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DIROFILARIA IMMITIS PREVALENCE IN CANIS LATRANS IN KENTUCKY

Melanie Ann Brandon

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***DIROFILARIA IMMITIS* PREVALENCE IN *CANIS LATRANS* IN KENTUCKY**

A thesis
Presented to
the Faculty of the Huston School of Agriculture
Murray State University
Murray, Kentucky

In Partial Fulfillment
of the Requirements for the Degree
of Master of Science

by Melanie Ann Brandon
May 2021

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Abstract

Dirofilaria immitis (canine heartworm) was found in forty-two (42) of the two hundred seventy-five (275) *Canis latrans* (coyote) necropsied in the state of Kentucky from November 27, 2019 through March 3, 2021. Thirty-five (35) of the positive cases were from western Kentucky region with the other seven spread across the state. With this group of coyotes, one hundred fifty-eight (158) were male and the other one hundred sixteen (116) were female. The estimated age ranged from a pup to senior dogs. A little over forty percent of the dogs were obtained through coyote/predator tournaments; the remaining were acquired from pest control, fur trappers, and vehicular accidents.

Keywords: heartworm, *Dirofilaria immitis*, coyote, *Canis latrans*, domestic dogs

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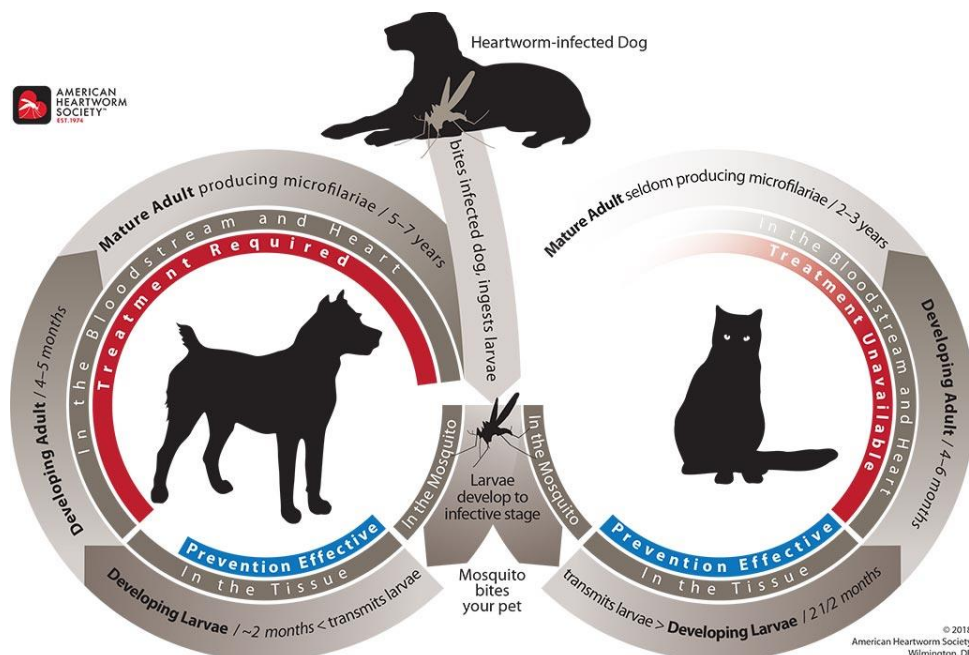
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Chapter I: Introduction

Dirofilaria immitis (*D. immitis*, canine heartworm) is a blood borne parasite that can set up in a variety of mammals and cause serious heart complications. Microfilariae (L1) are present in an infected specimen's bloodstream and are picked up by mosquitoes when taking a blood meal (Strickland & Hoch, 2008). While in the mosquito, the larvae require 8 to 17 days (depending on the weather) to molt two different times (L1 to L2 to L3). The L3 stage is transmitted into another host when the mosquito takes another blood meal from a different mammalian host (Strickland & Hoch, 2008).

