and 5 females. It was discovered that 4 out of the 22 samples were positive for *C. felis* infections, resulting in an overall infection rate of 18.2% (Table 1). The positive samples consisted of three males that were 5, 10, and 11 years of age and one female that was 6 years of age. These cats were all from Washington County, Arkansas, and all contributed by the same clinic. The infection rate for the samples contributed by this clinic exclusively was 23.5%.

Survey Results

The goal of the broadcasted online survey was to identify general trends for outdoor cats including the predominant physical attributes, living environments/lifestyles, and health conditions/practices that could be associated with cytauxzoonosis risk. The survey was also used as a tool to determine the extent of owner awareness in regards to cytauxzoonosis and the conditions that brought them to be aware of the disease. A total of 240 domestic cat owners completed surveys with 43.3% (n=104) from Washington County, 32.5% (n=78) from Benton County, 9.7% (n=22) from Carroll County, 6.7% (n=16) from other counties in Arkansas, and the remaining 8.3% (n=20) from counties in Missouri, Oklahoma, and Tennessee (Figure 2). Clearly, the survey collected a broad-range of responses that provided insight to the current cytauxzoonosis risk factors for outdoor cats in these areas. It should be noted that many of the questions throughout the survey allowed owners to select more than one response due to our request for them to answer the questions based on all the cats they own or care for on their property. Therefore, the added percentages for the recorded answers of some questions exceed 100%.

To determine the predominant physical attributes of outdoor cats in the area, the survey asked owners about the gender, age and breed of the cat(s) they currently own or care for on their

property. Of the 240 completed survey responses, 193 owners (80.4%) reported having at least one male cat, 190 owners (79.2%) reported having at least one female cat, and 6 owners (2.5%) reported the gender of their owned cat(s) as unknown (Figure 3). In regards to age, 12 owners (5%) reported that their owned cat(s) consisted of at least one kitten (less than 6 months of age), 154 owners (64.2%) reported that their owned cat(s) consisted of at least one young adult (between 6 months and 5 years of age), 168 owners (70%) reported that their cat(s) consisted of at least one adult (greater than 5 years of age), and 7 owners (2.9%) reported the age of their owned cat(s) as unknown (Figure 4). Finally, in regards to breed, 197 owners (82.1%) reported having at least one Domestic Short Hair, 86 owners (35%) reported having at least one Domestic Long hair, 6 owners (2.5%) reported having at least one purebred, and 38 owners (15.8%) reported the breed of their cat(s) as unknown (Figure 5).

To gather information on the predominant lifestyles/living conditions of cats in the area, the survey included questions concerning the number of cats owned on each property, amount of time spent outdoors, ownership status, and state/county of residence. After running calculations using Microsoft Excel on the survey data concerning the number of cats owned on each property, it was found that each owner had an average of 3.9 cats on their property with a standard deviation of 4.73. After further analysis, the data presented that 20% of responders were single-cat owners, 55% of responders owned 2-4 cats, 15% of responders owned 5-7 cats, 4% of responders owned 8-10 cats, 4% of responders owned 11-15 cats, and 2% of responders owned more than 15 cats (Figure 6). Considering the question about time spent outdoors, 117 owners (48.8%) had at least one cat that spends 0-25% of their time outdoors, 61 owners (25.4%) had at least one cat that spends 25-50% of their time outdoors, 48 owners (20%) had at least one cat that spends 50-75% of their time outdoors, 89 owners (37%) had at least one cat that spends 75-

100% of their time outdoors, and 2 owners (0.8%) reported the amount of time spent outdoors as unknown (Figure 7). In regards to ownership status of the cats being represented in the survey, 184 owners (76.7%) reported having at least one owned cat, 51 owners (21.3%) reported having at least one feral cat, and 118 owners (49.2%) reported having at least one rescued cat (Figure 8). Finally, the states and counties of the represented cats in the survey are previously listed and displayed in Figure 2.

The largest portion of questions from the survey were focused on identifying the predominant health conditions of cats in the area and asked questions concerning spay/neuter tendencies, vaccination practices, Feline Immunodeficiency Virus (FIV)/Feline Leukemia (FeLV) status, use of flea/tick prevention, and tick observation frequency. For the question asking whether or not owners have spayed/neutered cat(s), 227 owners (94.5%) answered yes, 38 owners (15.8%) answered no, and 6 owners (2.5%) answered unknown for the cat(s) they own on their property (Figure 9). For FIV and FeLV status, 9 and 0 owners (3.8%, 0%) answered positive respectively, 174 and 179 owners (72.5%, 74.5%) answered negative respectively, and 87 and 82 owners (36.3%, 34.2%) answered unknown for the respective statuses (Figures 10 & 11). In regards to vaccination practices for the represented cats in the survey, 24 owners (10%) never get their cat(s) vaccinated, 66 owners (27.5%) rarely get their cat(s) vaccinated, 22 owners (9.2%) occasionally get their cat(s) vaccinated, 120 owners (50%) regularly get their cat(s) vaccinated, and 33 owners (13.8%) always attentively had their cat(s) vaccinated. (Figure 12). Flea/tick prevention usage was divided into three separate questions which identified that 130 owners (54.2%) are currently using flea/tick prevention on their cats, with the most common brands being the Seresto Collar, Advantage, and Frontline (Figures 13 & 14). The number of cats on flea/tick prevention throughout the year gradually increase from January-June, peak in the

month of July, and then gradually decrease from August-December (Figure 15). Finally, in regards to how often owners find ticks on their cat(s), the most common answers were never (34.6%) and rarely (32.5%), with the remaining answers decreasing in responses as tick observation frequency increases (Figure 16).

Lastly, the survey was designated to gain a general understanding of owner awareness of cytauxzoonosis and the circumstances in which survey participants acquired their knowledge about the disease. The majority of owners that took the survey (64%) had heard of cytauxzoonosis prior to taking the survey (Figure 17). In response to the awareness question, 63 owners indicated that they knew of someone who has had a cat diagnosed with cytauxzoonosis, 48 owners indicated that they have lost a cat due to cytauxzoonosis, and 26 owners indicated that they have had a cat survive cytauxzoonosis (Figure 18).

Conclusions and Discussion

This study on *C. felis* prevalence in northwest Arkansas provided valuable information on *C. felis* prevalence and potential risk factors for domestic cats in the area. We discovered an overall infection rate of 18.2% in our sampled population, allowing us to conclude that there is a reasonably large population of *C. felis* 'carriers' in the northwest Arkansas area that are serving as transmission reservoirs in nature. These results suggest that naïve cats in northwest Arkansas are at high risk of *C. felis* infection if bitten by an infected tick vector. Additionally, we can confirm that cytauxzoonosis infections exhibit more complexity than simply the acute stages of clinical illness and death. Clearly, there are many cats that survive the initial infection and indefinitely remain 'carriers' of *C. felis*.

Even with a smaller than desired sample population of only 22 cats, the infection rate that we discovered is only 10% lower than the 28% infection rate found in healthy feral cats

statewide (Cohn & Sherrill, 2015). In comparison to the *C. felis* prevalence study in 33 cats from the Arkansas River Valley, our discovered infection rate of 18.2% exceeds that of the Arkansas River Valley by 5.2% (Jacobs, 2018). In the comparative study among Arkansas, Missouri, and Oklahoma, domestic cats from the state of Arkansas had the highest *C. felis* prevalence of 15.3% (25/161) (Birkenheuer *et al*. 2015). Our discovered infection rate of 18.2% in northwest Arkansas exceeds this statewide infection rate by 2.9%, further proving that *C. felis* exhibits the highest prevalence in domestic cats from Arkansas. In addition, it is evident that infection rates are especially high in northwest Arkansas compared to other regions. A very similar study on regional *C. felis* prevalence discovered an infection rate of 25.8% in domestic cats from eastern Kansas (Wikander, Anantatat, & Reif, 2020). Even with a significantly smaller sample size, shorter project duration, and smaller area of focus, the prevalence rate in northwest Arkansas was only 7.6% less than that of half the state of Kansas. This comparison demonstrates the high concentration of *C. felis* infections in northwest Arkansas and the possibility of an even higher infection. This could be determined in a more extensive and comprehensive study.