Figure 1

American Heartworm Society: Lifecycle of *Dirofilaria immitis*



Once in the mammalian's muscular tissue, the L3 molts into L4 within 12 days of infection (Strickland & Hoch, 2008). In another 50 to 68 days, the L4 will molt into the final larval stage (L5), which is an immature adult. At this stage, the immature adult moves into the vascular system to navigate its way to the heart and the pulmonary

arteries. There they mature over the next 99 to 152 days. Females will grow to about 25 to 30 cm long while the males only grow to about 15 to 18 cm with a corkscrew tail (Strickland & Hoch, 2008).

Since the mosquito is such a vital host in the lifecycle of this parasite it is important to understand its lifecycle as well. Eggs are laid near or on water one at a time or in “rafts” (up to 500 eggs stuck together) (Terminix, 2019). The eggs will not develop in running water or water that has not been standing for at least a week. Once the egg hatches, it turns into a larvae known as a “wiggler”. This stage last for a few days but can vary depending on species and weather. During this stage, they feed on various organic material. Next they molt into pupae called “tumblers”. At this stage, pupa stay near the surface of the water to breathe. They take one to four days to develop into adults. Once an adult emerges from the pupa casing in about twenty-eight, hours they begin breeding. Males will seek out sweet nectar and plant juices while females seek both nectars and blood. A blood meal contains necessary proteins needed to produce eggs (Terminix, 2019).

The canine heartworm can be found in domestic dogs all across the nation, but more often in the southeastern United States. According to the American Heartworm Society (AHS) in 2016, Kentucky did not have any clinics that reported more than one hundred cases that year (American Heartworm Society, 2018). However, most of western Kentucky had 25-100 cases reported, while the rest of the state reported only 1-25 cases per clinic (American Heartworm Society, 2018).

The canine heartworm can also infect other species, including wildlife, given the right environmental conditions. *Canis latrans* (coyote) is one of those species of animal

that the canine heartworm can infect. Coyotes migrated to Kentucky within the last 50 years, breeding with the local domestic dogs as well as the gray and red wolves that were once native to the area (William F. Ekstrom Licrary, n.d.). Due to their ability to adapt to just about any environment, the coyote population has begun to encroach suburb residences.

Unfortunately, this allows for closer proximity between coyotes and domestic dogs (pets). While a number of dog owners keep their pets on heartworm preventives, there is no way to keep the coyote population on preventives, nor is there a way to eradicate the mosquito population (the host of heartworms).

Chapter II: Methodology

Coyote carcasses were obtained through four main revenues from all across the state of Kentucky: fur trapping, coyote calling tournaments, pest control, or vehicular accident. The majority of carcasses collected for fur trapping came from one trapper in Monroe county. There were three calling tournaments that carcasses were collected from: Kentucky Predator Hunting (2020, 2021), West Kentucky Howlers (2020, 2021), and Terry Brother's Hunting Club (2021). The carcasses collected from pest control or vehicular accidents were mostly western Kentucky dogs from locals that knew about the project. Kentucky Fish and Wildlife contributed a couple of coyotes from locals in the Lexington area that had been removed from horse and cow farms.

All carcasses were brought to Breathitt Veterinary Center in Hopkinsville, Kentucky for processing, they were required to be dead before arrival. Due to coyotes being deceased before laboratory staff handled them, an IACUC (Institutional Animal Care and Use Committee) protocol was not needed. Once a carcasses was submitted, the animal nor any parts were allowed to be give back to the submitter. Animals were marked according to county and trapper/hunter before being brought to the facility. Everyone was required to fill out a survey detailing information on the submitted carcass: location, date and time of death, the reason for death, and any unusual behavior before death. Any carcasses that were not from the western Kentucky area or picked up same day from tournaments, were frozen to preserve the integrity of the carcass. Once in the facility, all animals were put into a cooler to wait processing. Being in the cooler slowed decomposition for those carcasses not frozen until a pathologist was able to process them.

Each coyote was given an internal accession number to allow for easy cataloging on the laboratory's system.

Necropsy

Coyotes were moved from the cooler individually, weighed and their fur coat was accessed. Some coyotes were submitted skinned (fur trappers), fur quality was notated on submission sheet. Fur coats were accessed on a scale of poor, fair, good, excellent. Poor coats were those with little (<50%) to no hair; fair coats were missing patches of hair, but more than 50% of the coat was present; good coats had all of the coat present; excellent coats had all the coat present and there was a shine to it like they had recently been groomed. If ticks were observed during examination of coat, they were removed and stored individually (per coyote) in alcohol. These ticks were marked with the internal accession number and county; these were to be used in another study to test what pathogens they might be carrying. Overall body condition (scored 1-9) was based on outward appearance as well as fat content around kidneys, heart, and intestines. Any noticeable bone deformities were notated.

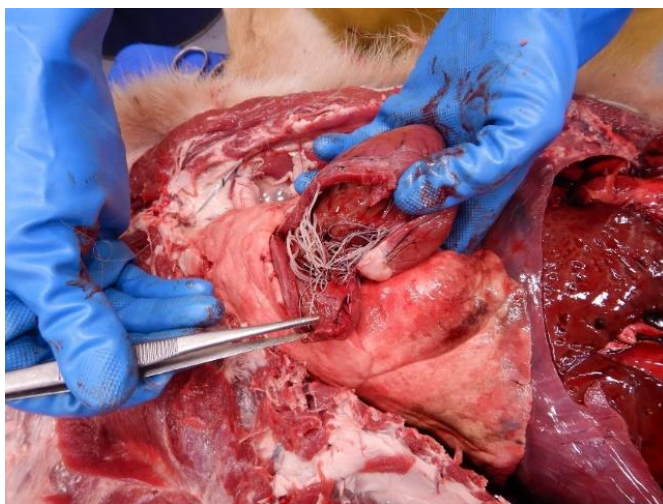
Sex was determined by looking at the genitalia of the coyote. An age estimate was given: juvenile, adult, or senior. This was determined by tooth wear. If they still had some or all of their baby teeth, they were considered to be juveniles. Those coyotes with adult bright white teeth with some to no wear were labeled adults. Coyotes that had numerous incisors missing and major wear to their canines were considered seniors. The lower jaw was removed to allow a technician to remove one or both lower canine teeth. These teeth were put into an individual paper envelopes and frozen to be mailed to Matson's Laboratory in Montana for age analysis at a later date.

Carcasses were opened to expose the chest and abdominal cavities. Any gun shots/injuries that might hinder the report were notated (heart shot, gut shot). The spleen was located and a portion was removed to be frozen for the same study as the ticks. Spleens will help determine what tick-borne diseases the coyote might be carrying. Fecal material was collected for the first hundred coyotes for fecal flotations. Fecal flotations were performed with sucrose solution and 2 grams of fecal material. The last hundred fifty coyotes' fecal material were frozen. Kentucky Fish and Wildlife requested frozen fecal material for a study on *Echinococcus* sp.

Ribs were removed on the top side to expose the heart and lungs. The heart was opened to observe the presence or absence of heartworms. Heartworms were removed, and the inferior vena cava and pulmonary artery branches were checked. If coyotes were shot in the chest, sometimes heartworms could be found in clots outside the heart but within the chest cavity. All heartworms (and pieces) were stored in formalin till they were fixed. The carcasses were to be incinerated with other waste from the laboratory.

Figure 2

Adult Male Coyote: Heartworms



Note. Numerous heartworms observed in right atrium of male coyote.

Fixed heartworms were stored for a week to three months before counting and sexing. Heartworms were removed from one container at a time to preserve the identity of the coyote. Male heartworms contain an obvious corkscrew tail. Female heartworms are approximately double the size of males. Once worms were separated by sex, they were counted. If pieces of heartworms were easily put together, they were counted as whole, if not they were notated as pieces.

Chapter III: Analysis

The prevalence of heartworms in coyotes from areas sampled around the state is relatively low at 15.27% (42/275), the western part of the state sits at 36.8% prevalence (35/97). The rest of the regions sit below the average for the state (1.64-11.76%).

Table 1

Percent with Heartworms (N=275)

Region in Kentucky	Per Region	Overall
Western	36.08	12.73
North Central	1.64	0.36
South Central	3.49	1.09
Eastern	11.76	0.73

Note. Mode is **bold**.