The data from the broadcasted online survey allowed us to identify general trends of outdoor cats in the area including the predominant physical attributes, living environments, and health practices for outdoor cats in northwest Arkansas. Male and female cats were almost evenly represented in the survey, with males having a slight majority. In regards to the physical attributes, the predominant age and breed were adult (greater than 5 years of age) and Domestic Short Hair. It was concluded that 80% of owners were multiple-cat owners, with the most predominant answer being 2-4 cats per household. This means that the large majority of cats in the area live in close proximity with other cats, making *C. felis* transmission much easier for tick vectors. The most common lifestyle conditions represented in the survey were owned cats from

Washington County that spend 0-25% of their time outdoors. It is crucial that cats in this area are limited in their time spent outdoors because the samples that tested positive for *C. felis* infections in this study were all from Washington County. The health conditions of cats in northwest Arkansas were found to be predominantly of high quality. The majority of cats represented in the survey were spayed/neutered, regularly receive vaccinations, and are negative for FIV and FeLV. This suggests that cats in the area are primarily healthy and have strong immune systems with the ability to fight off infectious diseases. However, only a little over half the represented cats are currently on flea/tick prevention. Especially during tick season, it is extremely important that cats in the northwest Arkansas area are on effective flea/tick preventatives to decrease the risk of *C. felis* infections. Based on the survey, the most common flea/tick preventative brand used on cats in the area is the Seresto Collar. This brand should be highly recommended for cats in northwest Arkansas because it has been proven to be 100% successful in preventing *C. felis* transmission by *A. americanum* (Reichard *et al.,* 2019). The majority of owners that took the survey reported that they have never found or rarely find ticks on their cat(s), which hopefully means they are either keeping their cat(s) on flea/tick prevention or are adamantly checking their cat(s) for ticks. This could also suggest that cats are fastidious groomers and removing ticks before owners notice them.

The extent of owner awareness of cytauxzoonosis was considerable, with 64% of owners having at least heard of the disease. The number of owners who have lost a cat due to cytauxzoonosis nearly doubled the number of owners who have had a cat survive cytauxzoonosis, so there is still much to learn about effective preventative methods for *C. felis* infections. Given the complex life cycle of *C. felis*, our inability to control transmission vector populations, and the difficulties that come with diagnosing and treating cytauxzoonosis, it is

important that we strongly advocate for use of tick-control products on cats that have been demonstrated effective at preventing *C. felis* transmission and infection. It is also important that we conduct more prevalence studies to gain a better understanding of *C. felis* risk factors and protect the feline populations in endemic areas. The best way to protect cats at risk of *C. felis* infections is by spreading awareness of the importance of using effective flea/prevention products and keeping regional veterinarians informed on prevalence rates. Once we have organized our collected data and conclusions from this study into a more concise format, we plan to develop and distribute an informational flyer on cytauxzoonosis to participating and regional veterinarians. We hope to communicate the risk that cytauxzoonosis poses for cats in their area and provide them with educational resources that will make diagnosing and treating cytauxzoonosis cases less difficult. Hopefully, these measures will adequately control *C. felis* infection rates and allow for more rapid diagnosis and treatment of acute cytauxzoonosis cases. Through further advancements in research and medicine, we will be able to gradually eliminate the risks of *C. felis* and preserve our feline populations**.**

Acknowledgements

There were several regional veterinarians that helped in the accomplishment of this study's objectives. These veterinarians were responsible for the collection and storage of blood samples at their respective veterinary care facilities and some also helped broadcast our online survey. These veterinarians include Dr. Mark Davis and Dr. Chase Davis from Davis Animal Hospital, Dr. Beth Stropes from Crossover Veterinary Clinic, Katy Thayer and the veterinarians from Animal Medical Center - Pinnacle Hills, and Dr. Valerie Henley from Fayetteville Animal Services.

All genomic DNA extractions and PCR assay procedures for sample testing were conducted at the Reif Lab at Kansas State's College of Veterinary Medicine. Sample testing was under the supervision of project leader Dr. Kathryn Reif, who was also involved in the planning, organization, and execution of the project.

The accomplishments of this project also would not have been possible without the help and guidance of Dr. Lauren Thomas from the University of Arkansas Department of Animal Science.

Statistical analysis on the survey data was assisted by Dr. Donald M. Johnson from the University of Arkansas Department of Agricultural Education, Communications, and Technology.

Appendix

Qualtrics Survey

Thank you for your generous contribution toward this research effort!

For the purpose of this survey, please select your responses based on the cat(s) you currently own or care for on your property

1. I consent to my recorded answers (excluding identifiable personal information) being used for research purposes.

a. Yes

b. No

2. Do one or more of your cats spend time outdoors?

a. Yes

b. No

3. What is the gender of your cat(s)? *If you own multiple cats, please select all that apply.*

a. Male

b. Female

c. Unknown

4. How old are your cat(s)? *If you own multiple cats, please select all that apply.*

a. Kitten (less than 6 months of age)

b. Young adult (6 months - 5 years of age)

c. Adult (greater than 5 years of age)

d. Unknown

5. What breed is your cat(s)? *If you own multiple cats, please select all that apply.*

a. Domestic Short Hair

b. Domestic Long Hair

c. Purebred

d. Unknown

6. Is your cat(s) spayed/neutered? *If you own multiple cats, please select all that apply.*

a. Yes

b. No

c. Unknown

7. Which best describes the ownership status of your cat(s)? *If you own multiple cats, please select all that apply.*

a. Owned

b. Feral

c. Rescue

8. Which best describes the FIV (Feline Immunodeficiency Virus) status of your cat(s)? *If you own multiple cats, please select all that apply.*

a. Positive

b. Negative

c. Unknown

9. Which best describes the FeLV (Feline Leukemia Virus) status of your cat(s)? *If you own multiple cats, please select all that apply.*

a. Positive

b. Negative

c. Unknown

10. Vaccination practices: Which best describes your vaccination practices provided to your cat(s)? *If you own multiple cats, please select all that apply.*

a. Never

b. Rarely, they've had at least one vaccination at some point in their life

c. Occasionally, when I think about it

d. Regularly, but I may be a little late sometimes

e. Once their vaccinations go out of date, we are at the vet the next day to get them updated!

11. Is your cat(s) CURRENTLY on flea/tick prevention? *If you own multiple cats, please select all that apply.*

a. Yes

b. No

c. Not currently, but sometimes

d. Unknown

12. If you answered YES to being on flea/tick prevention, what month(s) do you use flea/tick prevention for your cat(s)? Please s*elect all that apply*

a. January

b. February

c. March

d. April

e. May

f. June

g. July

h. August

i. September

j. October

k. November

l. December

13. If you answered YES to being on flea/tick prevention, what brand(s) of flea/tick prevention have you used most recently? Please s*elect all that apply*

a. Bravecto

b. Revolution

c. Seresto Collar

d. Advantage

e. Frontline

f. Other

g. Unknown

14. Over the course of a year, which best describes how much time your cat(s) spend outdoors? *If you own multiple cats, please select all that apply.*

a. 0-25%

b. 25-50%

c. 50-75%

d. 75-100%

e. Unknown

15. What is the state and county of residence of your cat(s)?

State:

County

16. How often are ticks observed on your cat(s)?

a. Gross! I've never found a tick on my cat!

b. Rarely (maybe once or twice ever)

c. Occasionally (at least once per year)

d. Frequently depending on the time of year

e. All the time, year round, my cat is a tick magnet!

17. Have you ever heard of the tick-borne disease cytauxzoonosis that affects domestic and wild cats?

a. Yes

b. No

18. If you answered YES to the previous question, select all of the following that apply to you.

a. I think I have heard of cytauxzoonosis somewhere

b. I have had a cat diagnosed with cytauxzoonosis

c. I have lost a cat due to cytauxzoonosis

d. I have had a cat survive cytauxzoonosis

e. I know someone who has had a cat diagnosed with cytauxzoonosis

19. Do you want to learn more about cytauxzoonosis?

a. Yes, please!

b. No thanks!

20. If you answered YES to the previous question and want to learn more about cytauxzoonosis, please provide your name and email address below and we will send you more information!

Please note: Your name and personal contact information will be separated from your survey responses upon receipt, and will kept confidential to the extent allowable by law. All survey responses will be saved anonymously and analyzed as such.

*****From the names and email addresses provided, four lucky individuals will be randomly selected to win a $50 Amazon Gift Card!**

Name:

Email:

Tables

Sample Testing Results

Sample ID	Cox3realtime PCR	Cq
01	*Negative*	
02	*Negative*	
03	*Negative*	
04	*Negative*	
05	*Negative*	
06	*Negative*	
07	*Positive*	21.28
08	*Negative*	
09	*Negative*	
10	*Negative*	
11	*Positive*	28.46
12	*Positive*	24.26
13	*Negative*	
14	*Negative*	
15	*Negative*	
16	*Negative*	
17	*Negative*	
18	*Negative*	
19	*Negative*	
20	*Negative*	
21	*Positive*	21.08
22	*Negative*	

Table 1: *C. felis* infection statuses and Cq values from the qPCR assays of the 22 tested whole blood samples.

Figures

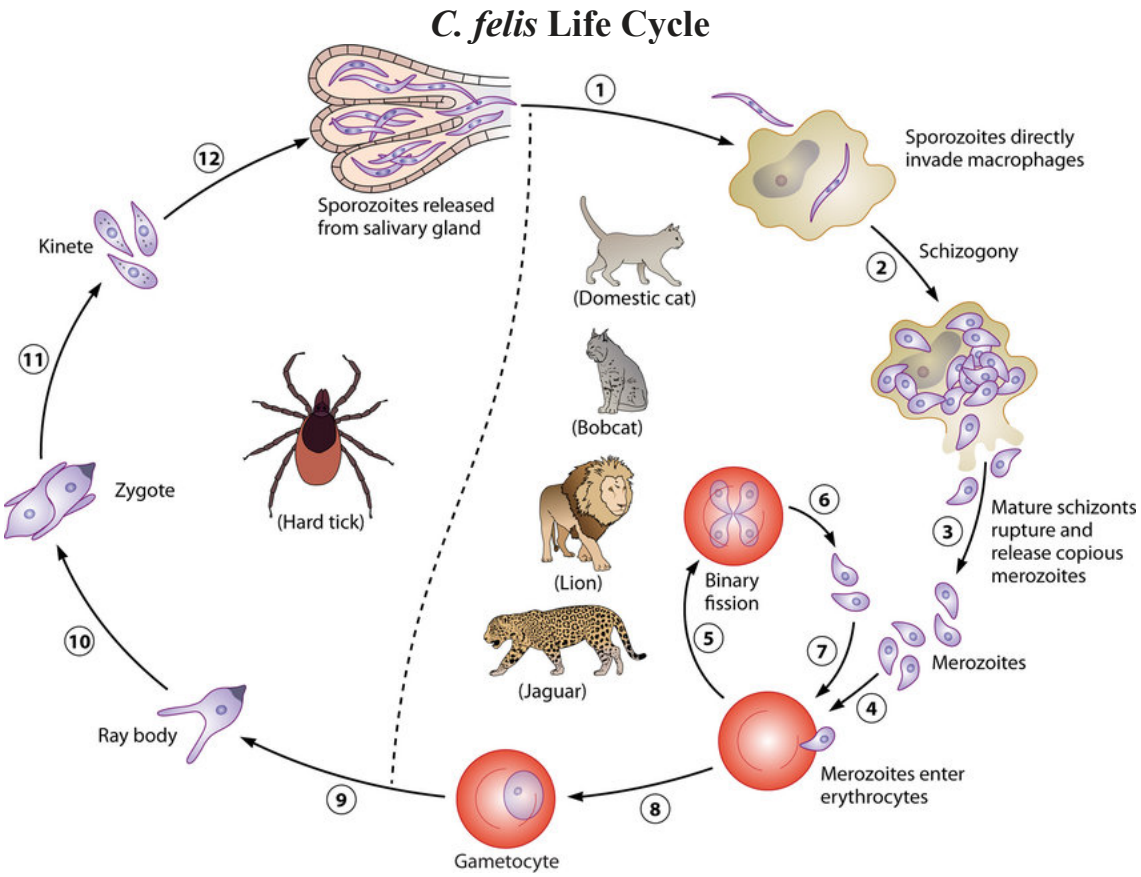

Figure 1: Flow chart displaying the complex life cycle of *Cytauxzoon felis.* Image retrieved from Wang *et al*. (2017)

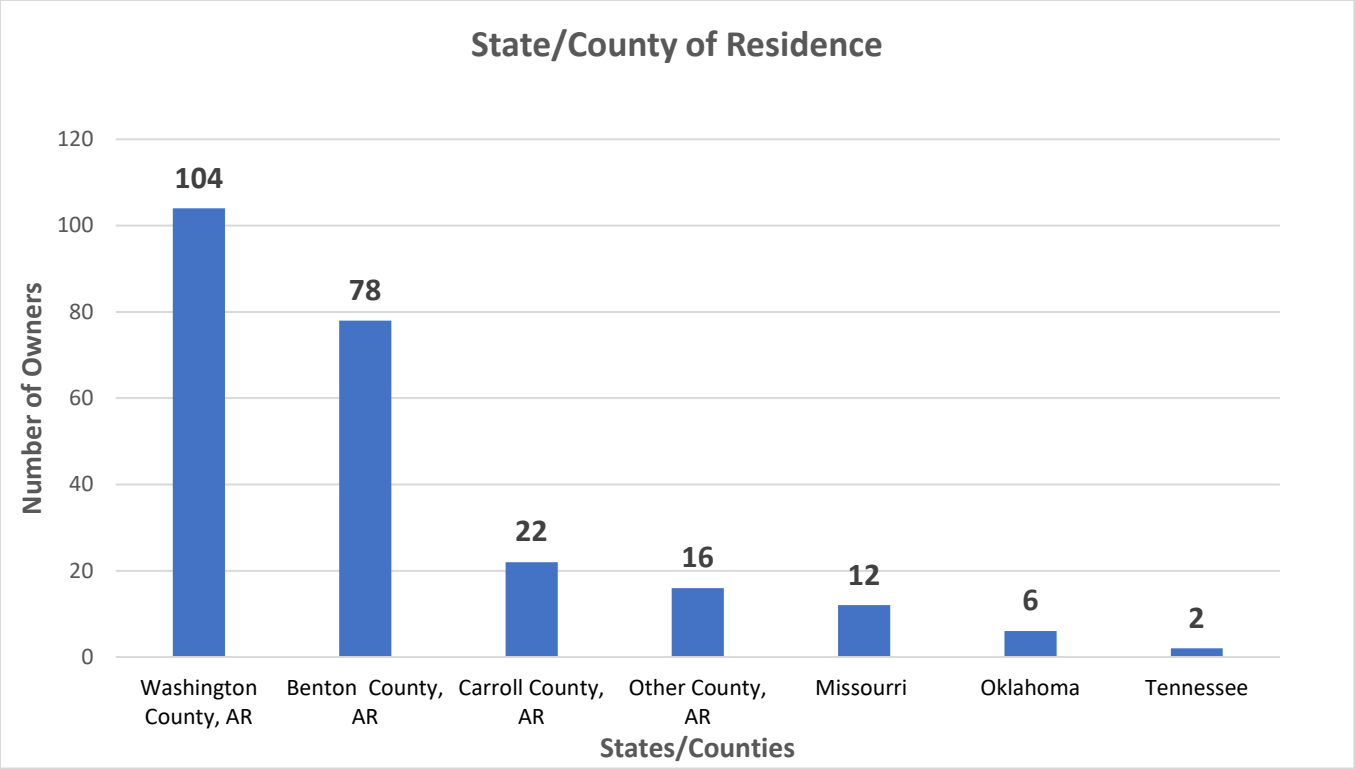

Figure 2: Results displaying the states and counties of residence represented in the online survey responses