Table 2

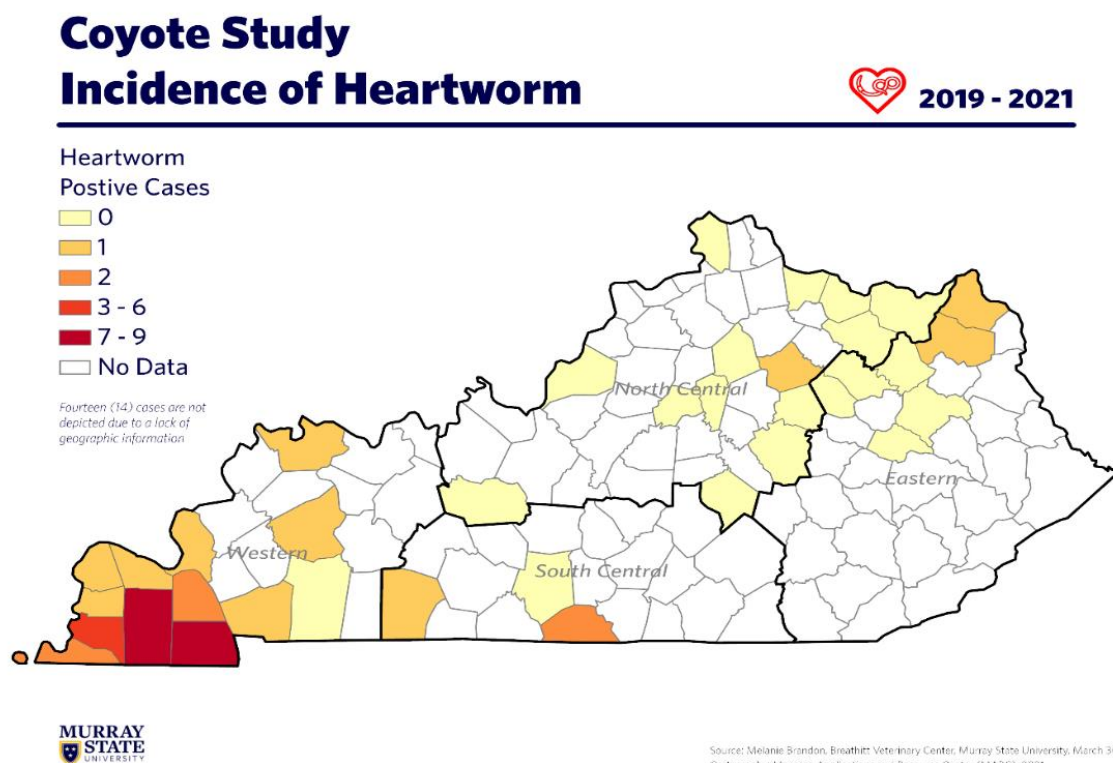
Number of Coyotes Per County (N=275)

County	Number	Number with heartworms
Anderson	3	0
Ballard	3	1
Barren	11	0
Bath	5	0
Boone	3	0
Bourbon	2	1
Bracken	3	0
Calloway	29	8
Carlisle	2	1
Carter	1	1
Christian	1	0
Clark	8	0
Fleming	7	0
Fulton	14	2
Grant	5	0
Grayson	1	0
Graves	19	9
Greenup	6	1
Hickman	8	6
Henderson	5	1
Hopkins	1	1
Jefferson	1	0

Table 2*Number of Coyotes Per County (N=275)*

<i>County</i>	<i>Number</i>	<i>Number with heartworms</i>
Lewis	3	0
Lincoln	1	0
Livingston	5	1
Logan	6	1
Madison	2	0
Marshall	6	2
Mason	2	0
McCracken	1	1
Montgomery	2	0
Monroe	69	2
Morgan	1	0
Muhlenberg	2	1
Nicholas	2	0
Owens	1	0
Rowan	1	0
Scott	1	0
Trigg	1	1
Wolfe	1	0
Woodford	16	0
Unknown	14	1
Total	275	42

Figure 3



Weights of the carcasses were noted as well as the weights of the hearts; the ratio of these numbers gave a cardiac weight. This number shows the overall health of the heart for each coyote. The range used was originally determined for domestic dogs. According to *Pathologic Basis of Veterinary Disease*, anything with ratio less than 0.75% was considered non-athletic specimens, while anything close or more than 1.25% was considered athletic (McGavin & Zachary, 2007). Many coyotes fell between these two numbers. There was no significance difference in cardiac weight between coyotes with heartworms and those without heartworms. There was also no noticeable regional pattern to cardiac weight.

Figure 4

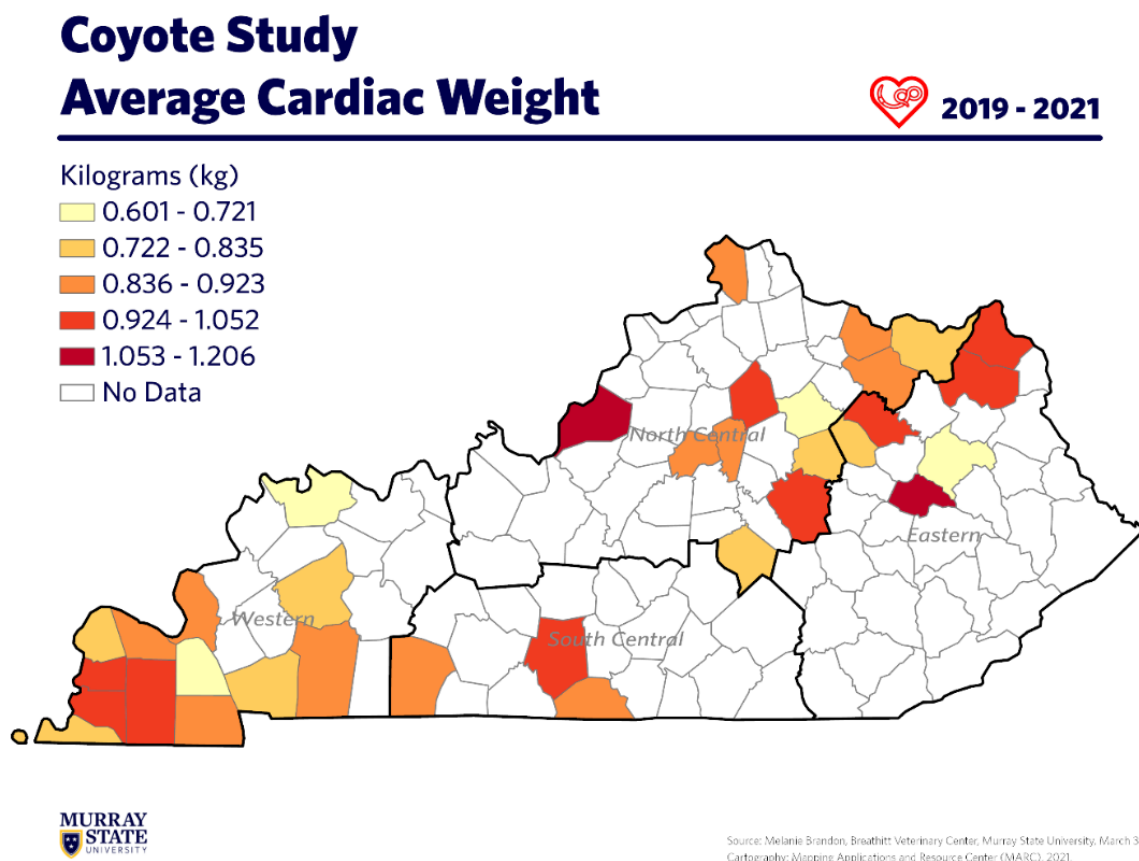
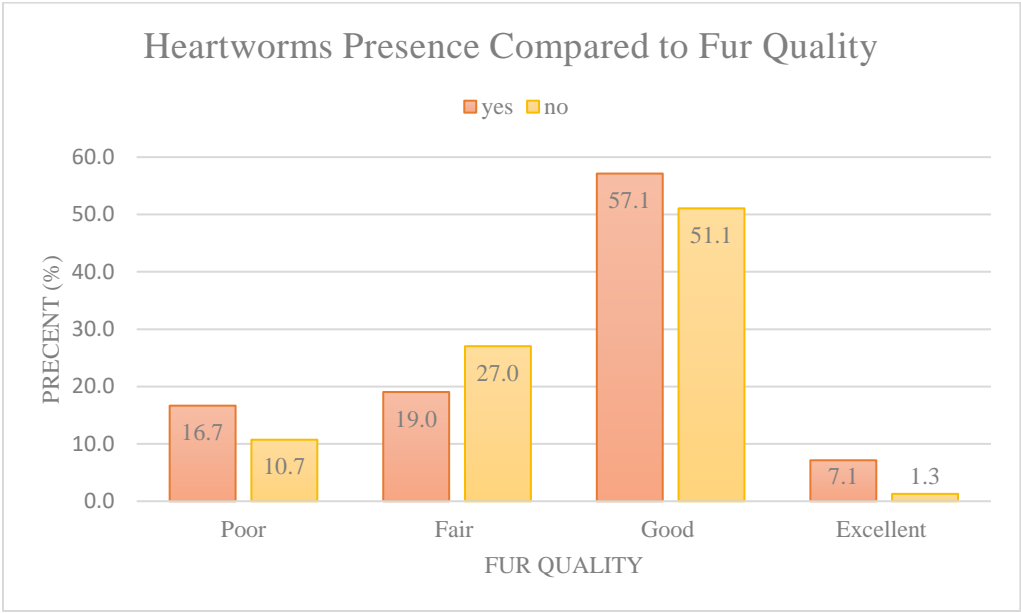