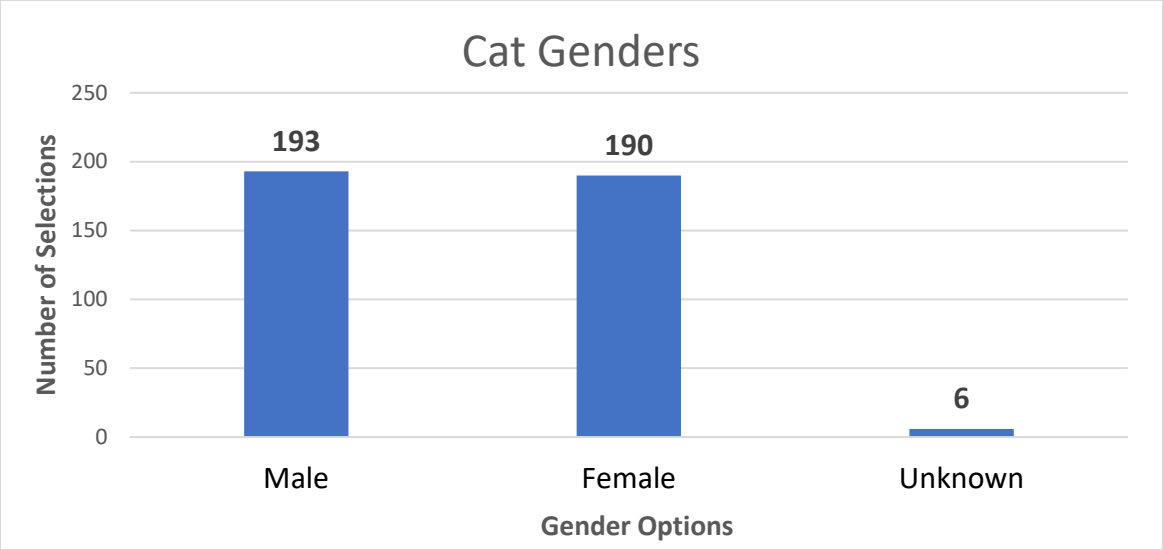

Figure 3: Results for the genders of cats represented in the online survey responses

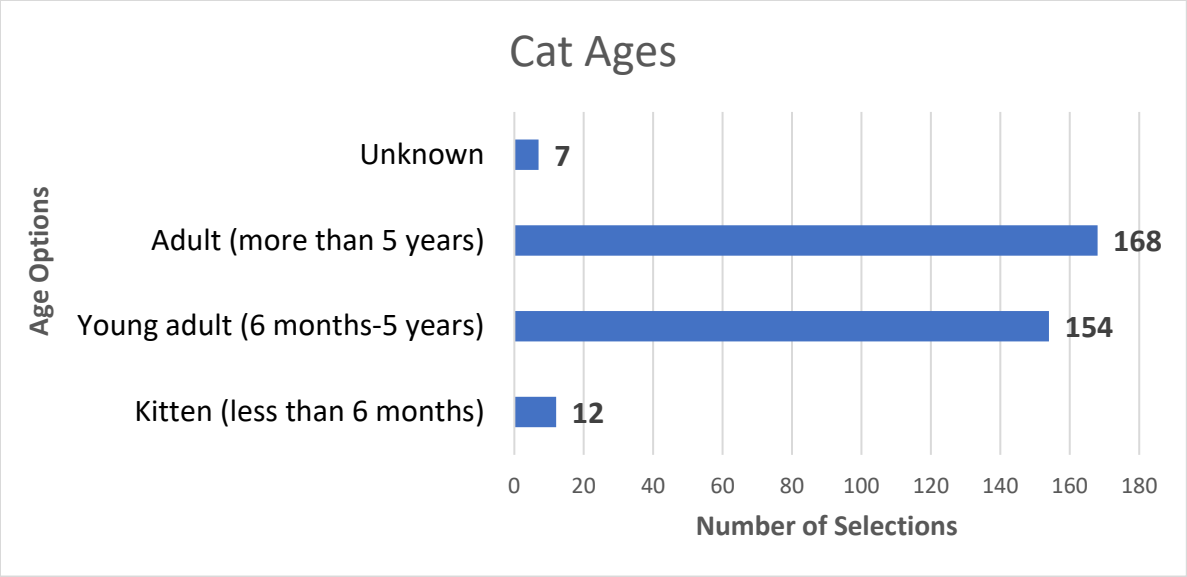

Figure 4: Results for the ages of cats represented in the online survey responses

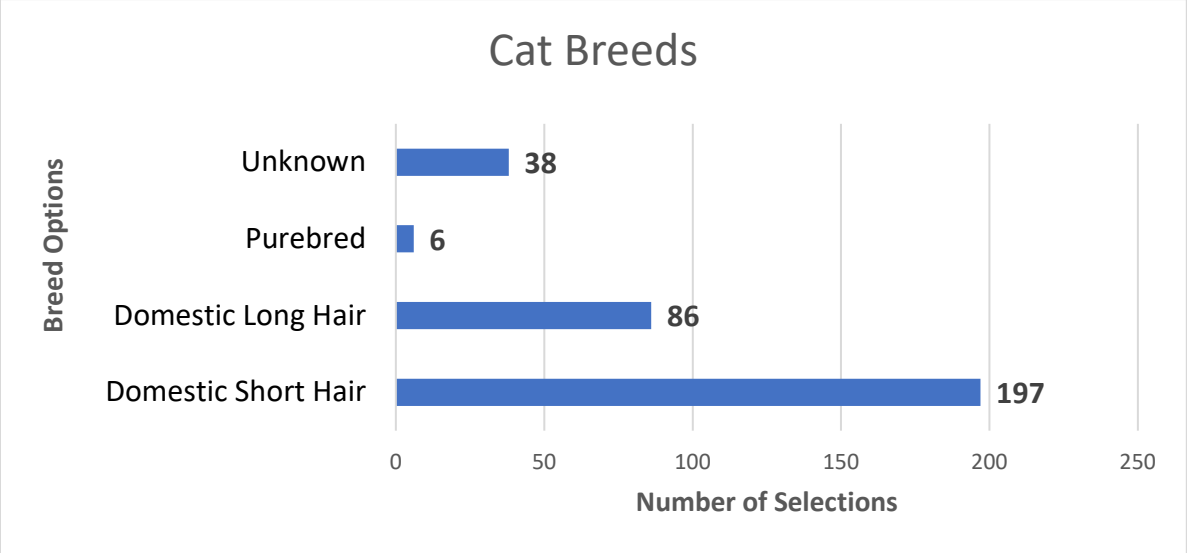

Figure 5: Results for the breeds of the cats represented in the online survey responses

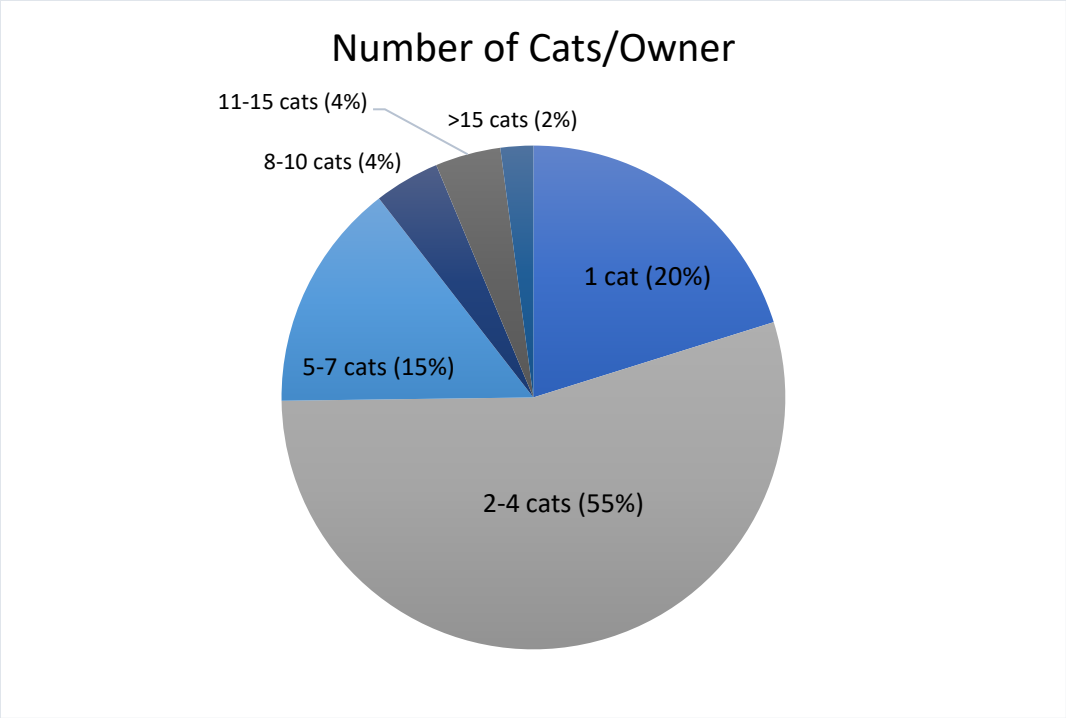

Figure 6: Results for the number of cats owned per property from the online survey responses