Table 3

<i>Cardiac Weight (N=275)</i>		
<i>Heartworms Present in Heart</i>	<i>Average (%)</i>	<i>Standard Deviation</i>
No	0.8909	0.15
Yes	0.8867	0.12

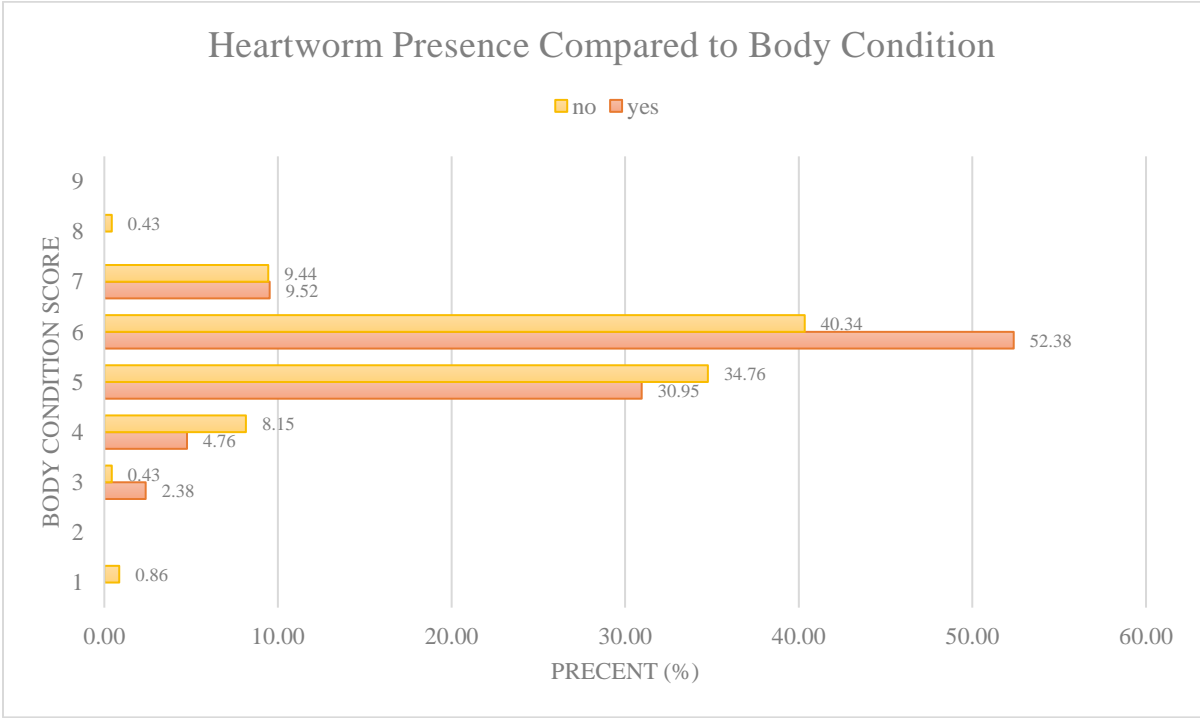
Fur quality was determined on most carcasses to help assess overall health. Most furs were considered to be in good (143) or excellent (6) condition. Seventy-one were marked fair and thirty-two were categorized poor. Body condition was determined to be between 5-7 for the majority of coyotes, both with and without heartworms.