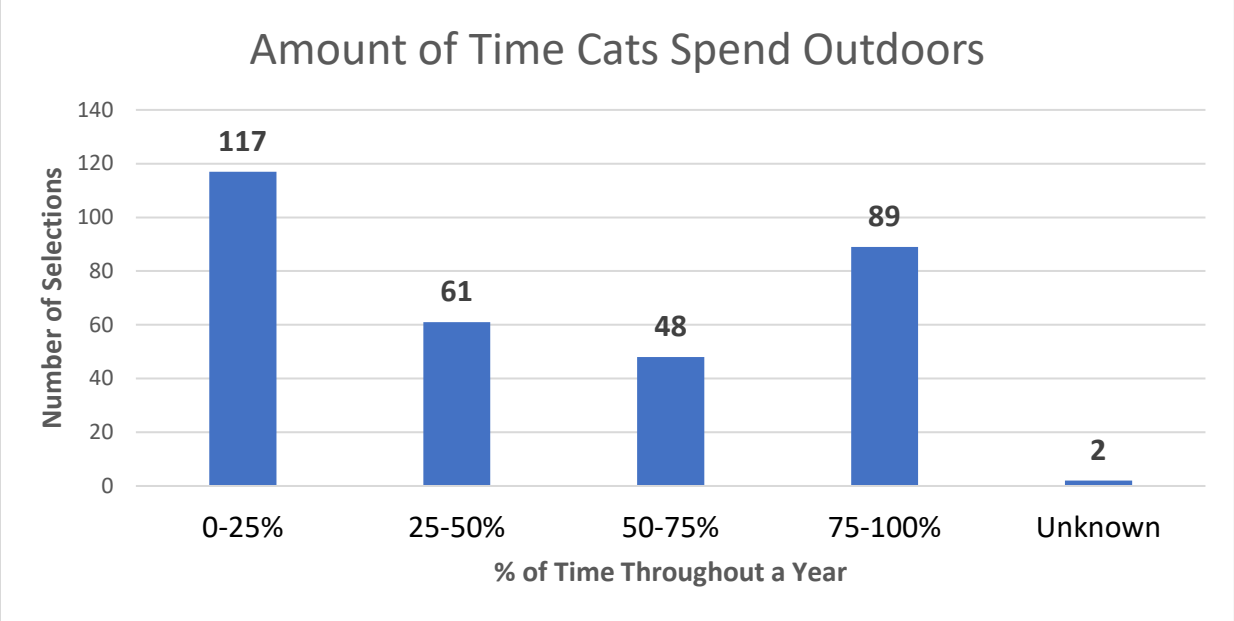

Figure 7: Results displaying the amount of time spent outdoors by cats represented in the online survey responses

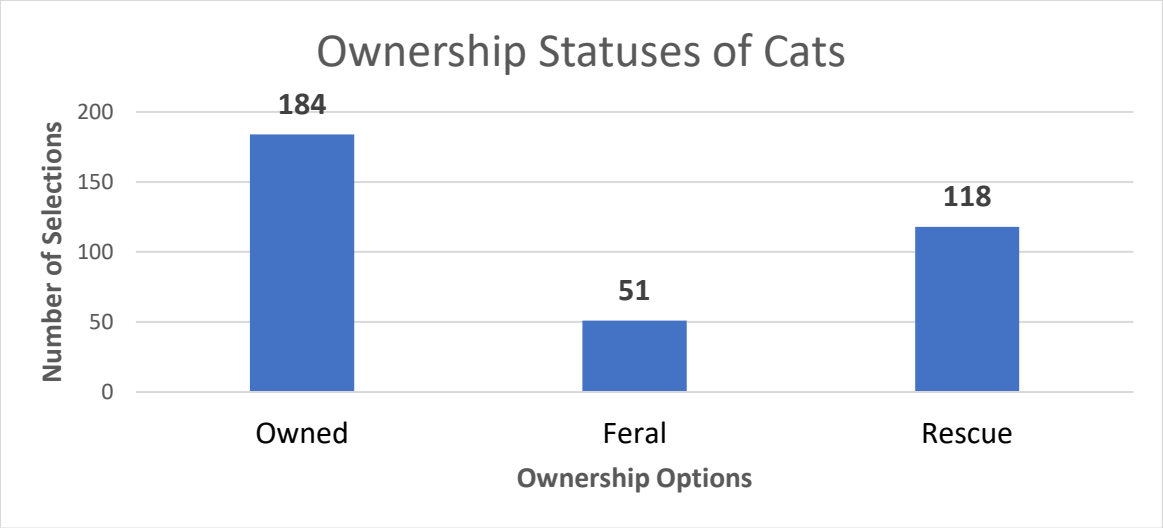

Figure 8: Results for the ownership statuses of cats represented in the online survey responses

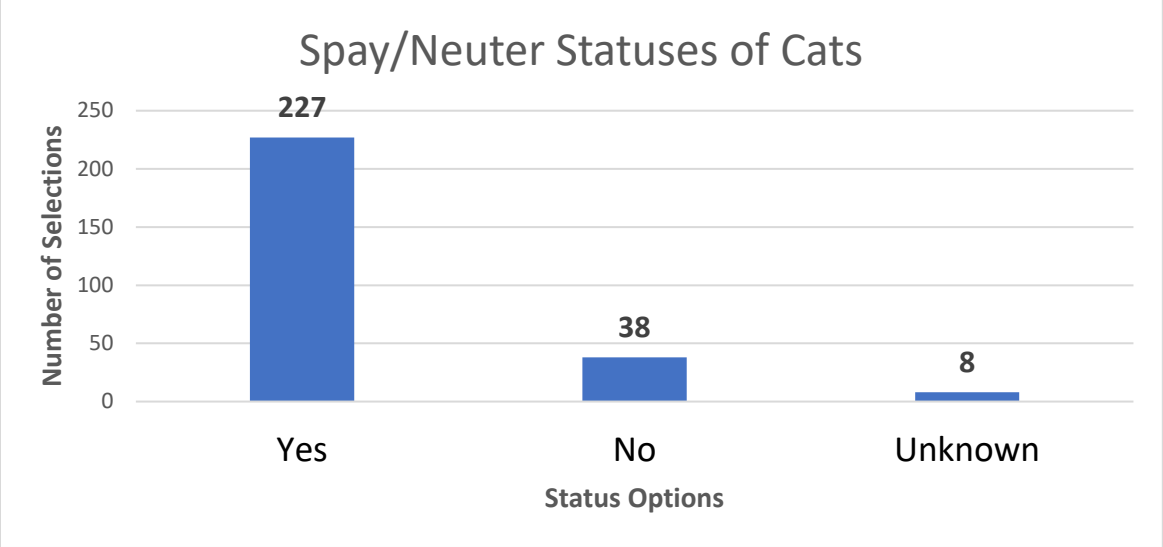

Figure 9: Results for the spay/neuter statuses of cats represented in the online survey responses

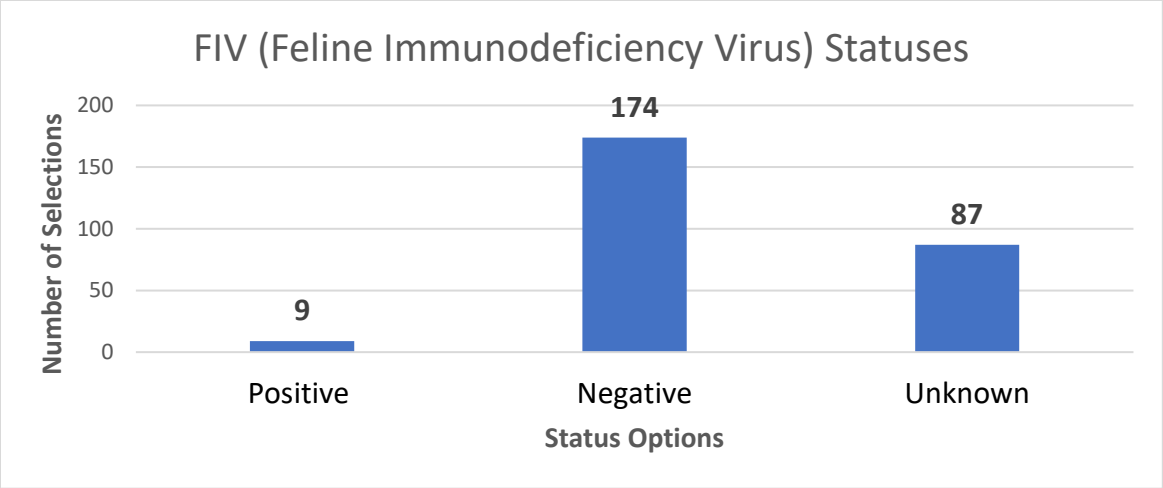

Figure 10: Results for the FIV statuses of cats represented in the online survey responses

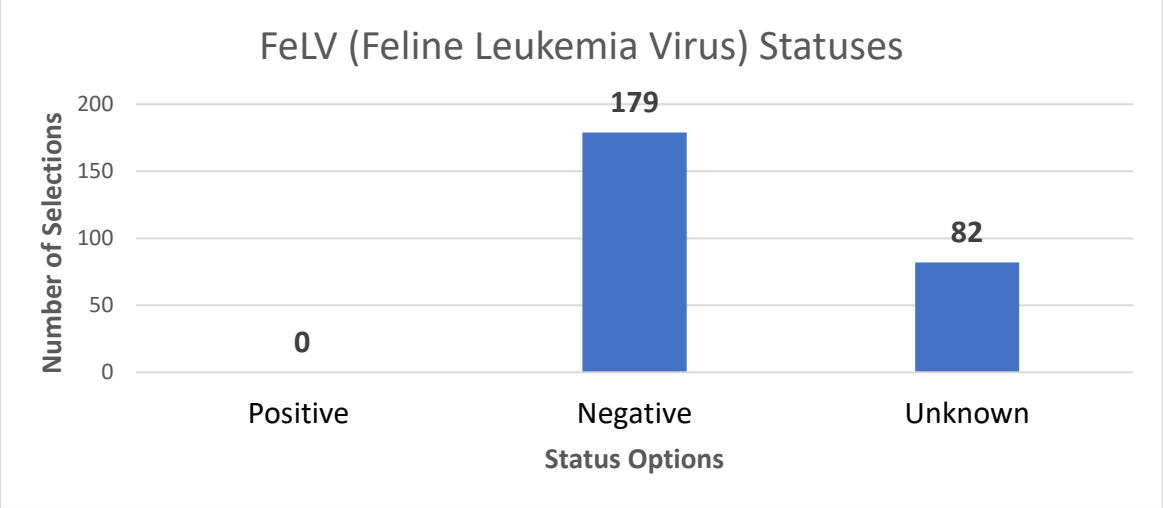

Figure 11: Results for the FeLV statuses of cats represented in the online survey responses

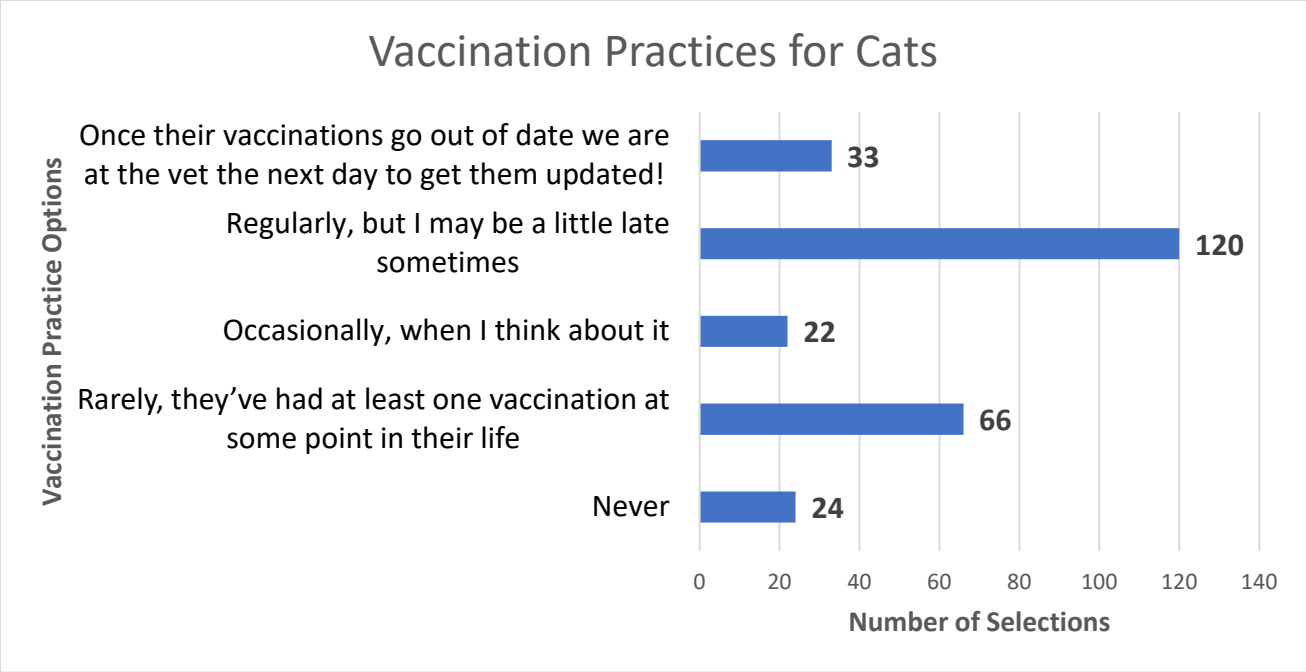

Figure 12: Results for the vaccination practices provided to the cats represented in the online survey responses

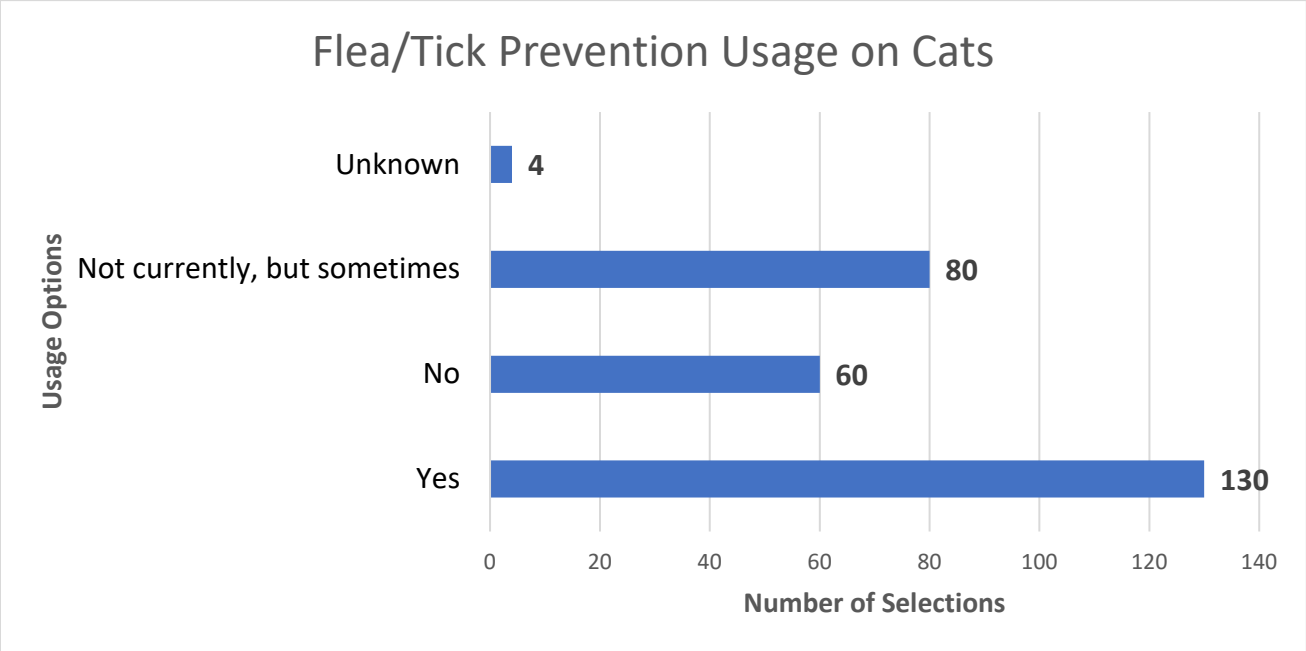

Figure 13: Results for flea/tick prevention usage on cats represented on the online survey responses