Figure 7



Note. N=275

Figure 8



Note. N=275

There were twenty-seven positive cases that were male (17.09%) and fifteen that were female (12.93%). There were forty-two more males collected than females. The males (15.2 kg) on average weighed two more kilograms than females (13.06 kg). Eighty-four coyotes were collected from fur-trappers, pest control cases accounted for seventy-two of the carcasses, two were brought in from being hit by a vehicle, and the rest (117) were from predator tournaments across the state.

There were a few coyotes with noticeable limb abnormalities, all of which seemed to hinder mobility to some degree. All three were found to have good body condition, and one female had even carried several litters of pups. Several old coyotes were found to have bad dentitions and worn/missing incisors as well as canine teeth.

Figure 5

Adult Male Coyote: Right Hind Leg



Note. Fibula and tibia were broken; bones completely remodeled and fused back together.

Figure 6

Adult (Senior) Female Coyote: Dentition



Note. Extremely poor dentition on female with numerous other health

Chapter IV: Conclusions

It was expected that the prevalence of heartworms in coyotes to be similar to the occurrence found in domestic dogs. After assessing two hundred seventy-five coyotes, there was only a heartworm prevalence of 15.27% for the entire state of Kentucky. This finding suggest that the state overall does not have a huge issue with heartworms.

However, assessing things on a county and regional level shows that the western part of the state carries the majority of that burden. This correlates with the American Heartworm Society's assessment of domestic dogs from veterinary clinics.

The thought for the regional prevalence is due to the nature of mosquitos' life cycle. Western Kentucky is mostly flat lands with some rolling hills that allow creeks and rivers to flood more easily and produce larger pools of standing water. The further east one moves across the state the more vertical land is encountered. Many creeks and rivers are constantly moving even when flooded due to the elevation change in the land. Also the east tends to have overall cooler weather so mosquitos have a shorter breeding season, and it is harder for pupa to develop.

The cardiac weight is supposed to give an idea about the overall health of an animal. For majority of the coyotes sampled the fell between 0.75-1.25% on cardiac weight. There was no significate difference between those with heartworms and those without. This suggest that heartworm burden does not affect the coyote's overall ability to prosper when infected with heartworms.

No noteworthy difference in either fur quality or body condition when heartworms were present was observed. With other parasitic infections, normally the parasite impedes on the hosts ability to thrive. The thought was with a heartworm burden

within a coyote that they might be more likely to exhibit poorer fur quality or body condition than a coyote without heartworms. The data shows that heartworm burden has little to no effect on the quality of life for coyotes. There was also no significant difference of heartworm burden found between males and females, which suggest both are equally favored to be infected.

Coyotes do not seem to deteriorate when infected with heartworms like domestic dogs. Cardiac weight, body condition, nor fur quality had any substantial difference between those with heartworms and those without heartworms. Now there is no way to prove that coyotes are a reservoir for heartworms, but the data suggest that even if owners were able to eradicate the disease in domestic dogs that preventives would still be needed to prevent infection from coyotes.

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