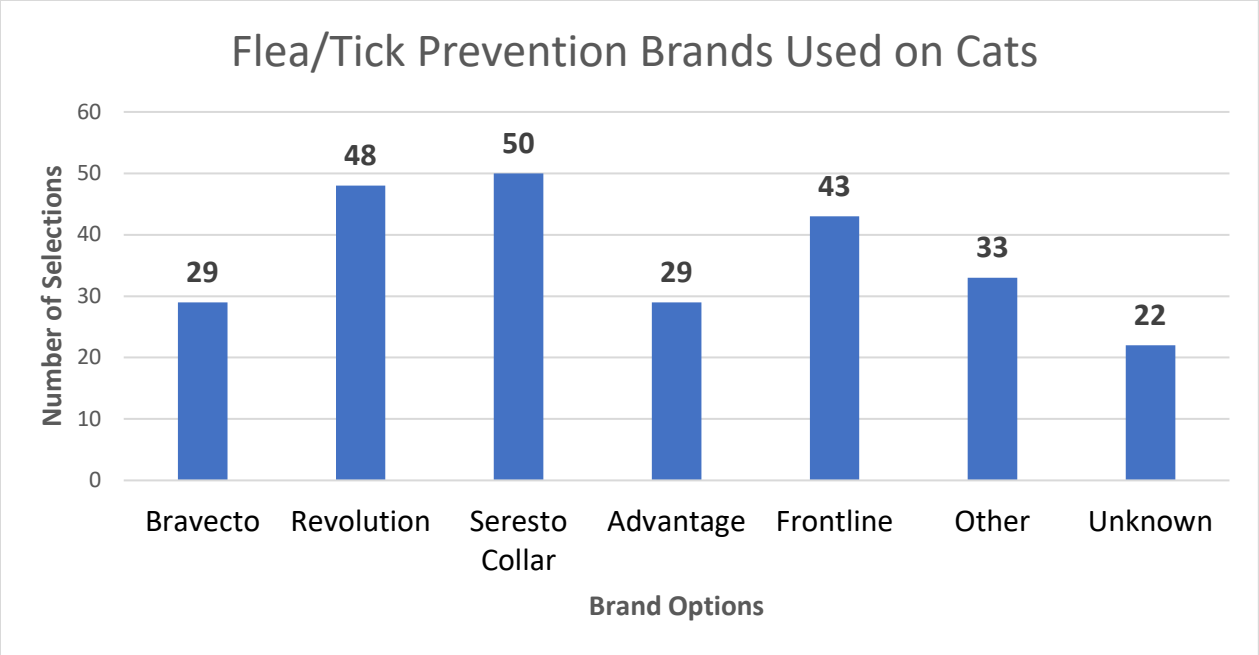

Figure 14: Results for the most commonly used brands of flea/tick prevention used on cats represented in the online survey responses

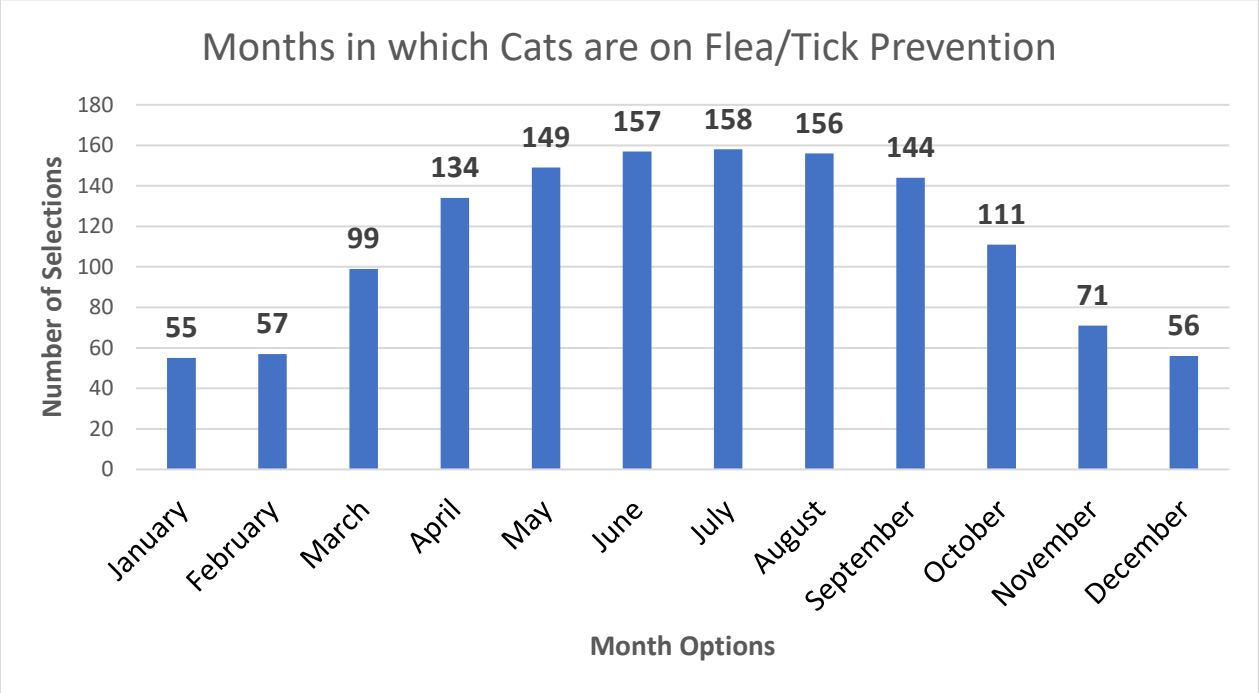

Figure 15: Results for the months out of the year that flea/tick prevention is used on the cats represented in the online survey responses

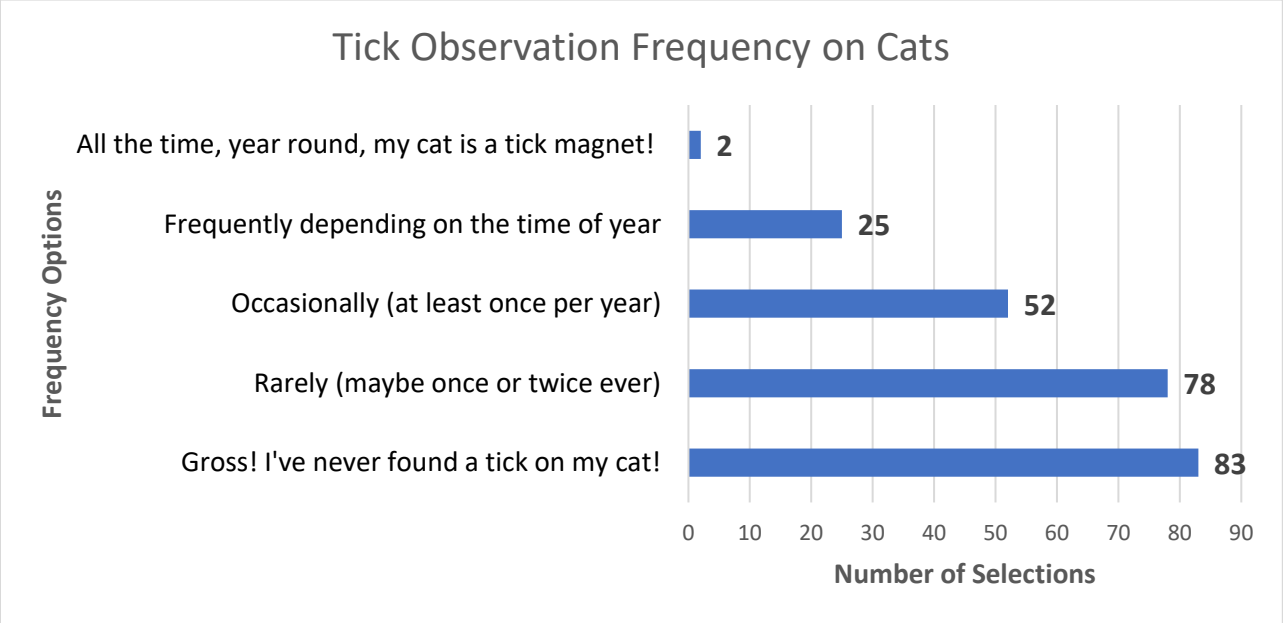

Figure 16: Results for the tick observation frequencies on the cats represented in the online survey responses

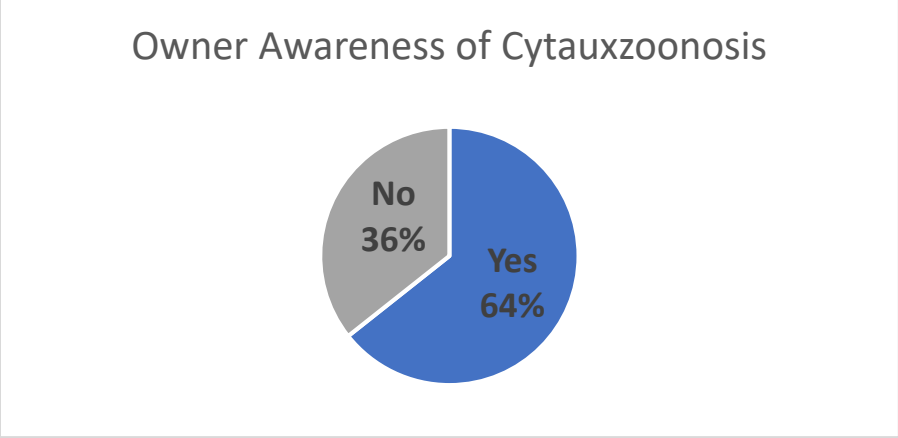

Figure 17: Pie chart displaying the percentages of owners who had and had not heard of cytauxzoonosis prior to taking the online survey

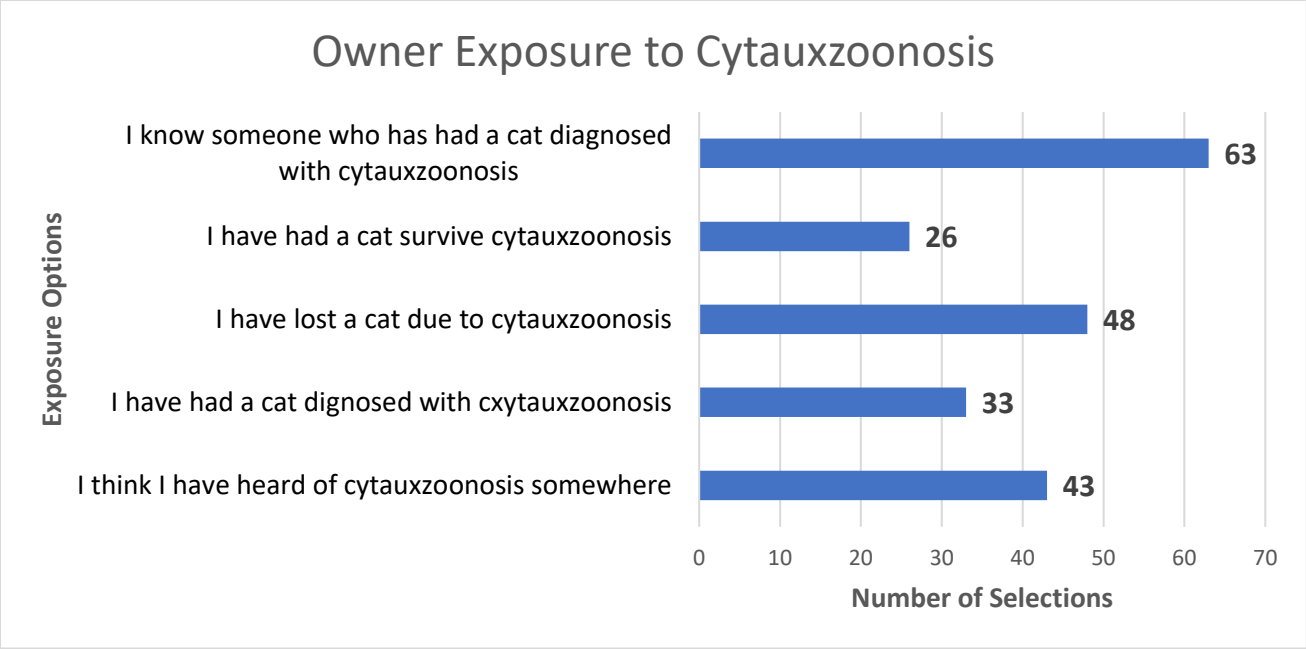

Figure 18: Results for the different owner exposure incidences to cytauxzoonosis from the online survey

References

Allen, K. E., Thomas, J. E., Wohltjen, M. L., & Reichard, M. V. (2019). Transmission of Cytauxzoon felis to domestic cats by Amblyomma americanum nymphs. *Parasites & vectors*, *12*(1), 28.

Cohn, L. A., Shaw, D., Shoemake, C., & Birkenheuer, A. J. (2020). Second illness due to subsequent Cytauxzoon felis infection in a domestic cat. *Journal of Feline Medicine and Surgery Open Reports, 6*(1).

Cohn, L., Birkenheuer, A., Brunker, J., Ratcliff, E., & Craig, A. (2010). Efficacy of Atovaquone and Azithromycin or Imidocarb Dipropionate in Cats with Acute Cytauxzoonosis. *Journal of Veterinary Internal Medicine, 25*(1), 55-60.

Dryden, M.W. (2009). Flea and tick control in the 21st century: challenges and opportunities. *Veterinary Dermatology*. 20(5-6), 435-440.

Jacobs, C. H. (2018). Prevalence of Cytauxzoon felis (Protista: Apicomplexa) in Feral Cats in Russellville Arkansas. *Journal of the Arkansas Academy of Science*. 72(21).

Lloret, A., Addie, D. D., Boucraut-Baralon, C., Egberink, H., Frymus, T., *et al*. (2015). Cytauxzoonosis in cats: ABCD guidelines on prevention and management. *Journal of Feline Medicine and Surgery, 17*(7), 637-641.

Nagamori, Y., Reichard, M.V. (2015). Feline Tick-Borne Diseases. *Today's Veterinary Practice, Parasitology Expertise from the NVCP.* 69-73.

Reichard, M.V., Baum, K.A., Cadenhead, S.C., & Snider, T.A. (2008). Temporal occurrence and environmental risk factors associated with cytauxzoonosis in domestic cats. *Veterinary Parasitology.* 152(3-4) 314-320.

Reichard, M. V., Rugg, Jady J., Thomas, K. E., *et al.* (2019). Efficacy of a topical formulation of selamectin plus sarolaner against induced infestations of Amblyomma americanum on cats and prevention of Cytauxzoon felis transmission. *Veterinary Parasitology*. 270(1), S31-S37.

Reichard, M.V., Thomas, J.E., Arther, R.G. *et al.* (2013). Efficacy of an Imidacloprid 10 % / Flumethrin 4.5 % Collar (Seresto®, Bayer) for Preventing the Transmission of Cytauxzoon felis to Domestic Cats by Amblyomma americanum. *Parasitology Research.* 112, 11–20.

Rizzi, T.E., Reichard, M.V., Cohn, L.A., Birkenheuer, A. J., Taylor, J. D., Meinkoth, J. H. (2015). Prevalence of *Cytauxzoon felis* infection in healthy cats from enzootic areas in Arkansas, Missouri, and Oklahoma. *Parasites Vectors.* 8(13).

Schreeg M.E., Marr H.S., Griffith E.H., Tarigo J.L., Bird D.M., Reichard M.V., Cohn L.A., Levy M.G., Birkenheuer A.J., Schrîg M.E. (2016). "PCR amplification of a multi-copy mitochondrial gene (cox3) improves detection of *Cytauxzoon felis* infection as compared to a ribosomal gene (18S)". *Veterinary Parasitology.* 225, 123–130.

Sherrill, M. K., & Cohn, L. A. (2015). Cytauxzoonosis: Diagnosis and treatment of an emerging disease. *Journal of Feline Medicine and Surgery, 17*(11), 940-948.

Thomas, J.E., Staubus, L., Goolsby, J. L., & Reichard, M.V. (2016). Ectoparasites of free-roaming domestic cats in the central United States. *Veterinary Parasitology.* 228, 17-22.

Wang, J., Li, T., Liu, G., Zhu, X., & Yao, C. (2017). Two Tales of Cytauxzoon felis Infections in Domestic Cats. *Clinical Microbiology Reviews, 30*(4), 861-885.

Wikander, Y. M., Anantatat, T., Kang, Q., & Reif, K. E. (2020). Prevalence of *Cytauxzoon felis* Infection-Carriers in Eastern Kansas Domestic Cats. *Pathogens (Basel, Switzerland)*, *9*(10), 854.

Zeiman, E. A., Nielson, C. K., & Agustín Jiménez, F. (2018). Chronic Cytauxzoon felis infections in wild-caught bobcats (Lynx rufus). *Veterinary Parasitology.* 252, 67-69.